ANNEX 2

Original: English

September/October 2013

REPORT OF THE MEETING OF THE OIE AQUATIC ANIMAL HEALTH STANDARDS COMMISSION

Paris (France), 30 September–4 October 2013

_____

EU comment
The EU would like to commend the OIE Aquatic Health Standard Commission for its work and for having taken into consideration EU comments on the Aquatic Code and Manual submitted previously.
The comments of the EU on the draft Aquatic Code and Manual chapters circulated for member country comment are inserted in the text of the report of the September/October meeting below, while some specific comments are inserted in the text of the respective annexes to the report.
The EU would like to stress again its continued commitment to participate in the work of the OIE and to offer all technical support needed by the Aquatic Animals Commission and its a*d hoc* groups for future work on the Aquatic Code and Manual.

The OIE Aquatic Animal Health Standards Commission (hereinafter referred to as the Aquatic Animals Commission) met at the OIE Headquarters from 30 September to 4th October 2013.

Details of participants and the adopted agenda are given at Annexes 1 and 2.

The Aquatic Animals Commission reviewed the documents identified in the agenda, addressing comments that Member Countries had submitted by 30 August 2013 and amended texts in the OIE *Aquatic Animal Health Code* (the *Aquatic Code*) and the OIE *Manual of Diagnostic Tests for Aquatic Animals* (the *Aquatic Manual*) where appropriate. The amendments are shown in the usual manner by double underline and strikethrough and may be found in the Annexes to the report.

Member Countries should note that, unless stated otherwise, texts submitted for comment may be proposed for adoption at the 82nd OIE General Session in May 2014. Depending on the comments received on each text, the

Commission will identify the texts proposed for adoption in May 2014 in the report of its February 2014 meeting.

The Aquatic Animals Commission strongly encourages Member Countries to participate in the development of the OIE's international standards by submitting comments on this report. It would be very helpful if comments were submitted as specific proposed text changes, supported by a scientific rationale. Proposed deletions should be indicated in 'strikethrough' and proposed additions with 'double underline'. Member Countries should not use the automatic 'track-change' function provided by word processing software as such changes are lost in the process of collating Member Countries' submissions into the Commission's working documents.

The table below summarises the texts presented in the Annexes. Annexes 3 to 12 are presented for Member Country comment; Annex 13 is presented for Member Countries' information.

Comments on this report must reach OIE Headquarters **prior to 24 January 2014** to be considered at the February 2014 meeting of the Commission. All comments should be sent to the OIE International Trade Department at: trade.dept@oie.int.

Texts for Member Countries' comments	**Annex number**
***Aquatic Code*:**	
Glossary	Annex 3
Notification of diseases and epidemiological information (Chapter 1.1.)	Annex 4
Criteria for listing aquatic animal diseases (Chapter 1.2.)	Annex 5
Necrotising hepatopancreatitis (Chapter 9.4.)	Annex 6
Infection with infectious salmon anaemia virus (Chapter 10.5.)	Annex 7
Model articles showing horizontal amendments	Annex 8
Criteria for determining susceptibility of aquatic animals to specific pathogenic agents (new chapter X.X.)	Annex 9
Infection with *Salmonid alphavirus* (New chapter 10.X.)	Annex 10
***Aquatic Manual*:**	
Infection with infectious salmon anaemia *(*Chapter 2.3.5.)	Annex 11
Infection with ostreid herpesvirus 1 microvariant (Chapter 2.4.9.)	Annex 12
Annexes for Member Countries' information	
Aquatic Animal Health Standards Commission Work Plan for 2012/2014	Annex 13

1. OIE *Aquatic Animal Health Code*

The Aquatic Animals Commission wished to emphasise that the issue of emerging diseases is important in light of the frequent emergence in aquaculture of new diseases some of which have significant impact on productivity, resource sustainability and trade. Diseases emerge regularly in aquatic animals due to newly domesticated species, the large number of species farmed and the rapid growth of aquaculture production.

During this meeting, the Aquatic Animals Commission touched upon several issues related to emerging diseases and how they are addressed in the *Aquatic Code*; this included the definition of emerging disease (Glossary), mechanisms for notification of an emerging disease (Chapter 1.1.) and the mechanism for listing emerging diseases (Chapter 1.2.). These sections of the *Aquatic Code* together assist Member Countries to recognise important emerging diseases and prevent their spread while continuing safe trade.

In this report, the relevant items are presented in Items 1.1, 1.2, and 1.3, (i.e. Glossary, Chapter 1.1. and Chapter 1.2., respectively). The Aquatic Animals Commission recommends that Member Countries comments are made taking into consideration the inter-relatedness of these items.

1.1. Glossary

Veterinarian

In light of the recently adopted amendment to the definition for 'veterinarian' in the *Terrestrial Code*, the Aquatic Animals Commission agreed to amend the definition in the *Aquatic Code* to align it with the *Terrestrial Code* definition.

Emerging disease

The Aquatic Animals Commission considered the proposal for a revised definition of 'emerging disease' developed by the Terrestrial Code Commission which primarily aimed at improving clarity.

The Aquatic Animals Commission noted two different circumstances of disease emergence: new pathogens or an epidemiological change of known pathogens. This is reflected in the amended definition.

In aquaculture, emergence of diseases caused by unknown pathogens is not uncommon, and therefore the definition in the aquatic context needs to include the situation where an infectious agent is strongly associated with a disease although not proven to be the aetiological agent. The Aquatic Animals Commission noted that this situation is currently captured in the criteria listing an emerging disease (Chapter 1.2.). In light of this, the Commission further amended the definition to read:means a *disease* ~~newly recognised~~ ~~or~~ *infection* ~~occurrence,~~ which has a significant impact on animal or public health~~, of a *disease*,~~ resulting from: ~~the evolution or~~

- a change of a~~n~~ known ~~existing~~ pathogenic agent~~, a known *infection*~~ or its spread~~ing~~ to a new geographic area or species ~~*population*,~~; or

- a newly ~~previously un~~recognised or suspected pathogenic agent ~~or *disease* diagnosed for the first time and which has a significant impact on aquatic animal or public health~~.

The Aquatic Animals Commission clarified that this definition excludes the diseases listed in the *Aquatic Code*.

The Aquatic Animals Commission wished to inform Member Countries that comments received on this amended definition will be submitted to the Terrestrial Code Commission at their next meeting (11-18 February, 2014) for their consideration to maintain alignment between the definition used in the two *Codes*, where relevant. Subsequently, the Aquatic Animals Commission will consider Member Countries comments along with the Terrestrial Code Commission proposal at their next meeting.

Other relevant items on this topic are presented in Items 1.2. and 1.3. (i.e. Chapters 1.1. and 1.2.). The Aquatic Animals Commission recommends that Member Countries comments are made taking into consideration the inter-relatedness of these items.

Susceptible species

The Aquatic Animals Commission considered that the second sentence of the current definition for 'susceptible species' does not contribute to defining this term. Consequently the Commission proposed to delete this sentence. In addition, the Commission proposed a change in the first sentence to improve clarity.

'means a species of aquatic animal in which infection has been demonstrated by natural cases or by experimental exposure~~s~~ to the pathogenic agent that mimics the natural pathways ~~for infection. Each disease chapter in the Aquatic Code and the Aquatic Manual contains a list of currently known susceptible species~~.'

The revised Glossary is presented at Annex 3 for Member Countries' comments.

EU comment

The EU in general supports the proposed the proposed changes to the glossary. However, some comments are inserted in the text of Annex 3.

1.2. Notification of diseases and epidemiological information (Chapter 1.1.)

The Aquatic Animals Commission considered the amendments to this chapter being proposed by the Terrestrial Code Commission which are primarily aimed at improving clarity. The Aquatic Animals Commission incorporated the Terrestrial Code Commission's proposed amendments into the revised chapter, with the exception in point e) of Article 1.1.3. For this point the Terrestrial Code Commission proposed the use of the words 'unusual host species'. However, the Aquatic Animals Commission proposed to use the word 'new host species' instead of 'unusual host species' because they considered that the term 'unusual' is ambiguous and that 'new host species' are important for trade and should be reported.

The Aquatic Animals Commission wished to emphasise some significant changes with regards to emerging diseases have been made. These include:

(i) the deletion of point f) in Article 1.1.3. (' ~~an *emerging disease* with significant morbidity or mortality, or zoonotic potential;~~') which clarifies that the definition for emerging disease excludes OIE listed diseases;

(ii) the development of a new Article 1.1.3.bis which clearly separates the different notification obligations for an emerging disease, i.e. the obligation to notify an emerging disease, and the circumstances under which reporting should be terminated.

The Aquatic Animals Commission noted that the information provided to Member Countries on an emerging disease is currently made via the OIE Disease Information Alert System. The Commission had concerns that this mechanism may overemphasise the impact or importance of some emerging disease events and discourage reporting. The Commission agreed to raise this matter with the OIE Director General.

Other relevant items on this topic are presented in Items 1.1 and 1.3. (i.e. Glossary and Chapter 1.2). The Commission recommended that Member Countries' comments are made taking into consideration the inter-relatedness of these items.

The revised Chapter 1.1. is presented at Annex 4 for Member Countries' comments.

EU comment

The EU thanks the OIE and in general supports the amendment to this chapter. Specific comments are inserted in the text of Annex 4.

1.3. Criteria for listing an emerging disease (Chapter 1.2.)

The Aquatic Animals Commission considered the importance of recognising emerging diseases at an early stage and communicating information about these events among Member Countries. In particular, the Commission considered recent examples such as Acute hepatopancreatic necrosis syndrome (AHPNS) and Infection with ostreid herpesvirus 1 microvariant (OsHv-1).

Despite the current notification and reporting obligations for emerging diseases described in point 1.f) of Article 1.1.3., notification and reporting have been inconsistent. The Aquatic Animals Commission considered that the revised definition for an emerging disease and revised Chapter 1.1., including Article 1.3.bis, both provided clarity on notification and reporting obligations for emerging diseases.

The Aquatic Animals Commission reconsidered the utility of the mechanism for listing emerging diseases (which utilises the criteria described in Article 1.2.3.). The Commission considered that this mechanism is not sufficiently responsive for effective communication of the occurrence of an emerging disease. This article provides an additional mechanism that requires Member Countries to report on specific listed

emerging diseases. However, this mechanism has established requirements for reporting only for a small number of listed emerging diseases.

The Aquatic Animals Commission also noted that the amended definition for emerging diseases now reflects the set of criteria in Article 1.2.3.

Considering the improved clarity on requirements for notification and reporting emerging diseases, clarified definition for emerging diseases, and the slow mechanism for listing an emerging disease, the Aquatic Animals Commission proposed that Article 1.2.3. be deleted.

Other relevant items on this topic are presented in Items 1.1. and 1.2. (i.e. Glossary and Chapter 1.1.). The Aquatic Animals Commission recommends that Member Countries comments are made taking into consideration the inter-relatedness of these items.

The revised Chapter 1.2. is presented at Annex 5 for Member Countries' comments..

EU comment

The EU thanks the OIE and strongly supports the proposed changes to this chapter.

1.4. Diseases listed by the OIE (Chapter 1.3.)

Acute hepatopancreatic necrosis syndrome

Following on from previous discussions held by the Aquatic Animals Commission at their March 2013 meeting on Acute hepatopancreatic necrosis syndrome (AHPNS), the Commission reviewed current information on this syndrome.

The Aquatic Animals Commission considered that the available information may be insufficient to assess the disease against the criteria in Article 1.2.2. In particular, uncertainty on pathogen identity (among strains of *Vibrio parahaemolyticus*) and lack of availability of a specific diagnostic test (to differentiate the causative agent from other strains of *V. parahaemolyticus*) means that criteria for listing are unlikely to be met. In light of the proposal to delete Article 1.2.3. for listing of an emerging disease, the Aquatic Animals Commission does not consider it appropriate to list AHPNS as an emerging disease.

However, the Aquatic Animals Commission agreed that the disease meets the definition of an emerging disease and that Member Countries should notify the occurrence of this disease in accordance with Article 1.1.3.

The Commission drafted an OIE Technical Factsheet on Acute Hepatopancreatic Necrosis Syndrome (AHPNS) with input from experts. This Factsheet will be uploaded onto the OIE web page and will be regularly reviewed as new knowledge becomes available.

The Commission invites Member Countries to provide any relevant information that would contribute to improving the Technical Factsheet on Acute Hepatopancreatic Necrosis Syndrome (AHPNS), and more specifically information on how to diagnose the disease.

EU comment

The EU in general supports the OIE position on AHPNS. The EU will, however, inquire establishing a central repository for confirmed infectious materials which can be used to investigate diagnostic methods and to carry out susceptibility trials with appropriate hosts. The EURL for crustacean diseases, which is ready to carry out such work and to contribute data arising from such work to the OIE for the Technical Factsheet as it becomes available, has experienced some reluctance from current expert laboratories to release materials for such work.

1.5. Control of hazards in aquatic animal feeds (Chapter 6.1.)

The Aquatic Animals Commission discussed how to review the purpose, scope and content of Chapter 6.1. The Commission agreed that the main objective of this chapter should be the prevention of spread of infectious diseases via aquatic animal feed. The Commission also agreed that this chapter should assist Member Countries in identifying risk pathways and assessing risk related to aquatic animal health hazards in aquatic animal feed.

The Commission will develop a revised chapter for consideration at their February 2014 meeting. The Commission invites Member Countries comments on this proposal.

EU comment

The EU supports this OIE proposal.

1.6. Necrotising hepatopancreatitis (Chapter 9.4.)

In light of the outcomes of the International Committee for Taxonomy of Bacteria regarding the new name for necrotising hepatopancreatitis bacteria (NHP-B) to Candidatus *Hepatobacter penaei*, the Aquatic Animals Commission proposed to amend Article 9.4.1. to incorporate this change.

The revised Chapter 9.4. is presented at Annex 6 for Member Countries' comments.

EU comment

As this seems not to be the definite name of the agent ["Candidatus"], the EU is of the opinion that it might be too early to change the pathogen name in the Aquatic Code. This should only be done once the change of the scientific name has been decided by the International Committee for Taxonomy of Bacteria, which might be in time before the next OIE General Session in May 2014. Otherwise it would be preferable to wait until May 2015 with this change.

1.7. Infection with infectious salmon anaemia virus (Chapter 10.5.)

In response to a Member Country comment at the May 2013, OIE General Session requesting clarification of the text in Article 10.5.5. point 1) regarding the absence of susceptible species and the declaration of country freedom from HPR-deleted ISAV, the Aquatic Animals Commission proposed to delete this point and the equivalent point in Article 10.5.7. (zone or compartment) as they considered that these points are redundant and these pathways to claim freedom are covered in the Articles 10.5.4. and 10.5.6., respectively.

Whilst reviewing this chapter, the Aquatic Animals Commission also realised that a specific article on the 'Importation of aquatic animal products from a country, zone or compartment declared free from infection with HPR-deleted infectious salmon anaemia virus' was missing. The Commission introduced a new Article 10.5.15bis to address this circumstance.

The Aquatic Animals Commission wished to note that horizontal amendments proposed in Item 1.8. have also been incorporated into this amended chapter.

The revised Chapter 10.5. is presented at Annex 7 for Member Countries' comments.

EU comment

The EU in general supports the amendment of this chapter. Specific comments are inserted in the text of Annex 7.

1.8. Horizontal issues

The Aquatic Animals Commission noted a number of inconsistencies in the inclusion of some relevant text in some of the disease specific chapters and proposed the following amendments in all disease specific chapters.

All disease specific chapters, amend:

Point 2 of Articles 10.X.9. and 10.X.10. as indicated below:

These proposed amendments were adopted in May 2013, OIE General Session in Chapter 10.2. This amendment to all disease specific chapters would ensure alignment across all chapters.

Article 10.X.9.

2) water used in transport and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of 'Disease X' or is disposed in a manner that prevents contact of waste with *susceptible species*.

Article 10.X.10.

2) water used in transport and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of 'Disease X'. This Article does not apply to *commodities* referred to in point 1 of Article 10.X.3.

Point 3 of Articles 10.X.4. and 10.X.5. as indicated below:

The Aquatic Animals Commission proposed these amendments to improve clarity on the circumstances of claiming freedom for these points. The reference to ten years and absence of conditions conductive to clinical expression are unnecessary and create ambiguity.

3) A country where ~~the last observed occurrence of the *disease* was within the past ten years or where~~ the disease ~~*infection*~~ status prior to *targeted surveillance* was unknown ~~(e.g. because of the absence of conditions conducive to clinical expression as described in the corresponding chapter of the *Aquatic Manual*)~~ may make a *self-declaration of freedom* from Disease X when:

   a) *basic biosecurity conditions* have been continuously met for at least the past two years; and

   b) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of Disease X.

Point 4d) of Articles 10.X.4. and 10.X.5., as indicated below:

The Aquatic Animals Commission proposed these articles be amended as they considered that implementation of modified 'basic biosecurity conditions' is relevant immediately upon eradication of disease and the 2 year period is not relevant to this point.

4d) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place since ~~for at least the past two years~~ eradication of the *disease*.

Articles 10.X.7. (all disease specific chapters) and Article 10.X.11. (in fish and amphibian chapters) or Article 10.X.10 (in mollusc and crustacean chapters)

The Aquatic Animals Commission noted that some articles were missing the correct cross reference to other relevant articles. They proposed that the relevant text be amended to ensure the correct cross referencing to other articles regarding maintenance of free status.

'Infection with pathogen X' approach

In addition, the Aquatic Animals Commission noted that a number of existing disease specific chapters are titled 'Infection with pathogen X' but this approach has not been applied consistently throughout the chapter. The Commission will ensure that these inconsistences will be corrected in all the relevant disease specific chapters in the 2014 edition of the *Aquatic Code*. The Commission agreed that the recently revised Chapters 10.2 and 10.5. should be used as the model for applying these changes to disease naming nomenclature.

Model articles are provided at Annex 8 for Member Countries' comments.

EU comment

The EU in general supports these proposed model articles. Some comments are inserted in the text of Annex 8.

1.9. Criteria for determining susceptibility of aquatic animals to specific pathogenic agents (new chapter X.X.)

Comments were received from: Australia, Canada, Chinese Taipei, European Union, Japan, Korea, New Zealand, Norway, Russia, Switzerland and the United States of America.

The Aquatic Animals Commission considered Member Countries' comments and amended the draft chapter, as appropriate.

The Aquatic Animals Commission agreed with several Member Countries requesting that this chapter should focus on criteria for listing species as susceptible for their inclusion in Article X.X.2. of disease specific chapters in the *Aquatic Code*. In order to clarify this, the Commission proposed to amend the title of the chapter to 'Criteria for listing species as susceptible to infection with specific pathogen'.

In response to Member Countries' comments, the Aquatic Animals Commission clarified that provisions in the disease specific chapters of the *Aquatic Code* only apply to species that are susceptible to infection which does not include mechanical vectors. In addition, the Commission clarified that 'disease' is defined in the *Aquatic Code* as clinical and non-clinical infection and therefore susceptibility of a species means susceptibility to infection with the causative agent. The proposed criteria are designed to assess susceptibility to infection.

In response to Member Countries' comments that only 'definite susceptible species' should be included in the *Aquatic Code*, the Aquatic Animals Commission recognised possible ambiguity in introducing 'definite susceptible species' and 'possible susceptible species'. Consequently, the Commission amended the chapter to remove these terms.

In addition, Article X.X.7. was amended to clearly indicate that in the situation where evidence exists but is insufficient to demonstrate susceptibility of a species, this species would be included in section 2.2.1. of the relevant disease chapter in the *Aquatic Manual*. The Aquatic Animals Commission included some text emphasising that where a species can reasonably be expected to pose a risk of transmission for the pathogen under consideration, Competent Authorities should assess the risk of introduction of the causative agent through imports of a possible susceptible species, in accordance with the recommendations in the *Aquatic Code*.Consistent with the Aquatic Animals Commission's intention to focus only on susceptible species, the Commission agreed that the proposed Article X.X.8. 'Taxonomic relationship of susceptible species' be deleted. The Aquatic Animals Commission clarified that once the new Chapter X.X. has been adopted, these criteria will be applied to determine susceptible species for each listed disease. The Commission proposed that an ad hoc Group would be convened to undertake assessments against the criteria which would then be circulated for Member Countries' comments. Any subsequent changes to the list of susceptible species in Article X.X.2. of specific disease chapters in the *Aquatic Code*, would then be circulated for Member Countries' comments prior to proposal for adoption.

If this draft chapter is adopted and once the species susceptibility determinations are completed, the Aquatic Animals Commission proposes that the following text in Article X.X.2 (Scope) of the disease specific chapters of the *Aquatic Code* 'These recommendations apply to any other susceptible species referred to in the *Aquatic Manual* when traded internationally' be deleted and be replaced with 'When the recommendations in this chapter are applied to any other species, *Competent Authorities* should conduct a risk analysis in accordance with the recommendations in the *Aquatic Code'*.

The revised Chapter X.X. is presented at Annex 9 for Member Countries' comments.

EU comment

The EU thanks the OIE and supports the proposed amendments to this chapter.

1.10. Infection with Salmonid alphavirus (New chapter 10.X.)

Given the adoption of 'Infection with *Salmonid alphavirus'* as an OIE listed disease, at the May 2013 OIE General Session, the Aquatic Animals Commission developed a new draft chapter on Infection with *Salmonid alphavirus* for inclusion in the *Aquatic Code*.

The Aquatic Animals Commission noted that the list of commodities in Articles 10.X.3 and 10.X.12, and 10.X.13. have been placed 'under study'. The Commission requested that the *ad hoc* Group on Safe Commodities be reconvened to conduct assessments on a range of commodities commonly traded internationally against the criteria provided in Chapter 5.4. and on the safety of disinfected eggs. The Commission requested that they receive the *ad hoc* Group report prior to their February 2014 meeting so that relevant articles can be updated.

The Aquatic Animals Commission wished to note that horizontal amendments proposed in Item 1.8. have been incorporated into this draft chapter.

The new Chapter 10.X. is attached as Annex 10 for Member Countries' comments.

EU comment

The EU commends the OIE and supports the adoption of this new chapter.

1.11. Future work on pathogen differentiation

The Aquatic Animals Commission reviewed all listed diseases and considered possible issues related to the pathogen differentiation approach. The Commission recognised that the Yellow head disease chapter in the *Aquatic Code* requires clarification with regards to scope, and alignment of the *Aquatic Manual* with the *Code*. The Commission will discuss this issue further at their February 2014 meeting.

1.12. *Aquatic Code* - Table of contents

The Aquatic Animals Commission reviewed the Table of contents of the *Aquatic Code* and agreed that Chapter 6.1. would be better placed within Section 4. General recommendations: disease prevention and control. Subsequent to this proposed change, the Commission agreed that the title of Section 6 should be changed in order to better reflect its contents. The Commission proposed that Section 6 'Veterinary Public Health' be renamed 'Recommendations For Antimicrobial Use In Aquatic Animals'. The Commission requested that the OIE International Trade Department implement this proposal in the 2014 edition of the *Aquatic Code*.

2. OIE *Manual of Diagnostic Tests for Aquatic Animals*

Ms Sara Linnane, Scientific Editor, from the Scientific and Technical Department, joined the meeting for this agenda item.

2.1. Review of the *Aquatic Manual* chapters

EU comment

As a general observation, the EU notes inconsistency in the confirmation of a suspected case of different viral diseases in the disease specific chapters of the Aquatic Manual. For some diseases (e.g. ISAV-deleted) culture is needed, for others it is not (e.g. IHN, where molecular tests e.g. sequencing is sufficient). It is not always clear why these differences exist, though it is recognised that for some pathogens culture is not possible.

The EU therefore kindly asks the OIE to perhaps revise the guidance to experts in order to ensure greater consistency in this point where possible.

Comments had been received from Australia and the European Union.

2.1.1. Chapter 2.3.5 Infection with infectious salmon anaemia

The Aquatic Animals Commission, in consultation with the OIE Designated Expert, reviewed Member Country comments and amended the text, as appropriate.

The revised Chapter 2.3.5. is presented at Annex 11, in English, for Member Countries' comments. Note, the Spanish version will be made available following adoption of the chapter in 2014.

EU comment

The EU thanks the OIE and in general supports the proposed amendments to this Manual chapter. Comments are inserted in the text of Annex 11.

2.1.2. Chapter 2.4.9 Infection with ostreid herpesvirus 1 microvariant

The Aquatic Animals Commission, in consultation with the chapter's authors, reviewed Member Country comments and amended the text, as appropriate.

The revised Chapter 2.4.9. is presented at Annex 12, in English, for Member Countries' comments. Note, the Spanish version will be made available following adoption of the chapter in 2014.

EU comment

The EU thanks the OIE and in general supports the proposed amendments to this Manual chapter. Comments are inserted in the text of Annex 12.

2.1.3. Chapter 2.1.4 Infection with salmonid alphavirus

The Aquatic Animals Commission noted that the draft chapter for the *Aquatic Manual* is still under development but will be submitted to the OIE by mid-November 2013. Once received, the draft chapter will then be sent to all Member Countries for comment with the request to submit comments prior to the February 2014 meeting to enable prompt adoption of this chapter.

EU comment

The EU thanks the OIE for having submitted this draft chapter separately in November. EU comments are included in the text of that chapter, which is appended at the end of this report.

2.2. Disease specific guidance documents on surveillance for a fish, a mollusc and a crustacean disease

The Aquatic Animals Commission considered the three stand-alone documents that provide examples of how to develop surveillance systems specific for a fish, a mollusc and a crustacean disease. Of these documents, the Commission agreed that the one for viral haemorrhagic septicaemia (VHS) be uploaded on the OIE website. The remaining examples for white spot disease and infection with *Bonamia ostreae* require further amendment prior to publication on the OIE website.

The Commission will undertake this work and will consider the revised documents at its next meeting in February 2014.

3. OIE Reference Centres

3.1. Feedback on Reference Laboratory quality management systems

Dr Min-Kyung Park, Scientific and Technical Department of the OIE, joined the meeting for this agenda item. At the previous meeting, Dr Park had presented an analysis of the activities of the OIE Reference Centres collected through the annual reports for 2012. The Aquatic Animals Commission noted that some laboratories are in the process of achieving an internationally recognised quality management system. The Commission noted that a letter of encouragement would be sent to these laboratories with a request that the OIE be provided with an update on any progress made during the year.

The Aquatic Animals Commission was concerned that some OIE Reference Laboratories do not have a quality management system nor plans to implement one, despite this being a requirement stated in the Terms of Reference for an OIE Reference Laboratory. The Commission noted that a letter would be sent to these laboratories indicating that failure to comply with Chapter 1.1.1 of the *Aquatic Manual* entitled: *Quality management in veterinary testing laboratories* could result in these laboratories being proposed for delisting.

3.2. Global Conference of the OIE Reference Centres, Seoul, Korea (Rep. of), 14–16 October 2014

The Third Global Conference of the OIE Reference Centres will be held in Seoul, Korea (Rep. of) from 14 to 16 October 2014. The main themes of the Conference will be: strengthening the OIE Reference Centre network, laboratory quality management systems, strengthening links between Reference Centres and Veterinary Services, and global databases of pathogen sequences.

The Commission agreed that the first three of these themes are of importance to Reference Centres for aquatic animals and welcomed the plan to conduct a breakout session specifically for Reference Centres for aquatic animals.

3.3. Withdrawal of OIE Reference Laboratories status

The Commission noted that the following OIE Reference Laboratories no longer wish to be designated as OIE Reference Laboratories and will be removed from the list:

- OIE Reference Laboratory for Spherical baculovirosis (*Penaeus monodon*-type baculovirus) at the National Taiwan University in Chinese Taipei.

- OIE Reference Laboratory for Infection with *Batrachochytrium dendrobatidis* at the Australian Animal Health Laboratory in Australia.

The Aquatic Animals Commission noted that there is now no OIE Reference Laboratory for Infection with *Batrachochytrium dendrobatidis* and invites applications for designation as an OIE Reference Laboratory for this listed disease.

3.4. Review nominations for replacement experts

The Aquatic Animals Commission reviewed and accepted the following nomination for a replacement expert at the OIE Reference Laboratory for Epizootic haematopoietic necrosis and Infection with ranavirus:

Dr Nick Moody to replace Dr Alex Hyatt at the Australian Animal Health Laboratory in Geelong, Victoria, Australia.

4. OIE Twinning Projects

Dr Gounalan Pavade, Scientific and Technical Department of the OIE, updated the Commission regarding three twinning proposals: USA/People's Republic of China for infectious haematopoietic necrosis; USA/Indonesia for crustacean/shrimp diseases; and Japan/Indonesia for Koi herpes virus.

5. Aquatic Animals Commission Work Plan 2013

The Aquatic Animals Commission reviewed and updated the work plan. The work plan provides Member Countries an overview of current and upcoming activities.

The detailed Commission's Work Plan is presented at Annex 13 for Member Countries information.

EU comment

The EU thanks the OIE Aquatic Animals Commission for submitting its detailed work plan, which it supports. Some suggestions for further work are provided below.

The EU would encourage the OIE to reconvene the ad hoc group on strain splitting, in order to progress work in this area.

The EU would ask the OIE to consider revising Article 7.3.6. number 2 letter c by deleting the words "commonly called lupara" which have been placed in brackets at the end of the sentence. Indeed, this type of gun is generally understood to be a shotgun with a wide spread to achieve maximum damage. The EU does thus not consider it appropriate in securing an accurate hit of the brain. Furthermore, it has no added value in ensuring the welfare of the fish and may be misleading as names for firearms may vary across the world.

Finally, the EU would like to inform the Aquatic Animals Commission of the comment made on the February 2013 report of the Code Commission as regards electronic certification (cf. http://ec.europa.eu/food/international/organisations/docs/eu_position_tahsc_report_feb2013_en.pdf, p. 427 and 430). As this would apply also to the aquatic code, the EU would encourage the Aquatic Animals Commission to liaise with the Code Commission so that whatever future amendments are envisaged for the Terrestrial Code could also be transferred to the Aquatic Code, in view of ensuring harmonisation between the two OIE Codes.

6. Other business

6.1. OIE Global Conference on Aquatic Animal Health

Dr Gillian Mylrea, Deputy Head, International Trade Department, informed the Aquatic Animals Commission that the OIE proposes to hold the third Global Conference on Aquatic Animal Health in January 2015. The location of the conference is pending confirmation from a possible host country.

6.2. Online commenting

The Aquatic Animals Commission discussed the use of on line commenting systems by the International Plant Protection Convention and CAC, and recommended the International Trade Department seek more information on how such a system could be used in the OIE, and report back to the Code Commission.

8. Next meeting

The Aquatic Animals Commission proposed to hold their next meetings respectively from 24th February to 28th February 2014 and from 29th September to 3rd October 2014.

_____

…/Annexes

Annex 1

MEETING OF THE OIE

AQUATIC ANIMAL HEALTH STANDARDS COMMISSION

Paris, 30 September–4 October 2013

_____

List of participants

MEMBERS OF THE COMMISSION

Dr Franck Berthe
President
European Food Safety Authority - EFSA
Head of Animal Health and Animal Welfare Unit
Via Carlo Magno 1, Parma
ITALY
Tel.: + 39 052 1 036 870
Fax: + 39 052 1 036 0870
Franck.Berthe@efsa.europa.eu

Dr Jie Huang
Vice President
Maricultural Organism Diseases Control & Molecular Pathology Laboratory,
Yellow Sea Fisheries Research Institute,
Chinese Academy of Fishery Sciences
106 Nanjing Road
Qingdao, SD 266071
PR CHINA
Tel.: +86 532 582 3062
Fax: +86-532-5811514
huangjie@ysfri.ac.cn

Dr Victor Manuel Vidal Martínez
Vice President
Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional
Carretera Antigua a Progreso Km. 6
Apartado Postal 73 Cordemex
Mérida,
Yucatán C.P. 97310
MÉXICO
Tel: +52 99 99 42 94 72
Fax: +52 99 81 29 17
vvidal@mda.cinvestav.mx

Dr Ingo Ernst
Director, Aquatic Animal Health
Animal Health Policy Branch
Australian Government Department of Agriculture
GPO Box 858
Canberra ACT 2601
Tel: 02 627 256 15
Fax: 02 627 231 50
ingo.ernst@daff.gov.au

Dr Brit Hjeltnes
Deputy Director, Fish and Shellfish Health
National Veterinary Institute
PO Box 750 Sentrum, N-0106
Bergen
NORWAY
Tel.: +47 918 893 76
brit.hjeltnes@vetinst.no

Dr Alicia Gallardo Lagno
Subdirectora nacional de acuicultura
Servicio Nacional de Pesca y Acuicultura
Calle Victoria 2832
CHILE
Tel.: +56 32 281 9282
agallardol@sernapesca.cl

OIE HEADQUARTERS

Dr Bernard Vallat
Director General
OIE
12, rue de Prony
75017 Paris
FRANCE
Tel.: 33 - (0)1 44 15 18 88
Fax: 33 - (0)1 42 67 09 87
oie@oie.int

Dr Derek Belton
Head
International Trade Department
OIE
d.belton@oie.int

Ms Sara Linnane
Scientific editor
Scientific and Technical Department
OIE
s.linnane@oie.int

Dr Gillian Mylrea
Deputy Head
International Trade Department
OIE
g.mylrea@oie.int

Flavia Godoy
Intern
International Trade Department
University of Sao Paulo
Brazil

Annex 2

MEETING OF THE OIE
AQUATIC ANIMAL HEALTH STANDARDS COMMISSION

Paris, 30 September–4 October 2013

_____

Adopted agenda

1. OIE *Aquatic Animal Health Code*

1.1. Glossary

1.2. Notification of Diseases and Epidemiological Information (Chapter 1.1.)

1.3. Criteria for listing an emerging disease (Chapter 1.2.)

1.4. Diseases listed by the OIE (Chapter 1.3.)

1.5. Control of hazards in aquatic animal feeds (Chapter 6.1.)

1.6. Necrotising hepatopancreatitis (Chapter 9.4.)

1.7. Infection with infectious salmon anaemia virus (Chapter 10.5.)

1.8. Horizontal issues

1.9. Criteria for determining susceptibility of aquatic animals to specific pathogenic agents (new chapter X.X.)

1.10. Infection with *Salmonid alphavirus* (new chapter 10.X.)

1.11. Future work on pathogen differentiation

1.12. *Aquatic Code* - Table of contents

2. OIE *Manual of Diagnostic Tests for Aquatic Animals*

2.1. Review of the *Aquatic Manual* chapters

2.1.1. Chapter 2.3.5 Infection with infectious salmon anaemia

2.1.2. Chapter 2.4.9 Infection with ostreid herpesvirus 1 microvariant

2.1.3. Chapter 2.1.4 Infection with salmonid alphavirus

2.2. Disease specific guidance documents on surveillance for a fish, a mollusc and a crustacean disease

Annex 2 (contd)

3. OIE Reference Centres

3.1. Feedback on Reference Laboratory quality management systems

3.2. Global Conference of the OIE Reference Centres, Seoul, Korea (Rep. of), 14–16 October 2014

3.3. Withdrawal of OIE Reference Laboratories status

3.4. Review nominations for replacement experts

4. OIE Twinning Projects

5. Cooperation with FAO

6. Aquatic Animals Commission Work Plan 2013

7. Other business

7.1. OIE Global Conference on Aquatic Animal Health

8. Next meeting

Annex 3

GLOSSARY

EU comment

The EU in general supports the proposed the proposed changes to the glossary. However, some comments are inserted in the text below.

Emerging disease

means a *disease* ~~newly recognised~~ *infection* which has a significant impact on animal or public health resulting from: ~~the evolution or~~

- a change of a~~n~~ known ~~existing~~ pathogenic agent~~, a known *infection*~~ or its spread~~ing~~ to a new geographic area or species ~~*population*~~,; or

- a newly ~~previously un~~recognised or suspected pathogenic agent ~~or *disease* diagnosed for the first time and which has a significant impact on aquatic animal or public health~~.

EU comment

The EU in general supports this proposed amendment to the definition of emerging disease, the intention being alignment to the proposed changes in the Terrestrial Code. However, the EU notes some differences to the proposed amended definition in the Terrestrial Code. In order to avoid any uncertainty and confusion, especially as regards notification obligations for emerging diseases, the EU suggests a complete alignment with the newly proposed Terrestrial Code definition. Indeed, the mentioning of "suspected pathogenic agents" would bring a lot of uncertainty, as only confirmed cases (and not unconfirmed suspected cases) fall under the notification obligation of member countries.

For ease of reference, this is the corresponding text proposed in the Terrestrial Code:

"*Emerging disease*

means a new occurrence, which has a significant impact on animal or public health, of a *disease*, *infection* or *infestation* resulting from: ~~the evolution or~~

- a change of a~~n~~ known ~~existing~~ pathogenic agent ~~, a known *infection* or *infestation*~~ or its spread~~ing~~ to a new geographic area or species ~~*population*~~,; or

- a previously unrecognised pathogenic agent or *disease* diagnosed for the first time ~~and which has a significant impact on animal or public health~~.".

Susceptible species

means a species of aquatic animal in which infection has been demonstrated by natural cases or by experimental exposure~~s~~ to the pathogenic agent that mimics ~~the~~ natural pathways ~~for infection. Each disease chapter in the Aquatic Code and the Aquatic Manual contains a list of currently known susceptible species~~.'

EU comment

The EU in general supports this amendment to the definition of susceptible species. However, the EU queries the OIE whether the principle described in the sentence being deleted is described somewhere else in the Aquatic Code, which perhaps could be useful (e.g. in the "Guide to the use of the *Aquatic Animal Health Code*").

Veterinarian

means a person with appropriate education, registered or licensed by the relevant *veterinary statutory body* of a country to practice veterinary medicine/science in that country.

EU comment

The EU in general supports the attempts to have as equal definitions as possible in the terrestrial and the aquatic codes, and thus supports the proposed amendment of the definition of veterinarian.

- - - - - - - - - - - - - -

— Text deleted

Annex 4

CHAPTER 1.1.

NOTIFICATION OF DISEASES AND EPIDEMIOLOGICAL INFORMATION

EU comment

The EU in general supports the proposed changes to this chapter and asks the OIE to consider changing the title as follows:

"NOTIFICATION OF DISEASES, AND PROVISION OF EPIDEMIOLOGICAL INFORMATION".

Indeed, whereas diseases are notified, epidemiological information is being provided to the OIE (please note that a similar comment is being made on the corresponding chapter of the Terrestrial Code).

Further comments are included in the text below.

Article 1.1.1.

For the purposes of the *Aquatic Code* and in terms of Articles 5, 9 and 10 of the OIE Organic Statutes, every Member Country of the OIE shall recognise the right of the *Headquarters* to communicate directly with the *Competent Authority* of its *territory* or *territories*.

All *notifications* and all information sent by the OIE to the *Competent Authority* shall be regarded as having been sent to the country concerned and all *notifications* and all information sent to the OIE by the *Competent Authority* shall be regarded as having been sent by the country concerned.

Article 1.1.2.

1) Member Countries shall make available to other Member Countries, through the OIE, whatever information is necessary to minimise the spread of important *diseases* of *aquatic animals* and their pathogenic agents and to assist in achieving better world-wide control of these *diseases*.

2) To achieve this, Member Countries shall comply with the *notification* requirements specified in Article 1.1.3.

3) To assist in the clear and concise exchange of information, reports shall conform as closely as possible to the current OIE *disease* reporting format.

4) Recognising that scientific knowledge concerning the relationship between *pathogenic agents* and clinical *disease* is constantly developing and that the presence of an infectious agent does not necessarily imply the presence of clinical *disease*, Member Countries shall ensure through their reports that they comply with the spirit and intention of point 1 above. This means that the detection of an infectious agent of a *listed disease* in an *aquatic animal* should be reported, even in the absence of clinical signs ~~of *disease*~~.

5) In addition to notifying findings in accordance with Article 1.1.3., Member Countries shall also provide information on the measures taken to prevent the spread of *diseases*, including possible *quarantine* measures and restrictions on the movement of *aquatic animals*, *aquatic animal products*, *biological products* and other miscellaneous objects which could by their nature be responsible for transmission of *disease*. In the case of *diseases* transmitted by vectors, the measures taken against such vectors shall also be specified.

Article 1.1.3.

The *Competent Authority* shall, under the responsibility of the Delegate, send to the *Headquarters* of the OIE:

1) in accordance with relevant provisions in the *disease*-specific chapters, *notification*, through the World

Animal Health Information System (WAHIS) or by fax or e-mail within 24 hours of any of the following events:

a) first occurrence of a *listed disease* in a country, a *zone* or a *compartment*;

b) re-occurrence of a *listed disease* in a country, a *zone* or a *compartment* following ~~a~~ the final report that declared ~~an~~ the *outbreak* ended;

c) first occurrence of a new strain of a pathogen of a *listed disease* new to a country, a *zone* or a *compartment*;

EU comment

In order to avoid any uncertainty and confusion related to notification obligations of member countries, the EU suggests a complete alignment of the point above with the corresponding wording in the Terrestrial Code, as follows:

"c) first occurrence of a new strain of a pathogen of a listed disease in a country, a zone or a compartment;"

d) a sudden and unexpected change ~~increase~~ in the distribution~~,~~ or increase in incidence or virulence of, or morbidity or mortality ~~of~~ caused by, the aetiological agent of a *listed disease*, ~~prevalent~~ present within a country, a *zone* or a *compartment*;

e) ~~evidence of change in the epidemiology~~ first occurrence of a *listed disease*~~,~~ in a new ~~unusual~~ host species ~~(including host range, pathogenicity, strain) in particular if there is a zoonotic impact;~~.

EU comment

As explained above, the terrestrial and Aquatic Codes should be aligned as far as possible. Therefore, the EU suggests deleting the word "first" in point e) above, as this does not appear in the Terrestrial Code.

On the other hand, the EU prefers the use of the term "new host species" instead of "unusual host species", and will therefore suggest to the Terrestrial Code Commission to align the Terrestrial Code to this suggestion in the Aquatic Code.

f) ~~an *emerging disease* with significant morbidity or mortality, or zoonotic potential;~~

In deciding whether findings justify immediate *notification* (within 24 hours), Member Countries must ensure that they comply with the obligations of Chapters 5.1. and 5.2. (especially Article 5.1.1.), to report developments that may have implications for *international trade*.

2) Weekly reports subsequent to a *notification* under point 1 above, to provide further information on the evolution of the event which justified the *notification*. These reports should continue until the *disease* has been eradicated or the situation has become sufficiently stable so that six-monthly reporting under point 3 will satisfy the obligation of the Member Country to the OIE; ~~in any~~ for each event notified ~~case~~, a final report on the event should be submitted.

3) Six-monthly reports on the absence or presence and evolution of *listed diseases* and information of epidemiological significance to other Member Countries.

4) Annual reports concerning any other information of significance to other Member Countries.

~~Although Member Countries are only required to notify *listed diseases* and *emerging diseases* according to points 1 to 4 above, they are encouraged to inform the OIE of other animal health events of epidemiological significance.~~

Article 1.1.3.bis

Veterinary Authorities shall, under the responsibility of the Delegate, send to the *Headquarters*:

1) a *notification* through the World Animal Health Information System (WAHIS) or by fax or e-mail, when an *emerging disease* has been detected in a country, a *zone* or a *compartment*;

2) periodic reports subsequent to a *notification* for *emerging disease*, as described under point 1). These should continue until the *disease* has been eradicated or the situation becomes sufficiently stable or scientific information is available to determine whether it meets the criteria for listing.

EU comment

The EU supports the new text proposed above, however point 2 should be amended as follows:

"2) periodic reports subsequent to a notification for *emerging disease,* as described under point 1). These should continue until the *disease, infection* or *infestation* has been eradicated or the situation becomes sufficiently stable for the event to be closed or it has been evaluated ~~scientific information is available~~ to determine whether it meets the criteria for listing."

Indeed, scientific information being available is too vague reference, while closing the event or evaluating it against the listing criteria is a better defined end point (please note that the same comment is being made on the corresponding chapter of the Terrestrial Code).

In addition, for reason of consistency, the EU would like to suggest that the words "Veterinary Authorities" be replaced by "Competent Authorities".

Article 1.1.4.

1) The *Competent Authority* of a country in which an *infected zone* or *compartment* was located shall inform the *Headquarters* when this *zone* or *compartment* is free from the *disease*.

2) An *infected zone* or *compartment* for a particular *disease* shall be considered as such until a period exceeding the *infective period* specified in the *Aquatic Code* has elapsed after the last reported *case* and when full prophylactic and appropriate animal health measures have been applied to prevent possible reappearance or spread of the *disease*. These measures will be found in detail in various chapters of the *Aquatic Code*.

3) A Member Country may be considered to regain freedom from a specific *disease* when all relevant conditions given in the *Aquatic Code* have been fulfilled.

4) The *Competent Authority* of a Member Country which sets up one or several *free zones* or *free compartments* shall inform the *Headquarters*, giving necessary details, including the criteria on which the free status is based, the requirements for maintaining the status and indicating clearly the location of the *zones* or *compartments* on a map of the territory of the Member Country.

Article 1.1.5.

1) Although Member Countries are only required to notify *listed diseases*, and *emerging diseases*, they are encouraged to inform the OIE of other important animal health events.

2) The *Headquarters* shall communicate by e-mail or World Animal Health Information Database (WAHID) to *Competent Authorities* all *notifications* received as provided in Articles 1.1.2. to 1.1.4. and other relevant information.

- - - - - - - - - - - - - -

— Text deleted

Annex 5

CHAPTER 1.2.

CRITERIA FOR LISTING AQUATIC ANIMAL DISEASES

EU comment

The EU thanks the OIE and strongly supports the proposed changes to this chapter.

Article 1.2.1.

Introduction

This chapter describes the criteria for listing *diseases* in Chapter 1.3. The objective of listing is to support Member Countries' efforts to prevent the transboundary spread of important *diseases* of *aquatic animals* through transparent and consistent reporting.

For the *diseases* listed in accordance with Article 1.2.2., the corresponding *disease*-specific chapters in the *Aquatic Code* provide standards for safe *international trade* in *aquatic animals* and their products.

~~The purpose of listing *diseases* in accordance with Article 1.2.3. is to recognise important *emerging diseases* and collect relevant epidemiological information. This information is collected to enable later consideration of listing of the *disease* in accordance with Article 1.2.2.*Diseases* listed in accordance with Article 1.2.3. do not have a corresponding *disease*-specific chapter in the *Aquatic Code* and thus no specific standards for *international trade*. Member Countries should only institute *disease* specific trade requirements where these are justified by a science based *risk assessment*.~~

The requirements for *notification* of *listed diseases* are detailed in Chapter 1.1.

Article 1.2.2.

Criteria for listing an aquatic animal disease

Diseases proposed for listing should meet the relevant criteria as set out in A. Consequences, B. Spread and C. Diagnosis. Therefore, to be listed, a *disease* should have the following characteristics: 1 or 2 or 3; and 4 or 5; and 6; and 7; and 8. Such proposals should be accompanied by a *case definition* for the *disease* under consideration.

No.		Criteria for listing	Explanatory notes
A. Consequences			
1.		The disease has been shown to cause significant production losses at a national or multinational (zonal or regional) level.	There is a general pattern that the disease will lead to losses in susceptible species, and that morbidity or mortality are related primarily to the infectious agent and not management or environmental factors. (Morbidity includes, for example, loss of production due to spawning failure.) The direct economic impact of the disease is linked to its morbidity, mortality and effect on product quality.
2.	Or	The disease has been shown to or scientific evidence indicates that it is likely to cause significant morbidity or mortality in wild aquatic animal populations.	Wild aquatic animal populations can be populations that are commercially harvested (wild fisheries) and hence are an economic asset. However, the asset could be ecological or environmental in nature, for example, if the population consists of an endangered species of aquatic animal or an aquatic animal potentially endangered by the disease.
3.	Or	The agent is of public health concern.	

Annex 5 (contd)

And B. Spread			
4.		Infectious aetiology of the disease is proven.	
5.	Or	An infectious agent is strongly associated with the disease, but the aetiology is not yet known.	Infectious diseases of unknown aetiology can have equally high-risk implications as those diseases where the infectious aetiology is proven. Whilst disease occurrence data are gathered, research should be conducted to elucidate the aetiology of the disease and the results be made available within a reasonable period of time.
6.	And	Likelihood of international spread, including via live animals, their products or fomites.	International trade in aquatic animal species susceptible to the disease exists or is likely to develop and, under international trading practices, the entry and establishment of the disease is likely.
7.	And	Several countries or countries with zones may be declared free of the disease based on the general surveillance principles outlined in Chapter 1.4.	Free countries/zones could still be protected. Listing of diseases that are ubiquitous or extremely widespread would render notification unfeasible. However, individual countries that run a control programme on such a disease can propose its listing provided they have undertaken a scientific evaluation to support their request. Examples may be the protection of broodstock from widespread diseases, or the protection of the last remaining free zones from a widespread disease.
And C. Diagnosis			
8.		A repeatable and robust means of detection/diagnosis exists.	A diagnostic test should be widely available and preferably has undergone a formal standardisation and validation process using routine field samples (See *Aquatic Manual*.) or a robust case definition is available to clearly identify cases and allow them to be distinguished from other pathologies.

~~Article 1.2.3.~~

~~**Criteria for listing an emerging aquatic animal disease**~~

~~An *emerging disease* may be proposed for listing if it meets the criteria 1 or 2, and 3 or 4. Such proposals should be accompanied by a *case definition* for the *disease* under consideration.~~

~~**No.**~~	~~**Criteria for listing**~~	~~**Explanatory notes**~~
~~1.~~	~~Infectious aetiology of the disease is proven.~~	
~~Or~~		
~~2.~~	~~An infectious agent is strongly associated with the disease, but the aetiology is not yet known.~~	~~Infectious diseases of unknown aetiology can have equally high risk implications as those diseases where the infectious aetiology is proven. Whilst disease occurrence data are gathered, research should be conducted to elucidate the aetiology of the disease and the results be made available within a reasonable period of time.~~

~~And~~		
~~3.~~	~~The agent is of public health concern.~~	
~~Or~~		
~~4.~~	~~Significant spread in naive populations of wild or cultured aquatic animals.~~	~~The disease has exhibited significant morbidity, mortality or production losses at a zone, compartment or country level. 'Naive' means animals previously unexposed either to a new disease or a new form of a known disease.~~

- - - - - - - - - - - - - -

— Text deleted

Annex 6

CHAPTER 9.4.

NECROTISING HEPATOPANCREATITIS

EU comment

As this seems not to be the definite name of the agent ["Candidatus"], the EU is of the opinion that it might be too early to change the pathogen name in the Aquatic Code. This should only be done once the change of the scientific name has been decided by the International Committee for Taxonomy of Bacteria, which might be in time before the next OIE General Session in May 2014. Otherwise it would be preferable to wait until May 2015 with this change.

Article 9.4.1.

For the purposes of the *Aquatic Code*, necrotising hepatopancreatitis (NHP) means *infection* with *Candidatus Hepatobacter penaei* ~~necrotising hepatopancreatitis bacteria~~ (NHP-B). This obligate intracellular bacterium is a member of the order α-Proteobacteria.

Information on methods for *diagnostic* is provided in the *Aquatic Manual*.

– Text deleted

Annex 7

CHAPTER 10.5.

INFECTION WITH INFECTIOUS SALMON ANAEMIA VIRUS

EU comment

The EU in general supports the amendment of this chapter.

The EU notes that the way the different options for freedom are presented in the chapter makes the chapter difficult to read. Therefore, the EU encourages the OIE to revise the structure of the chapter in order to improve readability. Furthermore, Article 10.5.3. could be restructured so as to clearly identify the safe commodities as such, which would be helpful for international trade purposes.

The EU would also prefer to have the case definition included in the Aquatic Code, and not in the Manual, as the Code is the relevant standard for international trade purposes.

Specific comments are inserted in the text below.

Article 10.5.1.

For the purposes of the *Aquatic Code*, infection with infectious salmon anaemia virus (ISAV) means *infection* with HPR0 (non-deleted highly polymorphic region) or HPR-deleted ISAV of the genus Isavirus of the family Orthomyxoviridae. Both genotypes should be notified in accordance with the *Aquatic Code*.

There is a link between non-pathogenic HPR0 ISAV and pathogenic HPR-deleted ISAV, with some outbreaks potentially occurring as a result of the emergence of HPR-deleted from HPR0.

The provisions in this chapter are provided in recognition of three possible levels of disease status with respect to ISAV:

1) HPR0 ISAV and HPR-deleted ISAV free;

2) HPR0 ISAV endemic (but HPR-deleted ISAV free);

3) HPR0 ISAV and HPR-deleted ISAV endemic.

Information on methods for diagnosis are provided in the *Aquatic Manual*.

Article 10.5.2.

Scope

The recommendations in this chapter apply to: Atlantic salmon (*Salmo salar*), brown trout (*S. trutta*) and rainbow trout (*Onchorynchus mykiss*). These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

EU comment

The EU would once again like to ask the OIE to consider adding coho salmon to the list of susceptible species, as it is already considered in the Manual chapter.

Furthermore, the EU suggests deleting the second sentence of the paragraph above. Indeed, the principle should be that susceptible species relevant for international trade

are listed in the Code chapter, instead of referring to the Manual. This would be relevant for all disease specific chapters.

Article 10.5.3.

Importation or transit of aquatic animals and aquatic animal products for any purpose from a country, zone or compartment not declared free from infection with infectious salmon anaemia virus

In this article, all statements referring to ISAV are for any detectable ISAV, including HPR0 ISAV.

1) *Competent Authorities* should not require any conditions related to infection with ISAV, regardless of the ISAV status of the *exporting country*, *zone* or *compartment* when authorising the importation or transit of the following *aquatic animals* and *aquatic animal products* from the species referred to in Article 10.5.2. intended for any purpose and complying with Article 5.4.1.:

   a) heat sterilised, hermetically sealed fish products (i.e. a heat treatment at 121˚C for at least 3.6 minutes or any time/temperature equivalent);

   b) pasteurised fish products that have been subjected to a heat treatment at 90˚C for at least 10 minutes (or to any time/temperature equivalent which has been demonstrated to inactivate ISAV);

   c) mechanically dried, eviscerated fish (i.e. a heat treatment at 100˚C for 30 minutes or any time/temperature equivalent which has been demonstrated to inactivate ISAV);

   d) fish oil;

   e) fish *meal*; and

   f) fish skin leather.

2) When authorising the importation or transit of *aquatic animals* and *aquatic animal products* of a species referred to in Article 10.5.2., other than those referred to in point 1 of Article 10.5.3., *Competent Authorities* should require the conditions prescribed in Articles 10.5.10. to 10.5.17. relevant to the ISAV status of the *exporting country*, *zone* or *compartment*.

3) When considering the importation or transit of *aquatic animals* and *aquatic animal products* from an *exporting country*, *zone* or *compartment* not declared free from infection with ISAV of a species not covered in Article 10.5.2. but which could reasonably be expected to pose a *risk* of transmission for ISAV, *Competent Authorities* should conduct a *risk analysis* in accordance with the recommendations in the *Aquatic Code*. The *exporting country* should be informed of the outcome of this assessment.

EU comment

To maintain consistency with the model chapter where 4 options to self-declare freedom are allowed, the EU suggests adding some explanation as to why the 4th option was not available at the end of Article 10.5.3., as follows:

"Self-declaration based on absence of observed disease and biosecurity conditions is not acceptable for ISA since infection with HPR0 is not likely to cause clinical signs (i.e. conditions are not conducive to clinical expression)."

Article 10.5.4.

Country free from infection with infectious salmon anaemia virus

In this article, all statements referring to a country free from infection with ISAV are for any detectable ISAV, including HPR0 ISAV.

A country may make a *self-declaration of freedom* from infection with ISAV if it meets the conditions in points 1, 2 or 3 below.

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from infection with ISAV if all the areas covered by the shared water are declared countries or *zones* free from infection with ISAV (see Article 10.5.6.).

1) A country where none of the *susceptible species* is present may make a *self-declaration of freedom* from infection with ISAV when *basic biosecurity conditions* have been continuously met in the country for at least the past two years.

OR

2) A country where the species referred to in Article 10.5.2. are present but there has been no detectable occurrence of infection with ISAV may make a *self-declaration of freedom* from infection with ISAV when:

   a) *basic biosecurity conditions* have been continuously met for at least the past two years; and

   b) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of infection with ISAV.

OR

3) A country that has made a *self-declaration of freedom* from infection with ISAV but in which infection with ISAV is subsequently detected may make a *self-declaration of freedom* from infection with ISAV again when the following conditions have been met:

   a) on detection of any infection with ISAV, the affected area was declared an *infected zone* and a *protection zone* was established; and

   b) infected populations have been destroyed or removed from the *infected zone* by means that minimise the *risk* of further spread of ISAV, and the appropriate *disinfection* procedures (see *Aquatic Manual*) have been completed; and

   c) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of infection with ISAV; and

   d) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place ~~for at least two years following~~ since eradication of the *disease*.

   In the meantime, part of the non-affected area may be declared a free *zone* provided that such part meets the conditions in point 3 of Article 10.5.6.

Article 10.5.5.

Country free from infection with HPR-deleted infectious salmon anaemia virus

In this article, all statements refer to a country free from infection with HPR-deleted ISAV but not necessarily free from infection with HPR0 ISAV.

A country may make a *self-declaration of freedom* from infection with HPR-deleted ISAV if it meets the conditions in points 1, 2~~,~~ or 3 ~~or 4~~ below.

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from infection with HPR-deleted ISAV if all the areas covered by the shared water are declared countries or *zones* free from infection with HPR-deleted ISAV (see Article 10.5.7.).

~~1) A country where none of the *susceptible species* is present may make a *self-declaration of freedom* from infection with HPR deleted ISAV when *basic biosecurity conditions* have been continuously met in the country for at least the past two years.~~

~~OR~~

1~~2~~) A country where the species referred to in Article 10.5.2. are present but there has been no observed occurrence of infection with HPR-deleted ISAV for at least the past ten years despite conditions that are

conducive to clinical expression, as described in the corresponding chapter of the *Aquatic Manual*, may make a *self-declaration of freedom* from infection with HPR-deleted ISAV when *basic biosecurity conditions* have been continuously met in the country for at least the past ten years.

OR

2~~3~~) A country where the last observed occurrence of infection with HPR-deleted ISAV was within the past ten years or where the disease status prior to *targeted surveillance* was unknown (e.g. because of the absence of conditions conducive to clinical expression as described in the corresponding chapter of the *Aquatic Manual*) may make a *self-declaration of freedom* from infection with HPR-deleted ISAV when:

a) *basic biosecurity conditions* have been continuously met for at least the past two years; and

b) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of infection with HPR-deleted ISAV.

OR

3~~4~~) A country that has made a *self-declaration of freedom* from infection with HPR-deleted ISAV but in which infection with HPR-deleted ISAV is subsequently detected may make a *self-declaration of freedom* from infection with HPR-deleted ISAV again when the following conditions have been met:

a) on detection of infection with HPR-deleted ISAV, the affected area was declared an *infected zone* and a *protection zone* was established; and

b) infected populations have been destroyed or removed from the *infected zone* by means that minimise the *risk* of further spread of HPR-deleted ISAV, and the appropriate *disinfection* procedures (see *Aquatic Manual*) have been completed; and

c) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of infection with HPR-deleted ISAV; and

d) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place ~~for at least two years following~~ since eradication of the *disease*.

In the meantime, part of the non-affected area may be declared a free *zone* provided that such part meets the conditions in point 3 of Article 10.5.7.

Article 10.5.6.

Zone or compartment free from infection with infectious salmon anaemia virus

In this article, all statements referring to a *zone* or *compartment* free from infection with ISAV are for any detectable ISAV, including HPR0 ISAV.

A *zone* or *compartment* within the *territory* of one or more countries not declared free from infection with ISAV may be declared free by the *Competent Authority(ies)* of the country(ies) concerned if the *zone* or *compartment* meets the conditions referred to in points 1, 2 or 3 below.

1) A *zone* or *compartment* where none of the *susceptible species* is present may be declared free from infection with ISAV when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past two years.

OR

2) A *zone* or *compartment* where the species referred to in Article 10.5.2. are present but there has been no detectable occurrence of infection with ISAV may be declared free from infection with ISAV when:

a) *basic biosecurity conditions* have been continuously met for at least the past two years; and

b) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of infection with ISAV.

OR

3) A *zone* previously declared free from infection with ISAV but in which any ISAV is detected may be declared free from infection with ISAV again when the following conditions have been met:

   a) on detection of infection with ISAV, the affected area was declared an *infected zone* and a *protection zone* was established; and

   b) infected populations have been destroyed or removed from the *infected zone* by means that minimise the *risk* of further spread of ISAV, and the appropriate *disinfection* procedures (see *Aquatic Manual*) have been completed; and

   c) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of infection with ISAV; and

   d) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place ~~for at least two years following~~ since eradication of the *disease*.

Article 10.5.7.

Zone or compartment free from infection with HPR-deleted infectious salmon anaemia virus

In this article, all statements refer to a *zone* or *compartment* free from infection with HPR-deleted ISAV but not necessarily free from infection with HPR0 ISAV.

A *zone* or *compartment* within the *territory* of one or more countries not declared free from infection with HPR-deleted ISAV may be declared free by the *Competent Authority(ies)* of the country(ies) concerned if the *zone* or *compartment* meets the conditions referred to in points 1, 2~~,~~ or 3 ~~or 4~~ below.

~~1) A *zone* or *compartment* where none of the *susceptible species* is present may be declared free from infection with HPR-deleted ISAV when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past two years.~~

~~OR~~

1~~2~~) A *zone* or *compartment* where the species referred to in Article 10.5.2. are present but there has been no observed occurrence of infection with HPR-deleted ISAV for at least the past ten years despite conditions that are conducive to its clinical expression, as described in the corresponding chapter of the *Aquatic Manual*, may be declared free from infection with HPR-deleted ISAV when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past ten years.

OR

2~~3~~) A *zone* or *compartment* where the last observed occurrence of infection with HPR-deleted ISAV was within the past ten years or where the disease status prior to *targeted surveillance* was unknown (e.g. because of the absence of conditions conducive to clinical expression as described in the corresponding chapter of the *Aquatic Manual*) may be declared free from infection with HPR-deleted ISAV when:

   a) *basic biosecurity conditions* have been continuously met for at least the past two years; and

   b) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of infection with HPR-deleted ISAV.

OR

3~~4~~) A *zone* previously declared free from infection with HPR-deleted ISAV but in which infection with HPR-deleted ISAV is detected may be declared free from infection with HPR-deleted ISAV again when the following conditions have been met:

Annex 7 (contd)

a) on detection of infection with HPR-deleted ISAV, the affected area was declared an *infected zone* and a *protection zone* was established; and

b) infected populations have been destroyed or removed from the *infected zone* by means that minimise the *risk* of further spread of HPR-deleted ISAV, and the appropriate *disinfection* procedures (see *Aquatic Manual*) have been completed; and

c) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of infection with HPR-deleted ISAV; and

d) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place ~~for at least two years following~~ since eradication of the *disease*.

Article 10.5.8.

Maintenance of free status for infection with infectious salmon anaemia virus

In this article, all statements referring to a country, *zone* or *compartment* free from ISAV are for any detectable ISAV, including HPR0 ISAV.

A country, *zone* or *compartment* that is declared free from infection with ISAV following the provisions of point 1 of Articles 10.5.4. or 10.5.6. (as relevant) may maintain its status as free from infection with ISAV provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from infection with ISAV following the provisions of point 2 of Articles 10.5.4. or 10.5.6. (as relevant) may maintain its status as free from infection with ISAV provided that *targeted surveillance* is continued at a level determined by the *Aquatic Animal Health Service* on the basis of the likelihood of *infection*, and *basic biosecurity conditions* are continuously maintained.

Article 10.5.9.

Maintenance of free status for infection with HPR-deleted infectious salmon anaemia virus

In this article, all statements refer to a country, *zone* or *compartment* free from infection with HPR-deleted ISAV, but not necessarily free from infection with HPR0 ISAV.

A country, *zone* or *compartment* that is declared free from infection with HPR-deleted ISAV following the provisions of points 1 or 2 of Articles 10.5.5. or 10.5.7. (as relevant) may maintain its status as free from infection with ISAV provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from infection with HPR-deleted ISAV following the provisions of point 3 of Articles 10.5.5. or 10.5.7. (as relevant) may discontinue *targeted surveillance* and maintain its free status provided that conditions that are conducive to clinical expression, as described in the corresponding chapter of the *Aquatic Manual*, exist and *basic biosecurity conditions* are continuously maintained.

However, for declared free *zones* or *compartments* in an infected country and in all cases where conditions are not conducive to clinical expression, *targeted surveillance* needs to be continued at a level determined by the *Aquatic Animal Health Service* on the basis of the likelihood of *infection*.

Article 10.5.10.

Importation of live aquatic animals from a country, zone or compartment declared free from infection with infectious salmon anaemia virus

In this article, all statements referring to a country, *zone* or *compartment* free from ISAV are for any detectable ISAV, including HPR0 ISAV.

When importing live *aquatic animals* of the species referred to in Article 10.5.2. from a country, *zone* or *compartment* declared free from infection with ISAV, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* certifying that, on the basis of the procedures described in Articles 10.5.4. or 10.5.6. (as applicable) and 10.5.8., the place of production of the *aquatic animal* is a country, *zone* or *compartment* declared free from infection with ISAV.

The *certificate* should be in accordance with the Model Certificate in Chapter 5.11. This Article does not apply to *commodities* referred to in point 1 of Article 10.5.3.

Article 10.5.11.

Importation of live aquatic animals from a country, zone or compartment declared free from infection with HPR-deleted infectious salmon anaemia virus

In this article, all statements refer to a country, *zone* or *compartment* free from infection with HPR-deleted ISAV, but not necessarily free from infection with HPR0 ISAV.

When importing live *aquatic animals* of the species referred to in Article 10.5.2. from a country, *zone* or *compartment* declared free from infection with HPR-deleted ISAV, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* certifying that, on the basis of the procedures described in Articles 10.5.5. or 10.5.7. (as applicable) and 10.5.9., the place of production of the *aquatic animal* is a country, *zone* or *compartment* declared free from infection with HPR-deleted ISAV.

The *certificate* should be in accordance with the Model Certificate in Chapter 5.11. This Article does not apply to *commodities* referred to in point 1 of Article 10.5.3.

Article 10.5.12.

Importation of live aquatic animals for aquaculture from a country, zone or compartment not declared free from infection with infectious salmon anaemia virus

In this article, all statements referring to infection with ISAV are for any detectable ISAV, including HPR0 ISAV.

1) When importing, for *aquaculture*, live *aquatic animals* of the species referred to in Article 10.5.2. from a country, *zone* or *compartment* not declared free from infection with ISAV, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, apply the following *risk* mitigation measures:

    a) the direct delivery to and lifelong holding of the consignment in biosecure facilities for continuous isolation from the local environment; and

    b) the treatment of all effluent and waste materials in a manner that ensures inactivation of ISAV.

Annex 7 (contd)

2) If the intention of the introduction is the establishment of a new stock, relevant aspects of the Code of Practice on the Introductions and Transfers of Marine Organisms of the International Council for the Exploration of the Seas (ICES) should be considered.

3) For the purposes of the *Aquatic Code*, relevant aspects of the ICES Code (full version see: http://www.ices.dk/publications/our-publications/Pages/Miscellaneous.aspx) may be summarised to the following points:

   a) identify stock of interest (cultured or wild) in its current location;

   b) evaluate stock health/disease history;

   c) take and test samples for ISAV, pests and general health/disease status;

   d) import and quarantine in a secure facility a founder (F-0) population;

   e) produce F-1 generation from the F-0 stock in *quarantine*;

   f) culture F-1 stock and at critical times in its development (life cycle) sample and test for ISAV and perform general examinations for pests and general health/disease status;

   g) if ISAV is not detected, pests are not present, and the general health/disease status of the stock is considered to meet the *basic biosecurity conditions* of the *importing country*, *zone* or *compartment*, the F-1 stock may be defined as infection with ISAV free or specific pathogen free (SPF) for ISAV;

   h) release SPF F-1 stock from *quarantine* for *aquaculture* or stocking purposes in the country, *zone* or *compartment*.

4) With respect to point 3e), *quarantine* conditions should be conducive to multiplication of the pathogen and eventually to clinical expression. If *quarantine* conditions are not suitable for pathogen multiplication and development, the recommended diagnostic approach might not be sensitive enough to detect low *infection* level.

This Article does not apply to *aquatic animals* referred to in point 1 of Article 10.5.3.

Article 10.5.13.

Importation of aquatic animals and aquatic animal products for processing for human consumption from a country, zone or compartment not declared free from infection with infectious salmon anaemia virus

In this article, all statements referring to infection with ISAV are for any detectable ISAV, including HPR0 ISAV.

When importing, for processing for human consumption, *aquatic animals* or *aquatic animal products* of species referred to in Article 10.5.2. from a country, *zone* or *compartment* not declared free from infection with ISAV, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, require that:

1) the consignment is delivered directly to and held in *quarantine* or containment facilities until processing into one of the products referred to in point 1 of Article 10.5.3., or products described in point 1 of Article 10.5.16., or other products authorised by the *Competent Authority*; and

2) water used in transport and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of ISAV or is disposed in a manner that prevents contact of waste with *susceptible species*.

Annex 7 (contd)

For these *commodities* Member Countries may wish to consider introducing internal measures to address the *risks* associated with the *commodity* being used for any purpose other than for human consumption.

Article 10.5.14.

Importation of live aquatic animals intended for use in animal feed, or for agricultural, industrial or pharmaceutical use from a country, zone or compartment not declared free from infection with infectious salmon anaemia virus

In this article, all statements referring to infection with ISAV are for any detectable ISAV, including HPR0 ISAV.

When importing, for use in animal *feed*, or for agricultural, industrial or pharmaceutical use, live *aquatic animals* of the species referred to in Article 10.5.2. from a country, *zone* or *compartment* not declared free from infection with ISAV, the *Competent Authority* of the *importing country* should require that:

1) the consignment is delivered directly to and held in *quarantine* for slaughter and processing to products authorised by the *Competent Authority*; and

2) water used in transport and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of ISAV.

This Article does not apply to *commodities* referred to in point 1 of Article 10.5.3.

Article 10.5.15.

Importation of aquatic animal products from a country, zone or compartment declared free from infection with infectious salmon anaemia virus

In this article, all statements referring to infection with ISAV are for any detectable ISAV, including HPR0 ISAV.

When importing *aquatic animal products* of the species referred to in Article 10.5.2. from a country, *zone* or *compartment* declared free from infection with ISAV, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* certifying that, on the basis of the procedures described in Articles 10.5.4., ~~10.5.5., 10.5.6.~~ or 10.5.~~7~~6.(as applicable) and 10.5.8.the place of production of the *commodity* is a country, *zone* or *compartment* declared free from infection with ISAV.

The *certificate* should be in accordance with the Model Certificate in Chapter 5.11. This Article does not apply to *commodities* referred to in point 1 of Article 10.5.3.

Article 10.5.15bis

Importation of aquatic animal products from a country, zone or compartment declared free from infection with HPR-deleted infectious salmon anaemia virus

In this article, all statements refer to a country, *zone* or *compartment* free from infection with HPR-deleted ISAV, but not necessarily free from infection with HPR0 ISAV.

When importing *aquatic animal products* of the species referred to in Article 10.5.2. from a country, *zone* or *compartment* declared free from infection with HPR-deleted ISAV, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* certifying that, on the basis of the procedures described in Articles 10.5.5. or 10.5.7.(as applicable) and 10.5.9., the place of production of the *commodity* is a country, *zone* or *compartment* declared free from infection with infection with HPR-deleted ISAV.

Annex 7 (contd)

The *certificate* should be in accordance with the Model Certificate in Chapter 5.11.

This Article does not apply to *commodities* referred to in point 1 of Article 10.5.3.

Article 10.5.16.

Importation of aquatic animals and aquatic animal products for retail trade for human consumption from a country, zone or compartment not declared free from infection with infectious salmon anaemia virus

In this article, all statements referring to infection with ISAV are for any detectable ISAV, including HPR0 ISAV.

1) *Competent Authorities* should not require any conditions related to infection with ISAV, regardless of the ISAV status of the *exporting country*, *zone* or *compartment* when authorising the importation or transit of the following *commodities* which have been prepared and packaged for retail trade and complying with Article 5.4.2.:

   a) fish fillets or steaks (frozen or chilled).

EU comment

The EU suggest replacing the "a)" in the point above by an "-", as there is no "b)" below.

Certain assumptions have been made in assessing the safety of *aquatic animals* and *aquatic animal products* listed above. Member Countries should refer to these assumptions at Article 5.4.2. and consider whether the assumptions apply to their conditions.

For these *commodities* Member Countries may wish to consider introducing internal measures to address the *risks* associated with the *commodity* being used for any purpose other than for human consumption.

2) When importing *aquatic animals* or *aquatic animal products*, other than those referred to in point 1 above, of the species referred to in Article 10.5.2. from a country, *zone* or *compartment* not declared free from infection with ISAV, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

Article 10.5.17.

Importation of disinfected eggs for aquaculture from a country, zone or compartment not declared free from infection with infectious salmon anaemia virus

In this article, all statements referring to infection with ISAV are for any detectable ISAV, including HPR0 ISAV.

1) When importing disinfected eggs of the species referred to in Article 10.5.2. for *aquaculture*, from a country, *zone* or *compartment* not declared free from infection with ISAV, the *Competent Authority* of the *importing country* should assess the *risk* associated with at least:

   a) the ISAV status of the water to be used during the *disinfection* of the eggs;

   b) the level of *infection* with ISAV in broodstock (ovarian fluid and milt); and

   c) the temperature and pH of the water to be used for *disinfection*.

2) If the *Competent Authority* of the *importing country* concludes that the importation is acceptable, it should apply the following *risk* mitigation measures including:

a) the eggs should be disinfected prior to importing, according to the methods described in Chapter 1.1.3. of the *Aquatic Manual* (under study) or those specified by the *Competent Authority* of the *importing country*; and

b) between *disinfection* and the import, eggs should not come into contact with anything which may affect their health status.

Member Countries may wish to consider internal measures, such as renewed *disinfection* of the eggs upon arrival in the *importing country*.

3) When importing disinfected eggs of the species referred to in Article 10.5.2. for *aquaculture*, from a country, *zone* or *compartment* not declared free from infection with ISAV, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that the procedures described in point 2 of this article have been fulfilled.

— Text deleted

Annex 8

MODEL DISEASE CHAPTER 10.X.
SHOWING HORIZONTAL AMENDMENTS

CHAPTER 10.X.

DISEASE X

EU comment

The EU in general supports these proposed model articles. Some comments are inserted in the text below.

[…]

Article 10.X.4.

Disease X

EU comment

Please insert the words "freedom form infection with" before "Disease X" in the title of this article.

A country may make a *self-declaration of freedom* from 'Disease X' if it meets the conditions in points 1, 2, 3 or 4 below.

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from 'Disease X' if all the areas covered by the shared water are declared 'Disease X' free countries or *zones* (see Article 10.X.5.).

1) A country where none of the *susceptible species* is present may make a *self-declaration of freedom* from 'Disease X' when *basic biosecurity conditions* have been continuously met in the country for at least the past two years.

OR

2) A country where the species referred to in Article 10.X.2. are present but there has been no observed occurrence of the *disease* for at least the past ten years despite conditions that are conducive to its clinical expression, as described in the corresponding chapter of the *Aquatic Manual*, may make a *self-declaration of freedom* from 'Disease X' when *basic biosecurity conditions* have been continuously met in the country for at least the past ten years.

OR

3) A country ~~where the last observed occurrence of the *disease* was within the past ten years or~~ where the disease *~~infection~~* status prior to *targeted surveillance* was unknown ~~(e.g. because of the absence of conditions conducive to clinical expression as described in the corresponding chapter of the *Aquatic Manual*)~~ may make a *self-declaration of freedom* from 'Disease X' when:

   a) *basic biosecurity conditions* have been continuously met for at least the past two years; and

   b) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of 'Disease X'.

OR

4) A country that has made a *self-declaration of freedom* from 'Disease X' but in which the *disease* is subsequently detected may make a *self-declaration of freedom* from 'Disease X' again when the following conditions have been met:

a) on detection of the *disease*, the affected area was declared an *infected zone* and a *protection zone* was established; and

b) infected populations have been destroyed or removed from the *infected zone* by means that minimise the *risk* of further spread of the *disease*, and the appropriate *disinfection* procedures (see *Aquatic Manual*) have been completed; and

c) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of 'Disease X'; and

d) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place since ~~for at least the past two years~~ eradication of the *disease*.

In the meantime, part of the non-affected area may be declared a free *zone* provided that such part meets the conditions in point 3 of Article 10.X.5.

EU comment

The EU notes that not all disease specific chapters include four options for self-declaration of disease freedom, but only three. Therefore, the structure of the model article above should be adapted accordingly in the different chapters (e.g. ISA, VHS, A. invadans).

On the other hand, a fourth point could be added in those chapters where currently only three options are proposed, and an explanation provided for the absence of the 4th option.

Article 10.X.5.

'Disease X' free zone or free compartment

A *zone* or *compartment* within the *territory* of one or more countries not declared free from 'Disease X' may be declared free by the *Competent Authority(ies)* of the country(ies) concerned if the *zone* or *compartment* meets the conditions referred to in points 1, 2, 3 or 4 below.

If a *zone* or *compartment* extends over more than one country, it can only be declared an 'Disease X' free *zone* or *compartment* if all the *Competent Authorities* confirm that the conditions have been met.

1) A *zone* or *compartment* where none of the *susceptible species* is present may be declared free from 'Disease X' when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past two years.

OR

2) A *zone* or *compartment* where the species referred to in Article 10.X.2. are present but there has been no observed occurrence of the *disease* for at least the past ten years despite conditions that are conducive to its clinical expression, as described in the corresponding chapter of the *Aquatic Manual*, may be declared free from 'Disease X' when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past ten years.

OR

3) A *zone* or *compartment* ~~where the last observed occurrence of the *disease* was within the past ten years or~~ where the disease ~~*infection*~~ status prior to *targeted surveillance* was unknown ~~(e.g. because of the absence of conditions conducive to clinical expression as described in the corresponding chapter of the *Aquatic Manual*)~~ may be declared free from 'Disease X' when:

a) *basic biosecurity conditions* have been continuously met for at least the past two years; and

b) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of 'Disease X'.

OR

4) A *zone* previously declared free from 'Disease X' but in which the *disease* is detected may be declared free from 'Disease X' again when the following conditions have been met:

a) on detection of the *disease*, the affected area was declared an *infected zone* and a *protection zone* was established; and

b) infected populations have been destroyed or removed from the *infected zone* by means that minimise the *risk* of further spread of the *disease*, and the appropriate *disinfection* procedures (see *Aquatic Manual*) have been completed; and

c) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of 'Disease X'; and

d) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place since ~~for at least the past two years~~ eradication of the *disease*

Article 10.X.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from 'Disease X' following the provisions of points 1 or 2 of Articles 10.X.4. or 10.X.5. (as relevant) may maintain its status as 'Disease X' free provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from 'Disease X' following the provisions of point 3 of Articles 10.X.4. or 10.X.5. (as relevant) may discontinue *targeted surveillance* and maintain its status as 'Disease X' free provided that conditions that are conducive to clinical expression of 'Disease X', as described in the corresponding chapter of the *Aquatic Manual*, exist and *basic biosecurity conditions* are continuously maintained.

However, for declared free *zones* or *compartments* in infected countries and in all cases where conditions are not conducive to clinical expression of 'Disease X', *targeted surveillance* needs to be continued at a level determined by the *Aquatic Animal Health Service* on the basis of the likelihood of *infection*.

Article 10.X.7

Importation of live aquatic animals from a country, zone or compartment declared free from 'Disease X'

When importing live *aquatic animals* of the species referred to in Article 10.X.2. from a country, *zone* or *compartment* declared free from 'Disease X', the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* certifying that, on the basis of the procedures described in Articles 10.X.4. or 10.X.5. (as applicable) and 10.X.6., the place of production of the *aquatic animal* is a country, *zone* or *compartment* declared free from 'Disease X'.

The *certificate* should be in accordance with the Model Certificate in Chapter 5.11. This Article does not apply to *commodities* referred to in point 1 of Article 10.X.3.

[…]

Annex 8 (contd)

Article 10.X.9.

Importation of aquatic animals and aquatic animal products for processing for human consumption from a country, zone or compartment not declared free from 'Disease X'

When importing, for processing for human consumption, *aquatic animals* or *aquatic animal products* of species referred to in Article 10.X.2. from a country, *zone* or *compartment* not declared free from 'Disease X', the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, require that:

1) the consignment is delivered directly to and held in *quarantine* or containment facilities until processing into one of the products referred to in point 1 of Article 10.X.3., or products described in point 1 of Article 10.X.12., or other products authorised by the *Competent Authority*; and

2) water used in transport and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of 'Disease X' or is disposed in a manner that prevents contact of waste with *susceptible species*.

For these *commodities* Member Countries may wish to consider introducing internal measures to address the *risks* associated with the *commodity* being used for any purpose other than for human consumption.

Article 10.X.10.

Importation of live aquatic animals intended for use in animal feed, or for agricultural, industrial or pharmaceutical use from a country, zone or compartment not declared free from from 'Disease X'

When importing, for use in animal *feed*, or for agricultural, industrial or pharmaceutical use, live *aquatic animals* of the species referred to in Article 10.X.2. from a country, *zone* or *compartment* not declared free from 'Disease X', the *Competent Authority* of the *importing country* should require that:

1) the consignment is delivered directly to and held in *quarantine* facilities for slaughter and processing to products authorised by the *Competent Authority*; and

2) water used in transport and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of 'Disease X'. This Article does not apply to *commodities* referred to in point 1 of Article 10.X.3.

Article 10.X.11. (in fish and amphibian chapters) and 10.X.10 (in mollusc and crustacean chapters)

Importation of aquatic animal products from a country, zone or compartment declared free from 'Disease X'

When importing *aquatic animal products* of the species referred to in Article 10.X.2. from a country, *zone* or *compartment* declared free from 'Disease X', the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* certifying that, on the basis of the procedures described in Articles 10.X.4. or 10.X.5. (as applicable) and 10.X.6, the place of production of the *commodity* is a country, *zone* or *compartment* declared free from 'Disease X'.

The *certificate* should be in accordance with the Model Certificate in Chapter 5.11. This Article does not apply to *commodities* referred to in point 1 of Article 10.X.3.

[…]

— Text deleted

Annex 9

CHAPTER X.X

CRITERIA FOR LISTING SPECIES AS SUSCEPTIBLE TO INFECTION WITH A SPECIFIC PATHOGEN ~~DETERMINING SUSCEPTIBILITY OF AQUATIC ANIMALS TO SPECIFIC PATHOGENIC AGENTS~~

EU comment

The EU commends the OIE Aquatic Animals Commission for taking into consideration the previous comments from the Member Countries and supports the amendments of this chapter.

Article X.X.1.

The purpose of this chapter is to provide criteria for determining ~~which *susceptible* *species*~~ that are listed as susceptible in Article X.X.2. of each *disease* specific chapter in the *Aquatic Code* and Article 2.2.1. of each *disease* specific chapter in the *Aquatic Manual*.

Article X.X.2.

Scope

~~This chapter provides criteria to determine which species should be listed as susceptible to *infection* with the aetiological agent of *listed diseases*.~~ Susceptibility may include clinical or non-clinical *infection*~~. This chapter~~ but does not ~~provide criteria for identifying~~ include mechanical vectors (i.e. species that may carry the ~~pathogen~~ aetiological agent without replication).

~~The decision to list a species as susceptible should be based on a finding that the evidence is definite. However, possible susceptibility of a species is also important information and this should also be included in Section 2.2.1. of the *disease* chapter of the *Aquatic Manual*.~~

Article X.X.3.

Approach

~~There~~ A~~are~~ three stage~~s~~ approach is outlined in this chapter to assess~~ing~~ susceptibility of a species to *infection* with a specified aetiological agent:

1) criteria to determine whether the route of *infection* used is consistent with natural pathways for the *infection* (as described in Article X.X.4.);

2) criteria to determine whether the aetiological agent has been identified using a technique (as described in Article X.X.5.);

3) criteria to determine whether the evidence indicates that presence of the aetiological agent constituted an *infection* (as described ~~using the criteria~~ in Article X.X.6.).

Article X.X.4.

Stage 1: criteria to determine whether the route of infection used is consistent with natural pathways for the infection ~~for transmission of infection~~

The evidence should be classified as transmission through: ~~a) natural occurrence, b) non-invasive experimental procedure, or c) invasive experimental procedure.~~

a) natural occurrence; includes all situations where infection has arisen without direct experimental intervention e.g. infection arising in wild or farmed populations;

b) non-invasive experimental procedure; includes cohabitation with infected hosts, infection by immersion or ingestion; or

c) invasive experimental procedure; includes injection, exposure to unnaturally high loads of pathogen, or exposure to stressors (e.g. temperature) not encountered in the host's natural or culture environment.

Consideration needs to be given to whether experimental procedures (e.g. inoculation, infectivity load, host stress) mimic natural pathways for *disease* transmission.

Article X.X.5.

Stage 2: criteria to determine whether the aetiological agent has been adequately identified ~~for identification of the aetiological agent~~

The aetiological agent should be identified and confirmed in accordance with the methods described in Section 7 (corroborative diagnostic criteria) of the relevant *disease* chapter in the *Aquatic Manual,* or other methods that have been demonstrated to be equivalent.

~~Under some circumstances the presumptive identification of the aetiological agent has~~ been ~~made but not~~ confirmed ~~in accordance with the *Aquatic Manual*~~.

Article X.X.6.

Stage 3: criteria to determine whether the evidence indicates that presence of the aetiological agent constituted an infection ~~to determine infection~~

A combination of t~~T~~he following criteria should be used to determine *infection* (see Article X.X.7):

A. the aetiological agent is multiplying in the host, or ~~that~~ developing ~~or latent~~ stages of the aetiological agent are present in or on the host;

B. viable aetiological agent is isolated from the proposed *susceptible species*, or ~~viability~~ infectivity is demonstrated ~~via~~ by way of transmission to naive individuals ~~(by natural routes)~~;

C. clinical ~~and/~~or pathological changes are associated with the *infection*;

D. the specific location of the pathogen corresponds with the expected target tissues.

The type of evidence to demonstrate *infection* will depend on the aetiological agent and potential host species under consideration.

Article X.X.7.

Outcomes of the assessment

The decision to list a species as susceptible should be based on a finding that the evidence is definite. Evidence should be provided for the following:

~~*Susceptible species* can be classified as 1) Possible or 2) Definite~~

~~1. Definite susceptible species:~~

1~~a~~) Transmission has been obtained ~~by~~ naturally or by experimental procedures that mimic natural pathways of *infection* in accordance with Article X.X.4.;

AND

2b) the identity of the aetiological agent has been confirmed in accordance with Article X.X.5;

AND

3c) there is evidence of *infection* with the aetiological agent in the suspect host species in accordance with criteria A to D in Article X.X.6. Evidence to support criterion A alone is sufficient to determine *infection*. In the absence of evidence to meet criterion A, satisfying at least two of criteria B, C or D would be required to determine *infection*.

Species for which there is incomplete evidence for susceptibility

Where evidence exists but is insufficient to demonstrate susceptibility of a species because either transmission does not mimic natural pathways of infection, or the identity of the aetiological agent has not been confirmed, or *infection* is only partially supported, the information will be included in the relevant disease chapter in the *Aquatic Manual*.

Where these species could reasonably be expected to pose a risk of transmission for the pathogen under consideration, Competent Authorities should conduct a risk analysis in accordance with the recommendations in the *Aquatic Code*.

2. Possible susceptible species:

a) The presumptive identification of the aetiological agent has been made but may not have been confirmed in accordance with Article X.X.5.;

AND

b) there is evidence of *infection* with the aetiological agent in the suspect species in accordance with Article X.X.6. At least one of criteria A, B, C or D in Article X.X.6. is required.

Article X.X.8.

Taxonomic relationship of susceptible species

Defining species as possible susceptible on the basis of a taxonomic relationship at levels higher than genus requires solid evidence that the pathogen has a very wide host range.

For aetiological agents with a wide host range, the taxonomic relationship of a species to other known *susceptible species* may be used to assume susceptibility. Species can be classified as 'possible' *susceptible species* if they reside in a genus that includes at least two *susceptible species* and in which there is no strong evidence of resistance to *infection*.

Evidence of resistance would include the following:

1) Appropriate testing reveals no evidence of *infection* when animals are exposed to the pathogen in natural setting where the pathogen is known to be present and to cause *disease* in *susceptible species*.

2) Appropriate testing reveals no evidence of *infection* when animals are exposed through controlled challenges by natural routes.

- - - - - - - - - - - - - -

— Text deleted

Annex 10

CHAPTER 10.X.

INFECTION WITH SALMONID ALPHAVIRUS

EU comment

The EU commends the OIE and supports the adoption of this new chapter.

Article 10.X.1.

General provisions

For the purposes of the *Aquatic Code*, infection with *Salmonid alphavirus* (SAV) means *infection* with any subtype of SAV of the genus Alphavirus of the family Togaviridae.

Information on methods for diagnostic tests is provided in the *Aquatic Manual*.

Article 10.X.2.

Scope

The recommendations in this chapter apply to: Atlantic salmon (*Salmo salar*), brown trout (*S. trutta*) and rainbow trout (*Onchorynchus mykiss*). These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

Article 10.X.3.

Importation or transit of aquatic animals and aquatic animal products for any purpose from a country, zone or compartment not declared free from infection with Salmonid alphavirus

1) *Competent Authorities* should not require any conditions related to infection with SAV, regardless of the SAV status of the *exporting country*, *zone* or *compartment* when authorising the importation or transit of the following *aquatic animals* and *aquatic animal products* from the species referred to in Article 10.X.2. intended for any purpose and complying with Article 5.4.1.:

   [a) heat sterilised, hermetically sealed fish products (i.e. a heat treatment at 121˚C for at least 3.6 minutes or any time/temperature equivalent);

   b) pasteurised fish products that have been subjected to a heat treatment at 90˚C for at least 10 minutes (or to any time/temperature equivalent which has been demonstrated to inactivate SAV);

   c) mechanically dried, eviscerated fish (i.e. a heat treatment at 100˚C for 30 minutes or any time/temperature equivalent which has been demonstrated to inactivate SAV);

   d) fish oil;

   e) fish *meal*; and

   f) fish skin leather] under study

2) When authorising the importation or transit of *aquatic animals* and *aquatic animal products* of a species referred to in Article 10.X.2., other than those referred to in point 1 of Article 10.X.3., *Competent Authorities* should require the conditions prescribed in Articles 10.X.7. to 10.X.12. relevant to the SAV status of the *exporting country*, *zone* or *compartment*.

3) When considering the importation or transit of *aquatic animals* and *aquatic animal products* from an *exporting country*, *zone* or *compartment* not declared free from infection with SAV of a species not covered in Article 10.X.2. but which could reasonably be expected to pose a *risk* of transmission for SAV, *Competent Authorities* should conduct a *risk analysis* in accordance with the recommendations in the *Aquatic Code*. The *exporting country* should be informed of the outcome of this assessment.

Article 10.X.4.

Country free from infection with Salmonid alphavirus

A country may make a *self-declaration of freedom* from infection with SAV if it meets the conditions in points 1, 2, 3 or 4 below.

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from infection with SAV if all the areas covered by the shared water are declared countries or *zones* free from infection SAV (see Article 10.X.5.).

1) A country where none of the *susceptible species* is present may make a *self-declaration of freedom* from infection with SAV when *basic biosecurity conditions* have been continuously met in the country for at least the past two years.

OR

2) A country where the species referred to in Article 10.X.2. are present but there has been no observed occurrence of infection SAV for at least the past ten years despite conditions that are conducive to clinical expression, as described in the corresponding chapter of the *Aquatic Manual*, may make a *self-declaration of freedom* from infection with SAV when *basic biosecurity conditions* have been continuously met in the country for at least the past ten years.

OR

3) A country where the disease status prior to *targeted surveillance* was unknown may make a *self-declaration of freedom* from infection with SAV when:

   a) *basic biosecurity conditions* have been continuously met for at least the past two years; and

   b) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of infection with SAV.

OR

4) A country that has made a *self-declaration of freedom* from infection with SAV but in which the disease is subsequently detected may make a *self-declaration of freedom* from infection with SAV again when the following conditions have been met:

   a) on detection of SAV, the affected area was declared an *infected zone* and a *protection zone* was established; and

   b) infected populations have been destroyed or removed from the *infected zone* by means that minimise the *risk* of further spread of the disease, and the appropriate *disinfection* procedures (see *Aquatic Manual*) have been completed; and

c) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of SAV; and

d) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place since eradication of the *disease*.

In the meantime, part of the non-affected area may be declared a free *zone* provided that such part meets the conditions in point 3 of Article 10.X.7.

Article 10.X.5.

Zone or compartment free from infection with salmonid alphavirus

In this article, all statements refer to a *zone* or *compartment* free from infection with SAV.

A *zone* or *compartment* within the *territory* of one or more countries not declared free from infection with SAV may be declared free by the *Competent Authority(ies)* of the country(ies) concerned if the *zone* or *compartment* meets the conditions referred to in points 1, 2, 3 or 4 below.

1) A *zone* or *compartment* where none of the *susceptible species* is present may be declared free from infection with SAV when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past two years.

OR

2) A *zone* or *compartment* where the species referred to in Article 10.X.2. are present but there has been no observed occurrence of infection with SAV for at least the past ten years despite conditions that are conducive to its clinical expression, as described in the corresponding chapter of the *Aquatic Manual*, may be declared free from infection with SAV when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past ten years.

OR

3) A *zone* or *compartment* where the disease status prior to *targeted surveillance* was unknown may be declared free from infection with SAV when:

a) *basic biosecurity conditions* have been continuously met for at least the past two years; and

b) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of infection with SAV.

OR

4) A *zone* previously declared free from infection with SAV but in which infection with SAV is detected may be declared free from infection with SAV again when the following conditions have been met:

a) on detection of infection with SAV, the affected area was declared an *infected zone* and a *protection zone* was established; and

b) infected populations have been destroyed or removed from the *infected zone* by means that minimise the *risk* of further spread of SAV, and the appropriate *disinfection* procedures (see *Aquatic Manual*) have been completed; and

Annex 10 (contd)

c) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of infection with SAV; and

d) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place since eradication of the *disease*.

Article 10.X.6.

Maintenance of free status for infection with salmonid alphavirus

A country, *zone* or *compartment* that is declared free from infection with SAV following the provisions of points 1 or 2 of Articles 10.X.4. or 10.X.5. (as relevant) may maintain its status as free from infection with SAV provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from infection with SAV following the provisions of point 3 of Articles 10.X.4. or 10.X.5. (as relevant) may discontinue *targeted surveillance* and maintain its free status provided that conditions that are conducive to clinical expression, as described in the corresponding chapter of the *Aquatic Manual*, exist and *basic biosecurity conditions* are continuously maintained.

However, for declared free *zones* or *compartments* in an infected country and in all cases where conditions are not conducive to clinical expression, *targeted surveillance* needs to be continued at a level determined by the *Aquatic Animal Health Service* on the basis of the likelihood of *infection*.

Article 10.X.7.

Importation of live aquatic animals from a country, zone or compartment declared free from infection with salmonid alphavirus

When importing live *aquatic animals* of the species referred to in Article 10.X.2. from a country, *zone* or *compartment* declared free from infection with SAV, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* certifying that, on the basis of the procedures described in Articles 10.X.4. or 10.X.5. (as applicable) and 10.X.6., the place of production of the *aquatic animal* is a country, *zone* or *compartment* declared free from infection with SAV.

The *certificate* should be in accordance with the Model Certificate in Chapter 5.11. This Article does not apply to *commodities* referred to in point 1 of Article 10.X.3.

Article 10.X.8.

Importation of live aquatic animals for aquaculture from a country, zone or compartment not declared free from infection with salmonid alphavirus

1) When importing, for *aquaculture*, live *aquatic animals* of the species referred to in Article 10.X.2. from a country, *zone* or *compartment* not declared free from infection with SAV, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, apply the following *risk* mitigation measures:

   a) the direct delivery to and lifelong holding of the consignment in biosecure facilities for continuous isolation from the local environment; and

   b) the treatment of all effluent and waste materials in a manner that ensures inactivation of SAV.

2) If the intention of the introduction is the establishment of a new stock, relevant aspects of the Code of Practice on the Introductions and Transfers of Marine Organisms of the International Council for the Exploration of the Seas (ICES) should be considered.

3) For the purposes of the *Aquatic Code*, relevant aspects of the ICES Code (full version see: http://www.ices.dk/publications/our-publications/Pages/Miscellaneous.aspx) may be summarised to the following points:

   a) identify stock of interest (cultured or wild) in its current location;

   b) evaluate stock health/disease history;

   c) take and test samples for SAV, pests and general health/disease status;

   d) import and quarantine in a secure facility a founder (F-0) population;

   e) produce F-1 generation from the F-0 stock in *quarantine*;

   f) culture F-1 stock and at critical times in its development (life cycle) sample and test for SAV and perform general examinations for pests and general health/disease status;

   g) if SAV is not detected, pests are not present, and the general health/disease status of the stock is considered to meet the *basic biosecurity conditions* of the *importing country*, *zone* or *compartment*, the F-1 stock may be defined as free from infections with SAV or specific pathogen free (SPF) for SAV;

   h) release SPF F-1 stock from *quarantine* for *aquaculture* or stocking purposes in the country, *zone* or *compartment*.

4) With respect to point 3e), *quarantine* conditions should be conducive to multiplication of the pathogen and eventually to clinical expression. If *quarantine* conditions are not suitable for pathogen multiplication and development, the recommended diagnostic approach might not be sensitive enough to detect a low *infection* level.

This Article does not apply to *aquatic animals* referred to in point 1 of Article 10.X.3.

Article 10.X.9.

Importation of aquatic animals and aquatic animal products for processing for human consumption from a country, zone or compartment not declared free from infection with salmonid alphavirus

When importing, for processing for human consumption, *aquatic animals* or *aquatic animal products* of species referred to in Article 10.X.2. from a country, *zone* or *compartment* not declared free from infection with SAV, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, require that:

1) the consignment is delivered directly to and held in *quarantine* or containment facilities until processing into one of the products referred to in point 1 of Article 10.X.3., or products described in point 1 of Article 10.X.12., or other products authorised by the *Competent Authority*; and

2) water used in transport and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of SAV or is disposed in a manner that prevents contact of waste with *susceptible species*.

For these *commodities* Member Countries may wish to consider introducing internal measures to address the *risks* associated with the *commodity* being used for any purpose other than for human consumption.

Annex 10 (contd)

Article 10.X.10.

Importation of live aquatic animals intended for use in animal feed, or for agricultural, industrial or pharmaceutical use from a country, zone or compartment not declared free from infection with salmonid alphavirus

When importing, for use in animal *feed*, or for agricultural, industrial or pharmaceutical use, live *aquatic animals* of the species referred to in Article 10.X.2. from a country, *zone* or *compartment* not declared free from infection with SAV, the *Competent Authority* of the *importing country* should require that:

1) the consignment is delivered directly to and held in *quarantine* for slaughter and processing to products authorised by the *Competent Authority*; and

2) water used in transport and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of SAV.

This Article does not apply to *commodities* referred to in point 1 of Article 10.X.3.

Article 10.X.11.

Importation of aquatic animal products from a country, zone or compartment declared free from infection with salmonid aphavirus

When importing *aquatic animal products* of the species referred to in Article 10.X.2. from a country, *zone* or *compartment* declared free from infection with SAV, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* certifying that, on the basis of the procedures described in Articles 10.X.4. or 10.X.5, (as applicable) and 10.X.6., the place of production of the *commodity* is a country, *zone* or *compartment* declared free from infection with SAV.

The *certificate* should be in accordance with the Model Certificate in Chapter 5.11.

This Article does not apply to *commodities* referred to in point 1 of Article 10.X.3.

Article 10.X.12.

Importation of aquatic animals and aquatic animal products for retail trade for human consumption from a country, zone or compartment not declared free from infection with salmonid alphavirus

1) *Competent Authorities* should not require any conditions related to infection with SAV, regardless of the SAV status of the *exporting country*, *zone* or *compartment* when authorising the importation or transit of the following *commodities* which have been prepared and packaged for retail trade and complying with Article 5.4.2.:

   [a) fish fillets or steaks (frozen or chilled)] under study.

   Certain assumptions have been made in assessing the safety of *aquatic animals* and *aquatic animal products* listed above. Member Countries should refer to these assumptions at Article 5.4.2. and consider whether the assumptions apply to their conditions.

   For these *commodities Member* Countries may wish to consider introducing internal measures to address the *risks* associated with the *commodity* being used for any purpose other than for human consumption.

2) When importing *aquatic animals* or *aquatic animal products*, other than those referred to in point 1 above, of the species referred to in Article 10.X.2. from a country, *zone* or *compartment* not declared free from infection with SAV, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

Article 10.X.13.

[Importation of disinfected eggs for aquaculture from a country, zone or compartment not declared free from infection with infectious salmonid alphavirus

1) When importing disinfected eggs of the species referred to in Article 10.X.2. for *aquaculture*, from a country, *zone* or *compartment* not declared free from infection with SAV, the *Competent Authority* of the *importing country* should assess the *risk* associated with at least:

   a) the SAV status of the water to be used during the *disinfection* of the eggs;

   b) the level of *infection* with SAV in broodstock (ovarian fluid and milt); and

   c) the temperature and pH of the water to be used for *disinfection*.

2) If the *Competent Authority* of the *importing country* concludes that the importation is acceptable, it should apply the following *risk* mitigation measures including:

   a) the eggs should be disinfected prior to importing, according to the methods described in Chapter 1.1.3. of the *Aquatic Manual* (under study) or those specified by the *Competent Authority* of the *importing country*; and

   b) between *disinfection* and the import, eggs should not come into contact with anything which may affect their health status.

   Member Countries may wish to consider internal measures, such as renewed *disinfection* of the eggs upon arrival in the *importing country*.

3) When importing disinfected eggs of the species referred to in Article 10.X.2. for *aquaculture*, from a country, *zone* or *compartment* not declared free from infection with SAV, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that the procedures described in point 2 of this article have been fulfilled.] under study.

Annex 11

CHAPTER 2.3.5.

INFECTION WITH INFECTIOUS SALMON ANAEMIA VIRUS

EU comment

The EU thanks the OIE for taking into consideration previous comments and in general supports the proposed amendments to this chapter. Comments are inserted in the text below.

1. Scope

For the purpose of this chapter, infection with infectious salmon anaemia virus (ISAV) means infection with highly polymorphic region (HPR)-deleted ISAV or HPR0 ISAV (with a non-deleted HPR) of the genus *Isavirus* of the family *Orthomyxoviridae*.

Infection with HPR-deleted ISAV may cause infectious salmon anaemia (ISA) in Atlantic salmon (*Salmo salar*), which is a generalised and lethal condition characterised by severe anaemia, and variable haemorrhages and necrosis in several organs. The disease course is prolonged with low daily mortality (0.05–0.1%) typically only in a few cages. Cumulative mortality may become very high for a period lasting several months if nothing is done to limit disease dissemination (Rimstad *et al*., 2011).

Detection of HPR0 ISAV has never been associated with ISA in Atlantic salmon. This virus genotype is known to cause transient subclinical infection and has mainly been detected localised to the gills. There is evidence of a link between non-pathogenic HPR0 ISAV and pathogenic HPR-deleted ISAV, with some outbreaks potentially occurring as a result of the emergence of HPR-deleted ISAV from HPR0 ISAV.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent, agent strains

ISAV is an enveloped virus, 100–130 nm in diameter, with a genome consisting of eight single-stranded RNA segments with negative polarity. The virus has haemagglutinating, receptor-destroying and fusion activity (Falk *et al*., 1997; Mjaaland *et al*., 1997; Rimstad *et al*., 2011).

The morphological, physiochemical and genetic properties of ISAV are consistent with those of the *Orthomyxoviridae,* and ISAV has been classified as the type species of the genus *Isavirus* (Kawaoka *et al*., 2005) within this virus family. The nucleotide sequences of all eight genome segments, encoding at least ten proteins, have been described (Cottet *et al*., 2011; Rimstad *et al*., 2011), including the 3' and 5' non-coding sequences (Kulshreshtha *et al.,* 2010). Four major structural proteins have been identified, including a 68 kDa nucleoprotein, a 22 kDa matrix protein, a 42 kDa haemagglutinin-esterase (HE) protein responsible for receptor-binding and receptor-destroying activity, and a 50 kDa surface glycoprotein with putative fusion (F) activity, encoded by genome segments 3, 8, 6 and 5, respectively. Segment 1, 2, and 4 encode the viral polymerases PB2, PB1 and PA. The two smallest genomic segments, segments 7 and 8, each contain two open reading frames (ORF). The ORF1 of segment 7 encodes a protein with type I interferon antagonistic properties, while ORF2 has been suggested to encode for a nuclear export protein (NEP). Whether the ORF1 gene product is nonstructural or a structural component of the virion remains to be determined. The smaller ORF1 of segment 8 encodes the matrix protein, while the larger ORF2 encodes an RNA-binding structural protein also with type I interferon antagonistic properties.

Annex 11 (contd)

Sequence analysis of various gene segments has revealed differences between isolates both within and between defined geographical areas. According to sequence differences in the 5'-region of the HE gene, ISAV isolates have been divided into two major groups, one European and one North American group. In the HE gene, a small HPR near the transmembrane domain has been identified. This region is characterised by the presence of gaps rather than single-nucleotide substitutions (Cunningham *et al*., 2002; Mjaaland *et al*., 2002). A full-length gene (HPR0) has been suggested to represent a precursor from which all ISAV HPR-deleted (pathogenic) variants of ISAV originate. The presence of non-pathogenic HPR0 ISAV has been reported in both apparently healthy wild and farmed Atlantic salmon, but has not been detected in diseased fish with clinical disease and pathological signs consistent with ISA (Christiansen *et al*., 2011; Cunningham *et al*., 2002; Lyngstad *et al*., 2012; Markussen *et al*., 2008; McBeath *et al*., 2009; Nylund *et al*., 2007). A mixed infection of HPR-deleted and HPR0 ISAV variants has been reported (Kibenge *et al.,* 2009). Recent studies show that HPR0 ISAV variants occur frequently in sea-reared Atlantic salmon. The HPR0 ISAV strain seems to be more seasonal and transient in nature and displays a tissue tropism with high prevalence in gills (Christiansen *et al.*, 2011; Lyngstad *et al*., 2011). To date there has been no direct evidence linking the presence of HPR0 ISAV to a subsequent clinical ISA outbreak. The risk of emergence of pathogenic HPR-deleted ISAV variants from a reservoir of HPR0 ISAV is considered to be low but not negligible (Christiansen *et al*., 2011; EFSA, 2012; Lyngstad *et al.,* 2012).

In addition to the variations seen in the HPR of the HE gene, other gene segments may also be of importance for development of ISA. A putative virulence marker has been identified in the fusion (F) protein. Here, a single amino acid substitution, or a sequence insertion, near the protein's putative cleavage site has been found to be a prerequisite for virulence (Kibenge *et al.,* 2007; Markussen *et al*., 2008). Aside from insertion/recombination, ISAV also uses gene segment reassortment in its evolution, with potential links to virulence (Devold *et al*., 2006; Markussen *et al*., 2008; Mjaaland *et al*., 2005).

2.1.2. Survival outside the host

ISAV has been detected by reverse-transcription polymerase chain reaction (RT-PCR) in seawater sampled at farming sites with ISAV-positive Atlantic salmon (Kibenge *et al.,* 2004). It is difficult to estimate exactly how long the virus may remain infectious in the natural environment because of a number of factors, such as the presence of particles or substances that may bind or inactivate the virus. Exposing cell culture-propagated ISAV to 15°C for 10 days or to 4°C for 14 days had no effect on virus infectivity (Falk *et al*., 1997).

2.1.3. Stability of the agent (effective inactivation methods)

ISAV is sensitive to UV irradiation (UVC) and ozone. A 3-log reduction in infectivity in sterile fresh water and seawater was obtained with a UVC dose of approximately 35 Jm^{-2} and 50 Jm^{-2}, respectively, while the corresponding value for ISAV in wastewater from a fish-processing plant was approximately 72 Jm^{-2}. Ozonated seawater (4 minutes with 8 mg ml^{-1}, 600–750 mV redox potential) may inactivate ISAV completely. Incubation of tissue homogenate from diseased fish at pH 4 or pH 12 for 24 hours inactivated ISAV infectivity. Incubation in the presence of chlorine (100 mg ml^{-1}) for 15 minutes also inactivated virus infectivity (Rimstad *et al*., 2011). Cell culture-isolated ISAV may survive for weeks at low temperatures, but virus infectivity is lost within 30 minutes of exposure at 56°C (Falk *et al*., 1997).

2.1.4. Life cycle

The main infection route is most likely through the gills for both HPR0 and HPR-deleted ISAV, but infection via the intestine or skin cannot be excluded. HPR-deleted ISAV has been used in the studies referred to below. Endothelial cells lining blood vessels seem to be the primary target cells for ISAV as demonstrated by electron microscopy immunohistochemistry and *in-situ* hybridisation. Virus replication has also been demonstrated in leukocytes, and sinusoidal macrophages in kidney tissue stain positive for ISAV using immunohistochemistry (IHC). As endothelial cells are the target cells (see Section 2.2.4), virus replication may occur in any organ (Aamelfot *et al.,* 2012; Rimstad *et al*., 2011).

The haemagglutinin-esterase (HE) molecule of ISAV, like the haemagglutinin (HA) of other orthomyxoviruses (influenza A, B and C viruses), is essential for binding of the virus to sialic acid residues on the cell surface. In the case of ISAV, the viral particle binds to glycoprotein receptors containing 4-*O*-acetylated sialic acid residues, which also functions as a substrate for the receptor-destroying enzyme. Further uptake and replication seem to follow the pathway described for influenza A viruses, indicated by demonstration of low pH-dependent fusion, inhibition of replication by actinomycin D and α-amanitin, early accumulation of nucleoprotein followed by matrix protein in the nucleus and budding of progeny virions from the cell surface (Cottet *et al.,* 2011; Rimstad *et al.,* 2011).

The route of shedding of ISAV from infected fish may be through natural excretions/secretions.

The HPR0 variant has hitherto not been isolated in cell culture, which hampers *in-vivo* and *in-vitro* studies of characteristics and the life cycle of this virus variant.

2.2. Host factors

2.2.1. Susceptible host species

Natural outbreaks of ISA have only been recorded in farmed Atlantic salmon, and in Coho salmon (*Oncorhynchus kisutch*) in Chile (Kibenge *et al*, 2001). Subclinically infected feral Atlantic salmon, brown trout and sea trout (*S. trutta*) have been identified by RT-PCR (Kibenge *et al*., 2004; Plarre *et al*., 2005). In marine fish, detection of ISAV by RT-PCR has been reported in tissues of pollock (*Pollachius virens*) and cod (*Gadus morhua*), but only in fish collected from cages with Atlantic salmon exhibiting ISA (reviewed in Kibenge *et al*., 2004). Following experimental infection by bath immersion, ISAV has been detected by RT-PCR in herring (*Clupea harengus*) and a subsequent transmission to Atlantic salmon. Attempts have been made to induce infection or disease in pollock, *Pollachius virens*, but with negative results. Replication of ISAV has also been demonstrated in several salmonid species but only after intraperitoneal injection of ISAV-infected material (reviewed in Rimstad *et al*., 2011).

2.2.2. Susceptible stages of the host

In Atlantic salmon, disease outbreaks are mainly reported in seawater cages, and only a few cases have been reported in the freshwater stage, including one case in yolk sac fry (Rimstad *et al*., 2011). ISA has been experimentally induced in both Atlantic salmon fry and parr kept in freshwater. Genetics may also play an important role in the susceptibility of Atlantic salmon to ISA, as differences in susceptibility among different family groups have been observed.

2.2.3. Species or subpopulation predilection (probability of detection)

ISA is primarily a disease of Atlantic salmon.

2.2.4. Target organs and infected tissue

For fish that have developed ISA: endothelial cells in all organs (gills, heart, liver, kidney, spleen and others) (Aamelfot *et al.,* 2012). HPR0 ISAV variants seem primarily to target the gills, but this variant has also been detected in kidney and heart (Christiansen *et al*., 2011; Lyngstad *et al.,* 2011).

2.2.5. Persistent infection with lifelong carriers

Persistent infection in lifelong carriers has not been documented in Atlantic salmon, but at the farm level, infection may persist in the population by continuous infection of new individuals that do not develop clinical signs of disease. This may include infection with the HPR0 ISAV variants, which seems to be only transient in nature (Christiansen *et al*., 2011; Lyngstad *et al*., 2011). Experimental infection of rainbow trout and brown trout with ISAV indicate that persistent infection in these species could be possible (Rimstad *et al.*, 2011).

Annex 11 (contd)

2.2.6. Vectors

Passive transfer of ISAV by salmon lice (*Lepeophtheirus salmonis*) has been demonstrated under experimental conditions. Although natural vectors have not been identified, several different vector groups could be possible vectors under certain defined conditions (reviewed in Rimstad *et al.*, 2011).

2.2.7. Known or suspected wild aquatic animal carriers

Wild Atlantic salmon, brown trout and sea trout (*S. trutta*) may be carriers of ISAV (Rimstad *et al.*, 2011). The importance of wild marine fish (see Section 2.2.1) as virus carriers needs to be clarified. The results from a study from the Faroe Islands point to the potential presence of an unknown marine reservoir for this virus (Christiansen *et al*., 2011).

2.3. Disease pattern

2.3.1. Transmission mechanisms

Studies of recurrent epidemics of ISA in different salmon-producing areas conclude that the virus spreads locally between adjoining sites. Proximity to sites with ISA outbreaks is a risk of primary importance, and the risk for a susceptible farm increases the nearer it is to an infected farm. Sequence analysis of ISAV from ISA outbreaks in Norway shows a high degree of similarity between viruses isolated from neighbouring ISA affected sites, further supporting ISAV transmission between proximate sites. The risk of transmission of ISAV is dependent on the level of biosecurity measures in place. Suggested pathways for ISAV transmission are through sea water, shipment of live fish, transmission through sea lice, and via infected wild salmonids (Aldrin *et al.*, 2011; Gustafson *et al*., 2007; Lyngstad *et al*., 2011; Mardones *et al.,* 2011; Rimstad *et al*., 2011).

Many ISA outbreaks in Norway appear to be isolated in space and time from other outbreaks with unknown sources of infection (Aldrin *et al*., 2011). A suggested hypothesis for disease emergence is occasional transition of HPR0 ISAV into HPR-deleted ISAV variants causing solitary outbreaks or local epidemics through local transmission (Lyngstad *et al.,* 2011; 2012). The risk of emergence of HPR-deleted ISAV variants from a reservoir of HPR0 ISAV is considered to be low but not negligible (EFSA, 2012). A direct link between HPR0 variants and HPR-deleted ISAV remains to be demonstrated.

As ISA has also been reported from smolt-producing sites with Atlantic salmon, transmission of ISAV from parent to progeny cannot be excluded. Even though there is no evidence of true vertical transmission, eggs and embryos could be a risk of transmission if ~~of~~ ISAV biosecurity measures are not adequate (Rimstad *et al*., 2011).

2.3.2. Prevalence

In a net pen containing diseased fish, the prevalence of HPR-deleted ISAV may vary widely, while in adjacent net pens ISAV may be difficult to detect, even by the most sensitive methods. Therefore, for diagnostic investigations it is important to sample from net pens containing diseased fish.

There is increasing evidence that the prevalence of the non-pathogenic HPR0 ISAV genotype may be high in Atlantic salmon production areas. HPR0 variants in Atlantic salmon appear to be a seasonal and transient infection (Christiansen *et al*., 2011). HPR0 variants of ISAV have also been detected in wild salmonids (reviewed in Rimstad *et al*., 2011).

2.3.3. Geographical distribution

Initially reported in Norway in the mid-1980s (Thorud & Djupvik, 1988), ISA in Atlantic salmon has since then been reported in Canada (New Brunswick in 1996; Mullins *et al.,* 1998), the United Kingdom (Scotland in 1998), the Faroe Islands (2000), the USA (Maine in 2001) and in Chile (2007) (Cottet *et al*., 2011; Rimstad *et al*., 2011). The presence of the HPR0 ISAV variant has been reported in all countries where ISA has occurred.

2.3.4. Mortality and morbidity

During ISA outbreaks, morbidity and mortality may vary greatly within and between different net pens in a seawater fish farm, and between different fish farms. Morbidity and mortality within a net pen may start at very low levels. Typically, daily mortality ranges from 0.5 to 1% in affected cages. Without intervention, mortality increases and seems to peak in early summer and winter. The range of cumulative mortality during an outbreak is from insignificant to moderate, but in severe cases, cumulative mortality exceeding 90% may be recorded during several months. Initially, an outbreak of ISA may be limited to one or two net pens over a long time period. In such cases, if net pens with clinical ISA are slaughtered immediately, further development of clinical ISA at the site may be prevented. In outbreaks where smolts have been infected in well boats during transport, simultaneous outbreaks may occur.

HPR0 ISAV has not been associated with ISA in Atlantic salmon.

2.3.5. Environmental factors

Generally, outbreaks of ISA tend to be seasonal with most outbreaks in late spring and late autumn. Handling of fish (e.g. sorting or treatment, splitting or moving of cages) may initiate disease outbreaks on infected farms, especially if long-term undiagnosed problems have been experienced in advance (Lyngstad *et al*., 2008).

2.4. Control and prevention

2.4.1. Vaccination

Vaccination against ISA has been carried out in North America since 1999 and the Faroe Islands since 2005. In Norway vaccination against ISA was carried out for the first time in 2009 in a region with a high rate of ISA outbreaks. Chile started vaccinating against ISA in 2010. However, the currently available vaccines do not seem to offer complete protection in Atlantic salmon.

2.4.2. Chemotherapy

Most recently, it has been demonstrated that the broad-spectrum antiviral drug Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) is effective in inhibiting ISAV replication both *in vitro* and *in vivo* (Rivas-Aravena *et al.,* 2011).

2.4.3. Immunostimulation

Not applicable.

2.4.4. Resistance breeding

Differences in susceptibility among different family groups of Atlantic salmon in fresh water have been observed in challenge experiments and in field tests, indicating the potential for resistance breeding.

2.4.5. Restocking with resistant species

Not applicable.

2.4.6. Blocking agents

Not applicable.

2.4.7. Disinfection of eggs and larvae

Disinfection of eggs according to standard procedures is suggested as an important control measure.

Annex 11 (contd)

2.4.8. General husbandry practices

The incidence of ISA may be greatly reduced by implementation of legislative measures or husbandry practices regarding the movement of fish, mandatory health control, transport and slaughterhouse regulations. Specific measures including restrictions on affected, suspected and neighbouring farms, enforced sanitary slaughtering, generation segregation ('all in/all out') as well as disinfection of offal and wastewater from fish slaughterhouses and fish processing plants may also contribute to reducing the incidence of the disease. The experience from the Faroe Islands, where the prevalence of HPR0 is high, demostrates that the combination of good biosecurity and husbandry reduces the risk of ISA outbreaks substantially.

3. Sampling

3.1. Selection of individual specimens

The following is primarily for verification of suspected cases based on clinical signs and gross pathology or positive RT-PCR for HPR-deleted ISAV.

For detection of HPR0 ISAV, gill tissue should be sampled in randomly selected individuals at different points of time through the production cycle. Only detection using RT-PCR is possible for this genotype.

3.2. Preservation of samples for submission

Haematology:	Heparin or EDTA (ethylene diamine tetra-acetic acid)
Cell culture:	Virus transport medium
Histology and immunohistochemistry:	Fixation in neutral phosphate-buffered 10% formalin
Immunofluorescense (smears):	Either submitted dried, or dried and fixed in 100% acetone
Molecular biology (RT-PCR and sequencing):	Appropriate medium for preservation of RNA

3.3. Pooling of samples

Pooling of samples may be acceptable under some circumstances, however, the impact on sensitivity and design prevalence must be considered ~~is not recommended for verification of ISAV as it is usually of interest to compare results from the various examinations for each individual. For surveillance purposes, pooling of samples for virological examination (PCR and/or cell culture) may be accepted. However, the number of fish to be pooled may depend on the suggested prevalence of ISAV in the population and of the method used~~.

3.4. Best organs or tissues

3.4.1. Detection of HPR-deleted ISAV

Blood is preferred for non-lethal sampling. Generally, as ISA is a generalised infection, internal organs not exposed to the environment should be used for diagnostic testing.

Virological examination (cell culture and PCR): heart (should always be included) and mid-kidney;

Histology (prioritised): mid-kidney, liver, heart, pancreas/intestine, spleen;

Immunofluorescense (smears): mid-kidney;

Immunohistochemistry: mid-kidney, heart (including valves and bulbus arteriosus).

3.4.2. Detection of HPR0 ISAV

Gills should be tested by RT-PCR

4. Diagnostic methods

4.1. Field diagnostic methods

4.1.1. Clinical signs

The most prominent external signs of ISA are pale gills (except in the case of blood stasis in the gills), exophthalmia, distended abdomen, blood in the anterior eye chamber, and sometimes skin haemorrhages especially of the abdomen, as well as scale pocket oedema.

Generally, naturally infected Atlantic salmon with HPR-deleted ISAV appear lethargic and may keep close to the wall of the net pen.

Nutritional status is usually quite normal, but diseased fish have no feed in the digestive tract.

4.2. Pathological evaluation

4.2.1. Gross pathology

Fish infected with HPR-deleted ISAV may show a range of pathological changes, from none to severe, depending on factors such as infective dose, virus strain, temperature, age and immune status of the fish. No lesions are pathognomonic to ISA, but anemia and circulatory disturbances are always present. The following findings have been described to be consistent with ISA, though all changes are seldom observed in one single fish.

- Yellowish or blood-tinged fluid in peritoneal and pericardial cavities.
- Oedema of the swim bladder.
- Small haemorrhages of the visceral and parietal peritoneum.
- Focal or diffusely dark red liver. A thin fibrin layer may be present on the surface.
- Swollen, dark red spleen with rounded margins.
- Dark redness of the intestinal wall mucosa in the blind sacs, mid- and hind-gut, without blood in the gut lumen of fresh specimens.
- Swollen, dark red kidney with blood and liquid effusing from cut surfaces.
- Pinpoint haemorrhages of the skeletal muscle.

4.2.2. Clinical chemistry

- Haematocrit <10 in end stages (25–30 often seen in less advanced cases). Haematocrit <10 should always be followed up by investigation for ISA in sea-water reared Atlantic salmon.
- Blood smears with degenerate and vacuolised erythrocytes and the presence of erythroblasts with irregular nuclear shape. Differential counts show a reduction in the proportion of leucocytes relative to erythrocytes, with the largest reduction being among lymphocytes and thrombocytes.

Liver pathology will lead to increased levels of liver enzymes in the blood.

4.2.3. Microscopic pathology

Histological changes in clinically diseased Atlantic salmon are variable, but can include the following:

- Numerous erythrocytes in the central venous sinus and lamellar capillaries where erythrocyte thrombi also form in the gills.
- Multifocal to confluent haemorrhages and/or hepatocyte necrosis at some distance from larger vessels in the liver. Focal accumulations of erythrocytes in dilated hepatic sinusoids.
- Accumulation of erythrocytes in blood vessels of the intestinal lamina propria and eventually haemorrhage into the lamina propria.
- Spleen stroma distended by erythrocyte accumulation.
- Slight multifocal to extensive diffuse interstitial haemorrhage with tubular necrosis in the haemorrhagic areas, erythrocyte accumulation in the glomeruli in the kidney.
- Erythrophagocytosis in the spleen and secondary haemorrhages in liver and kidney.

4.2.4. Wet mounts

Not applicable.

4.2.5. Smears

See Section 4.3.1.1.2

4.2.6 Fixed sections

See Section 4.3.1.1.3

4.2.7. Electron microscopy/cytopathology

Virus has been observed in endothelial cells and leukocytes by electron microscopy of tissue preparations, but this method has not been used for diagnostic purposes.

4.2.8. Differential diagnoses

Other anaemic and haemorrhagic conditions, including erythrocytic inclusion body syndrome, winter ulcer and septicaemias caused by infections with *Moritella viscosa*. Disease cases in Atlantic salmon with haematocrit values below 10 is not a unique finding for ISA, however cases with such low haematocrit values without any obvious explanation should always be tested for the presence of ISAV.

4.3. Agent detection and identification methods

4.3.1. Direct detection methods

With the exception of molecular techniques (see 4.3.1.2.3), these direct detection methods are only recommended for fish with clinical signs of infection with HPR-deleted ISAV.

4.3.1.1. Microscopic methods

4.3.1.1.1. Wet mounts

Not applicable.

4.3.1.1.2. Smears

4.3.1.1.2.1 Indirect fluorescent antibody test

An indirect fluorescent antibody test (IFAT) using validated monoclonal antibodies (MAbs) against ISAV haemagglutinin-esterase (HE) on kidney smears (imprints) or on frozen tissue sections of kidney, heart and liver has given positive reactions in both experimentally and naturally infected Atlantic salmon. Suspected cases (see Section 7.1) may be confirmed with a positive IFAT.

i) Preparations of tissue smears (imprints)

A small piece of the mid-kidney is briefly blotted against absorbent paper to remove excess fluid, and several imprints in a thumbnail-sized area are fixed on poly-L-lysine-coated microscope slides. The imprints are air-dried, fixed in chilled 100% acetone for 10 minutes and stored either at 4°C for a few days or at –80°C until use.

ii) Staining procedure

After blocking with 5% non-fat dry milk in phosphate-buffered saline (PBS) for 30 minutes, the preparations are incubated for 1 hour with an appropriate dilution of anti-ISAV MAb, followed by three washes. For the detection of bound antibodies, the preparations are incubated with fluoescein isothiocyanate (FITC)-conjugated anti-mouse Ig for 1 hour. PBS with 0.1% Tween 20 is used for washing. All incubations are performed at room temperature.

4.3.1.1.2. Fixed sections

4.3.1.1.3.1 Immunohistochemistry (IHC)

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.

i) Preparation of tissue sections

The tissues are fixed in neutral phosphate-buffered 10% formalin for at least 1 day, dehydrated in graded ethanol, cleared in xylene and embedded in paraffin, according to standard protocols. Approximately 5 µm thick sections (for IHC sampled on poly-L-lysine-coated slides) are heated at 56–58°C (maximum 60°C) for 20 minutes, dewaxed in xylene, rehydrated through graded ethanol, and stained with haematoxylin and eosin for pathomorphology and IHC as described below.

ii) Staining procedure for IHC

All incubations are carried out at room temperature on a rocking platform, unless otherwise stated.

a) Antigen retrieval is done by boiling sections in 0.1 M citrate buffer pH 6.0 for 2 × 6 minutes followed by blocking with 5% non-fat dry milk and 2% goat serum in 50 mM TBS (TBS; Tris/HCl 50 mM, NaCl 150 mM, pH 7.6) for 20 minutes.

b) Sections are then incubated overnight with primary antibody (monospecific rabbit antibody against ISAV nucleoprotein) diluted in TBS with 1% non-fat dry milk, followed by three washes in TBS with 0.1% Tween 20.

c) For detection of bound antibodies, sections are incubated with Alkaline phosphatase-conjugated antibodies to rabbit IgG for 60 minutes. Following a final wash, Fast Red (1 mg ml–1) and Naphthol AS-MX phosphate (0.2 mg ml^{-1}) with 1 mM Levamisole in 0.1 M TBS (pH 8.2) is added to develop for 20 minutes. Sections are then washed in tap water before counterstaining with Harris haematoxylin and mounted in aqueous mounting medium. ISAV positive and ISAV negative tissue sections are included as controls in every setup.

iii) Interpretation

Negative control sections should not have any significant colour reactions. Positive control sections should have clearly visible red-coloured cytoplasmic and intranuclear staining of endothelial cells in blood vessels or heart endocardium. A test sample section should only be regarded as positive if clear, intranuclear red staining of endothelial cells is found. The intranuclear localisation is particular to the orthomyxovirus nucleoprotein during a stage of virus replication. Concurrent cytoplasmic staining is often dominant. Cytoplasmic and other staining patterns without intranuclear localisation must be considered as nonspecific or inconclusive.

The strongest positive staining reactions are usually obtained in endothelial cells of heart and kidney. Endothelial staining reactions within very extensive haemorrhagic lesions can be slight or absent, possibly because of lysis of infected endothelial cells.

4.3.1.2. Agent isolation and identification

4.3.1.2.1. Cell culture

ASK cells (Devold *et al*., 2000) are recommended for primary ISAV isolation, but other susceptible cell lines, such as SHK-1 (Dannevig *et al*., 1995), may be used. However, strain variability and the ability to replicate in different cell lines should be taken into consideration. The ASK cells seem to support isolation and growth of the hitherto known virus isolates. A more distinct cytopathic effect (CPE) may appear in ASK cells. Both the SHK-1 and ASK cell lines appear to lose susceptibility for ISAV with increasing passage level.

The SHK-1 and ASK cells are grown at 20°C in Leibovitz's L-15 cell culture medium supplemented with fetal bovine serum (5% or 10%), L-glutamine (4 mM), gentamicin (50 µg ml^{-1}) and 2-mercapto-ethanol (40 µM) (this latter may be omitted).

For virus isolation, cells grown in 25 cm^2 tissue culture flasks or multi-well cell culture plates, which may be sealed with parafilm or a plate sealer to stabilise the pH of the medium, may be used. Cells grown in 24-well plates may not grow very well into monolayers, but this trait may vary between laboratories and according to the type of cell culture plates used. Serially diluted ISAV-positive controls should be inoculated in parallel with the tissue samples as a test for cell susceptibility to ISAV (this should be performed in a separate location from that of the test samples).

i) Inoculation of cell monolayers

Prepare a 2% suspension of tissue homogenate using L-15 medium without serum or other medium with documented suitability. Remove growth medium from actively growing monolayers (1–3 day old cultures or cultures of 70–80 % confluency) grown in 25 cm^2 tissue culture flasks or multi-well cell culture plates (see above). Inoculate monolayers (25 cm^2 tissue culture flasks) with 1.5 ml of the 2% tissue homogenate. Adjust volume to the respective surface area in use. Allow 3–4 hours incubation at 15°C followed by removal of the inoculum, and addition of fresh, L-15 medium supplemented with 2–5% FCS. Alternatively, a 1/1000 dilution and direct inoculation without medium replacement can be used.

When fish samples come from production sites where infectious pancreatic necrosis virus (IPNV) is regarded as endemic, the tissue homogenate supernatant should be incubated (for a minimum of 1 hour at 15°C) with a pool of antisera to the indigenous serotypes of IPNV prior to inoculation.

ii) Monitoring incubation

Inoculated cell cultures (kept at 15°C) are examined at regular intervals (at least every 7 days) for the occurrence of CPE. Typical CPE due to ISAV appears as vacuolated cells that subsequently round up and loosen from the growth surface. If CPE consistent with that described for ISAV or IPNV appears, an aliquot of the medium for virus identification, as described below, must be collected. In the case of an IPNV infection, re-inoculate cells with tissue homogenate supernatant that has been incubated with a lower dilution of IPNV antisera. If no CPE has developed after 14 days, subculture to fresh cell cultures.

iii) Subcultivation procedure

Aliquots of medium (supernatant) from the primary cultures are collected 14 days (or earlier when obvious CPE appears) after inoculation. Supernatants from wells inoculated with different dilutions of identical samples may be pooled for surveillance purposes.

Supernatants are inoculated into fresh cell cultures as described for the primary inoculation: remove growth medium, inoculate monolayers with a small volume of diluted supernatant (1/5 and higher dilutions) for 3–4 hours before addition of fresh medium. Alternatively, add supernatants (final dilutions 1/10 and higher) directly to cell cultures with growth medium.

Inoculated cell cultures are incubated for at least 14 days and examined at regular intervals, as described for the primary inoculation. At the end of the incubation period, or earlier if obvious CPE appears, the medium is collected for virus identification, as described below. Cell cultures with no CPE should always be examined for the presence of ISAV by immunofluorescence (IFAT), haemadsorption or by PCR because virus replication may occur without development of apparent CPE.

The procedure described below has been successful for isolation of HPR-deleted ISAV from fish with clinical signs or from suspected cases. HPR0 has hitherto not been isolated in cell culture.

4.3.1.2.2. Antibody-based antigen detection methods

4.3.1.2.2.1 Virus identification by IFAT

All incubations are carried out at room temperature unless otherwise stated.

i) Prepare monolayers of cells in appropriate tissue culture plates (e.g. 96-well or 24-well plates), in slide flasks or on cover-slips dependent on the type of microscope available (an inverted microscope equipped with UV light is necessary for monolayers grown on tissue culture plates). SHK-1 cells grow rather poorly on glass cover-slips. The necessary monolayers for negative and positive controls must be included.

ii) Inoculate the monolayers with the virus suspensions to be identified in tenfold dilutions, two monolayers for each dilution. Add positive virus control in dilutions known to give a good staining reaction. Incubate inoculated cell cultures at 15°C for 7 days or, if CPE appears, for a shorter time.

iii) Fix in 80% acetone for 20 minutes after removing cell culture medium and rinsing once with 80% acetone. Remove the fixative and air dry for 1 hour. The fixed cell cultures may be stored dry for less than 1 week at 4°C or at –20°C for longer storage.

iv) Incubate the cell monolayers with anti- ISAV MAb in an appropriate dilution in PBS for 1 hour. and rinse twice with PBS/0.05% Tween 20. If unspecific binding is observed, incubate with PBS containing 0.5% dry skimmed milk.

Annex 11 (contd)

v) Incubate with FITC-conjugated goat anti-mouse immunoglobulin for 1 hour (or if antibody raised in rabbits is used as the primary antibody, use FITC-conjugated antibody against rabbit immunoglobulin), according to the instructions of the supplier. To increase the sensitivity, FITC-conjugated goat anti-mouse Ig may be replaced with biotin-labelled anti-mouse Ig and FITC-labelled streptavidin with the described rinsing in between the additional step. Rinse once with PBS/0.05% Tween 20, as described above. The nuclei can be stained with propidiumiodid (100 µg ml^{-1} in sterile distilled water). Add PBS (without Tween 20) and examine under UV light. To avoid fading, the stained plates shoul be kept in dark until examination. For long periods of storage (more than 2–3 weeksa solution of 1,4-diazabicyclooctane (DABCO 2.5% in PBS, pH 8.2) or similar reagent may be added as an anti-fade solution. .

4.3.1.2.3. Molecular techniques

4.3.1.2.3.1 Reverse-transcription polymerase chain reaction (RT-PCR)

The primers described below for RT-PCR and real-time RT-PCR will detect both European and North-American HPR-deleted ISAV, and also HPR0 ISAV.

RT-PCR may be used for detection of ISAV from total RNA (or total nucleic acid) extracted from recommended organs/tissues (see Section 3.4). The real-time RT-PCR for the detection of ISAV is recommended as it increases the specificity and, probably, also the sensitivity of the test. Though several primer sets for ISAV real-time RT-PCR have been reported, recommended primer sets are presented in the table below. The primer sets derived from genomic segment 8 and segment 7 have been used by several laboratories and have been found suitable for detection of ISAV during disease outbreaks and in apparently healthy carrier fish

With the widespread occurrence of HPR0 ISAV variants, it is essential to follow up any positive PCR results based on segment 7 or 8 primer sets by sequencing the HPR of segment 6 in order to determine the ISAV HPR variant present (HPR-deleted or HPR0 or both). Adequate primers, designed and validated by the OIE Reference Laboratory are given in the table below. Validation of the HPR primer set for the North American isolates is restricted by the limited sequence data available in the Genbank for the 3' end of ISAV segment 6.

The primers for segment 7 and 8 as well as sequencing primers for segment 6 HPR, are listed below and may also be used for conventional RT-PCR if necessary.

Real-time RT-PCR: Primer and probe sequences	**Named**	**Genomic segment**	**Product size**	**Reference**
5'-CAG-GGT-TGT-ATC-CAT-GGT-TGA-AAT-G-3' 5'-GTC-CAG-CCC-TAA-GCT-CAA-CTC-3' *5'-6FAM*-CTC-TCT-CAT-TGT-GAT-CCC-*MGBNFQ-3'*	forward primer reverse primer Taqman®probe	7	155 nt	Snow *et al*., 2006
5'-CTA-CAC-AGC-AGG-ATG-CAG-ATG-T-3' 5'-CAG-GAT-GCC-GGA-AGT-CGA-T-3' *5'-6FAM*-CAT-CGT-CGC-TGC-AGT-TC-*MGBNFQ-3'*	forward primer reverse primer Taqman®probe	8	104 nt	Snow *et a*l., 2006
5'-GAC-CAG-ACA-AGC-TTA-GGT-AAC-ACA-GA-3' 5'-GAT-GGT-GGA-ATT-CTA-CCT-CTA-GAC-TTG-TA-3'	forward primer reverse primer	6 (HPR)	304 nt if HPR0	Designed by OIE Ref. Lab.

4.3.1.2.4. Agent purification

ISAV propagated in cell culture can be purified by sucrose gradient centrifugation (Falk *et al*., 1997) or by affinity purification using immunomagnetic beads coated with anti-ISAV MAb.

4.3.2. Serological methods

Both Atlantic salmon and rainbow trout develop a humoral immune response to the ISAV infection. Enzyme-linked immunosorbent assays (ELISAs) with either purified virus or lysates from ISAV-infected cell cultures have been established for detection of ISAV-specific antibodies. ELISA titres can be very high and appear to be quite specific for the nucleoprotein in Western blots (K. Falk, pers. comm.). The test is not standardised for surveillance or diagnostic use, but may be used as a supplement to direct virus detection and pathology in obscure cases. Furthermore, the level and distribution of seroconversion in an ISAV-infected population may give some information about the spread of infection, particularly in cases where vaccination is not practised, and in wild fish.

5. Rating of tests against purpose of use

As an example, the methods currently available for targeted surveillance for infection with HPR-deleted ISAV and diagnosis of ISA are listed in Table 5.1. For surveillance of infection with HPR0 ISAV, real-time RT-PCR followed by sequencing is the only recommended method (not included in the table). The designations used in the Table indicate: a = the method is the recommended method for reasons of availability, utility, and diagnostic specificity and sensitivity; b = the method is a standard method with good diagnostic sensitivity and specificity; c = the method has application in some situations, but cost, accuracy, or other factors severely limits its application; and d = the method is presently not recommended for this purpose. These are somewhat subjective as suitability involves issues of reliability, sensitivity, specificity and utility. Although not all of the tests listed as category a or b have undergone formal standardisation and validation, their routine nature and the fact that they have been used widely without dubious results, makes them acceptable.

Table 5.1. *Methods for targeted surveillance and diagnosis**

Method	Targeted surveillance for infection with HPR-deleted ISAV				Presumptive diagnosis	Confirmatory diagnosis
	Larvae	PLs	Juveniles	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
RT-PCR or real-time RT-PCR followed by sequencing	a	a	a	a	b	a

*As the diagnosis of ISA is not based on the results of a single method, the information in this Table should be used with care. See Section 7 for the criteria for ISA diagnosis.
PLs = postlarvae; IFAT = indirect fluorescent antibody test; EM = electron microscopy;
RT-PCR = reverse-transcription polymerase chain reaction.

Annex 11 (contd)

6. Test(s) recommended for targeted surveillance to declare freedom from infectious salmon anaemia virus

Regular health inspections combined with investigation for ISA when increased mortality is associated with one of the given clinical signs and/or pathological changes consistent with ISA is an efficient way of obtaining data on the occurrence of ISA in farmed populations. In addition to regular health inspections, testing for HPR-deleted ISAV, preferentially by PCR-based methodology, at certain intervals may be carried out. However, due to the uneven spread of infection within a farm, large numbers of samples need to be tested. The significance of positive findings of ISAV by PCR alone for the risk of developing ISA disease is not clear, and therefore any positive findings would have to be followed up by either further testing and/or surveillance of the production site.

Because of the transient nature of HPR0 ISAV, large sample sizes need to be tested at time points through the production cycle to be able to document freedom of this infection.

7. Corroborative diagnostic criteria

Reasonable grounds to suspect fish of being infected with ISAV (HPR-deleted or HPR0) are outlined below. The Competent Authority should ensure that, following the suspicion of fish infected with ISAV on a farm, an official investigation to confirm or rule out the presence of the disease will be carried out as quickly as possible, applying inspection and clinical examination, as well as collection and selection of samples and using the methods for laboratory examination as described in Section 4.

7.1. Definition of suspect case (HPR-deleted ISAV)

ISA or infection with HPR-deleted ISAV would be suspected if at least one of the following criteria is met:

i) Clinical signs consistent with ISA or pathological changes consistent with ISA (Section 4.2) whether or not the pathological changes are associated with clinical signs of disease;

ii) Isolation and identification of ISAV in cell culture from a single sample (targeted or routine) from any fish on the farm, as described in Section 4.3.1.2.1;

iii) Evidence for the presence of ISAV from two independent laboratory tests such as RT-PCR (Section 4.3.1.2.3) and IFAT on tissue imprints (Section 4.3.1.1.2.1) or IHC (Section 4.3.1. 1.3.1)

7.2. Definition of confirmed case (HPR-deleted ISAV)

7.2.1. Definition of confirmed ISA

The following criteria should be met for confirmation of ISA: Mortality, clinical signs and pathological changes consistent with ISA (Section 4.2), and detection of ISAV in tissue preparations by means of specific antibodies against ISAV (IHC on fixed sections [Section 4.3.1.1.3.1] or IFAT on tissue imprints [Section 4.3.1.1.2] or fixed sections as described in Section 4.3.1.1.3) in addition to either:

EU comment

The EU suggests deleting the words "Mortality, clinical signs and pathological changes consistent with ISA (Section 4.2), and". Indeed, this seems irrelevant in the definition of ISA now that the distinction can be made between HPR0 and HPR-deleted ISAV by PCR. There should be no need for clinical signs or pathological changes as a criterion for detecting the disease.

i) Isolation and identification of ISAV in cell culture from at least one sample from any fish on the farm, as described in Section 4.3.1.2.1

or

ii) Detection of ISAV by RT-PCR by the methods described in Section 4.3.1.2.3;

7.2.2 Definition of confirmed HPR-deleted ISAV infection

The criteria given in i) or ii) should be met for the confirmation of infection with HPR-deleted ISAV.

i) Isolation and identification of ISAV in cell culture from at least two independent samples (targeted or routine) from any fish on the farm tested on separate occasions as described in Section 4.3.1.2.1.

ii) Isolation and identification of ISAV in cell culture from at least one sample from any fish on the farm with corroborating evidence of ISAV in tissue preparations using either RT-PCR (Section 4.3.1.2.3) or IFAT/IHC (Sections 4.3.1.1.2 and 4.3.1.1.3).

7.3. Definition of confirmed infection with HPR0 ISAV

7.3.1. Definition of confirmed infection with HPR0 ISAV

The criteria given in i) and ii) should be met for the confirmation of HPR0 ISAV infection.

i) An absence of clinical signs consistent with ISA disease or mortality (= apparently healthy fish).

EU comment

For the same reasons as explained above, the EU suggests deleting point i) above, as the absence of clinical signs would not be relevant in defining HPR0 ISAV, and this would be inconsistent with other diseases. On the contrary, should clinical signs be present in fish while only HPR0 is detected, e.g. because of co-infections with other pathogens, the definition of confirmed infection with HPR0 would not be met and therefore no disease would have to be notified.

ii) Detection of ISAV by RT-PCR followed by independent amplification and sequencing of the HPR region of segment 6 to confirm the presence of HPR0 only.

8. References

AAMELFOT M., DALE O.B., WELI S., KOPPANG E.O. & FALK K. (2012). Expression of 4-O-acetylated sialic acids on Atlantic salmon endothelial cells correlates with cell tropism of Infectious salmon anemia virus. *J.Virol.,* **86**, 10571–10578.

ALDRIN M., LYNGSTAD T.M., KRISTOFFERSEN A.B., STORVIK B., BORGAN O. & JANSEN P.A. (2011). Modelling the spread of infectious salmon anaemia among salmon farms based on seaway distances between farms and genetic relationships between infectious salmon anaemia virus isolates. *J.R. Soc. Interface*, **8**, 1346–1356.

CHRISTIANSEN D.H., ØSTERGAARD P.S., SNOW M., DALE O.B & FALK K. (2011). A low-pathogenic variant of infectious salmon anemia virus (ISAV1 - HPR0) is highly prevalent and causes a non-clinical transient infection in farmed Atlantic salmon (*Salmo salar* L.) in the Faroe Islands. *J. Gen. Virol.,* **92**, 909–918.

COTTET L., RIVAS-ARAVENA A., CORTEZ-SAN MARTIN M., SANDINO A.M. & SPENCER E. (2011) Infectious salmon anemia virus – genetics and pathogenesis. *Virus Res.,* **155**, 10-19.

CUNNINGHAM C.O., GREGORY A., BLACK J., SIMPSOM I. & RAYNARD R.S. (2002). A novel variant of the infectious salmon anaemia virus (ISAV) haemagglutinin gene suggests mechanisms for virus diversity. *Bull. Eur. Assoc. Fish Pathol*., **22**, 366–374.

DANNEVIG, B.H., FALK, K. & NAMORK E. (1995). Isolation of the causal virus of infectious salmon anemia (ISA) in a long-term cell line from Atlantic salmon head kidney. *J. Gen. Virol.*, **76**, 1353–1359.

DEVOLD M., KARLSEN M. & NYLUND A. (2006). Sequence analysis of the fusion protein gene from infectious salmon anemia virus isolates: evidence of recombination and reassortment. *J. Gen. Virol.*, **87**, 2031–2040.

DEVOLD M., KROSSOY B., ASPEHAUG V. & NYLUND A. (2000). Use of RT-PCR for diagnosis of infectious salmon anaemia virus (ISAV) in carrier sea trout *Salmo trutta* after experimental infection. *Dis. Aquat. Org*., **40**, 9–18.

EUROPEAN FOOD SAFETY AUTHORITY (EFSA) (2012) EFSA Panel on Animal Health and Welfare (AHAW); Scientific Opinion on infectious salmon anaemia. *EFSA Journal*, **10** (11), 2971.

FALK K., NAMORK E., RIMSTAD E., MJAALAND S. & DANNEVIG B.H. (1997). Characterization of infectious salmon anemia virus, an orthomyxo-like virus isolated from Atlantic salmon (*Salmo salar L*). *J. Virol*., **71**, 9016–9023.

GUSTAFSON L.L., ELLIS S.K., BEATTIE M.J., CHANG B.D., DICKEY D.A., ROBINSON T.L., MARENGHI F.P., MOFFETT P.J. & PAGE F.H. (2007). Hydrographics and the timing of infectious salmon anemia outbreaks among Atlantic salmon (*Salmo salar* L.) farms in the Quoddy region of Maine, USA and New Brunswick, Canada. *Prev. Vet. Med.*, **78**, 35–56.

KAWAOKA Y., COX N.J., HALLER O., HONGO S., KAVERIN N., KLENK H.D., LAMB R.A., MCCAULEY J., PALESE P., RIMSTAD E. & WEBSTER R.G. (2005). Infectious Salmon Anaemia Virus. *In:* Virus Taxonomy – Eight Report of the International Committee on Taxonomy Viruses, Fauquet C.M., Mayo M.A., Maniloff J., Desselberger U., Ball L.A., eds. Elsevier Academic Press, New York, USA, pp 681–693.

KIBENGE F.S.B., GARATE O.N. JOHNSON G., ARRIAGADA K., KIBENGE M.J.T. & WADOWAKA D. (2001). Isolation and identification of infectious salmon anaemia virus (ISAV) from Coho salmon in Chile. *Dis. Aquat. Org.*, **45,** 9–18.

KIBENGE F.S.B., GODOY M.G., WANG Y., KIBENGE M.J.T., GHERARDELLI V., MANSILLA S., LISPERGER A., JARPA M., LARROQUETE G., AVENDAÑO F., LARA M. & GALLARDO A. (2009). Infectious salmon anaemia virus (ISAV) isolated from the ISA disease outbreaks in Chile diverged from ISAV isolates from Norway around 1996 and was disseminated around 2005, based on surface glycoprotein gene sequences. *Virol. J*., **6**, 88.

KIBENGE F.S.B., KIBENGE M.J.T., WANG Y., QIAN B., HARIHARAN S. & MCGEACHY S. (2007). Mapping of putative virulence motifs on infectious salmon anaemia virus surface glycoprotein genes. *J. Gen. Virol*., **88**, 3100–3111.

KIBENGE F.S.B., MUNIR K., KIBENGE M.J.T., MONEKE T.J. & MONEKE E. (2004). Infectious salmon anemia virus: causative agent, pathogenesis and immunity. *Anim. Health Res. Rev.*, **5**, 65–78.

KULSHRESHTHA V., KIBENGE M., SALONIUS K., SIMARD N., RIVEROLL A. & KIBENGE F. (2010). Identification of the 3' and 5' terminal sequences of the 8 RNA genome segments of European and North American genotypes of infectious salmon anaemia virus (an orthomyxovirus) and evidence for quasispecies based on the non-coding sequences of transcripts. *Virol. J.,* **7**, 338.

LYNGSTAD T.M., HJORTAAS M.J, KRISTOFFERSEN A.B, MARKUSSEN T., KARLSEN E.T., JONASSEN C.M. & JANSEN P.A. (2011). Use of molecular epidemiology to trace transmission pathways for infectious salmon anaemia virus (ISAV) in Norwegian salmon farming. *Epidemics,* **3**, 1–11.

LYNGSTAD T.M., ., KRISTOFFERSEN A. B., HJORTAAS M. J., DEVOLD, M., ASPEHAUG, V., LARSSEN, R. B. & JANSEN, P. A. (2012). Low virulent infectious salmon anaemia virus (ISAV-HPR0) is prevalent and geographically structured in Norwegian salmon farming. *Dis. Aquat. Org*., **101**, 197–206.

LYNGSTAD T.M., JANSEN P.A., SINDRE H., JONASSEN C.M., HJORTAAS M.J., JOHNSEN S. & BRUN E. (2008). Epidemiological investigation of infectious salmon anaemia (ISA) outbreaks in Norway 2003–2005. *Prev. Vet. Med.,* **84**, 213–227.

MARDONES F.O., PEREZ A.M., VALDES-DONOSO P. & CARPENTER T.E. (2011). Farm-level reproduction number during an epidemic of infectious salmon anaemia virus in southern Chile in 2007–2009. *Prev. Vet. Med.*, **102** (3), 175–184.

MARKUSSEN T., JONASSEN C.M., NUMANOVIC S., BRAAEN S., HJORTAAS M., NILSEN H. & MJAALAND S. (2008). Evolutionary mechanisms involved in the virulence of infectious salmon anaemia virus (ISAV), a piscine orthomyxovirus. *Virology,* **374**, 515–527.

MCBEATH A. J., BAIN N. & SNOW M. (2009). Surveillance for infectious salmon anaemia virus HPR0 in marine Atlantic salmon farms across Scotland. *Dis. Aquat. Org.,* **87**, 161–169.

MJAALAND S., HUNGNES O., TEIG A., DANNEVIG B.H., THORUD K. & RIMSTAD E. (2002). Polymorphism in the infectious salmon anemia virus hemagglutinin gene; importance and possible implications for evolution and ecology of infectious salmon anemia disease. *Virology*, **302**, 379–391.

MJAALAND S., MARKUSSEN T., SINDRE H., KJOGLUM S., DANNEVIG B.H., LARSEN S. & GRIMHOLT U. (2005). Susceptibility and immune responses following experimental infection of MHC compatible Atlantic salmon (*Salmo salar* L.) with different infectious salmon anaemia virus isolates. *Arch. Virol*., **150**, 2195–2216.

MJAALAND S., RIMSTAD E., FALK K. & DANNEVIG B.H. (1997). Genomic characterisation of the virus causing infectious salmon anemia in Atlantic salmon (*Salmo salar* L): an orthomyxo-like virus in a teleost. *J. Virol*., **71**, 7681–7686.

MULLINS J.E, GROMAN D.B & WADOWSKA D (1998) Infectious salmon anaemia in salt water Atlantic salmon (*Salmo salar* L.) in New Brunswick, Canada. *Bull. Eur. Assoc. Fish Pathol.,* **18**, 110–114.

NYLUND A., PLARRE H., KARLSEN M., FRIDELL F., OTTEM K.F., BRATLAND A.,& SAETHER P.A. (2007). Transmission of infectious salmon anaemia virus (ISAV) in farmed populations of Atlantic salmon (*Salmo salar*). *Arch. Virol.*, **152**, 151–179.

PLARRE H., DEVOLD M., SNOW M. & NYLUND A. (2005). Prevalence of infectious salmon anaemia virus (ISAV) in wild salmonids in western Norway. *Dis. Aquat. Org.*, **66**, 71–79.

RIMSTAD E., DALE O.B., DANNEVIG B.H. & FALK K. (2011). Infectious Salmon Anaemia. *In*: Fish Diseases and Disorders, Volume 3: Viral, Bacterial and Fungal Infections, Woo P.T.K. & Bruno D., eds. CAB International, Oxfordshire, UK, 143–165.

RIVAS-Aravena A., VALLEJOS-VIDAL E., MARTIN M.C., REYES-LOPEZ F., TELLO M., MORA P., SANDINO A.M., SPENCER E. (2011). Inhibitory effect of a nucleotide analog on ISAV infection. *J. Virol*., **85**, 8037–8045.

SNOW M., MCKAY P., MCBEATH A. J. A., BLACK J., DOIG F., KERR R., CUNNINGHAM C. O., NYLUND A. & DEVOLD M. (2006). Development, application and validation of a taqman® real-time RT-PCR assay for the detection of infectious salmon anaemia virus(ISAV) in Atlantic salmon (*Salmo salar*), Vannier P. & Espeseth D., eds. New Diagnostic Technology: Applications in Animal Health and Biologics Controls. *Dev. Biol*., Basel, Karger. **126**, 133–145.

THORUD K.E. & DJUPVIK H.O. (1988). Infectious salmon anaemia in Atlantic salmon (*Salmo salar* L)*. Bull. Eur. Assoc. Fish Pathol.,* **8**, 109–111.

*
* *

NB: There is an OIE Reference Laboratory for Infection with infectious salmon anaemia virus
(see Table at the end of this *Aquatic Manual* or consult the OIE Web site for the most up-to-date list:
http://www.oie.int/en/our-scientific-expertise/reference-laboratories/list-of-laboratories/).
Please contact the OIE Reference Laboratory for any further information on
Infection with infectious salmon anaemia virus

Annex 12

CHAPTER 2.4.9

INFECTION WITH OSTREID HERPESVIRUS 1 MICROVARIANTS

EU comment

The EU thanks the OIE Aquatic Animals Commission for taking into consideration the previous comments from the EU and in general supports the proposed amendments to this chapter.

The EU would suggest revising this chapter as regards the list of susceptible species in accordance with the new proposed chapter on *criteria for determining susceptibility of aquatic animals to specific pathogenic agents* once adopted.

Further comments are inserted in the text below.

1. Scope

For the purpose of this chapter, infection with ostreid herpesvirus 1 microvariants is considered a viral infection of bivalve molluscs caused by ostreid herpesvirus 1 microvariants, variants of OsHV-1 (ostreid herpesvirus 1) defined by a deletion in a microsatellite locus upstream from ORF4 (Renault *et al.,* 2012; Segarra *et al.,* 2010) when compared with the reference type OsHV-1. µVar is a microvariant ~~Ostreid herpesvirus 1 µVar is strictly~~ defined by a 12 bp deletion in a microsatellite locus upstream of the ORF4 and additional mutations in ORF4 and ORF2/43; however, the scope of this chapter includes related variants with a deletion of around 12 base pairs in ~~this~~ the microsatellite locus region. The term OsHV-1 microvariants is used in this chapter to refer to the OsHV-1 ~~µVar~~ microvariant and these related variants. Until now, mortality associated with OsHV-1 microvariants has only been reported in the Pacific cupped oyster *Crassostrea gigas* and and in the Portuguese cupped oyster *C. ~~angulata~~.* ~~Infection with OsHV-1 microvariant mainly affects the Pacific cupped oyster, *Crassostrea gigas*.~~

EU comment

The EU suggests slightly amending the paragraph above, in order to clarify the scope, as detailed below:

"[…]; however, the scope of this chapter includes all related variants with a deletion of around 12 base pairs in ~~this~~ the microsatellite locus region (with or without additional mutation in ORF4 and ORF 2/43). The term […]".

Furthermore, the EU suggests including a Genebank citation for the sequence of the reference type of OsHV1.

2. Disease information

2.1. Agent factors

OsHV-1 is the aetiological agent of a contagious viral disease of Pacific cupped oysters, *Crassostrea gigas*, also affecting other bivalve species. The genome of the virus was sequenced from infected Pacific oyster larvae collected in France in 1995 (Davison *et al.,* 2005). As this specimen was the first to be described (through complete genome sequencing), it can be considered as the reference type.

2.1.1. Aetiological agent, agent strains

OsHV-1 particles have been purified from French *C. gigas* larvae (Le Deuff & Renault, 1999) and were observed by transmission electron microscopy to be enveloped icosahedral with electron dense cores and a diameter around 120 nm. The intranuclear location of the virus particles, their size and ultrastructure are characteristic of members of the *Herpesvirales*.

The genome structure and sequence, and the capsid morphology (Davison *et al.*, 2005) have been further studied in order to assess OsHV-1 phylogenetic status in relation to vertebrate herpesviruses. The entire virus DNA was sequenced (GenBank accession number AY509253) and OsHV-1 capsids appear structurally similar to those of other herpes viruses that have been studied (Davison *et al.*, 2005). The virus was classified under the name *Ostreid herpesvirus* 1 (OsHV-1) as the first known species in the family *Malacoherpesviridae* (Davison *et al.*, 2009).

A variant of OsHV-1 has been identified in France (Arzul *et al.*, 2001b) in *C. gigas*, *Ruditapes philippinarum* and *Pecten maximus*. Friedman *et al.* (2005) and Moss *et al.* (2007) also described differences in the sequences of OsHV-1 from California and Asia, respectively. Moss *et al.* (2007) suggested that there are at least two strains in Japan, one in South Korea and two in China (the People's Rep. of). One of the strains that occurred in China and South Korea was similar in sequence to the OsHV-1 strain from California described by Friedman *et al.* (2005), and the other strain from China was similar to OsHV-1 from France.

More recently, polymerase chain reactions (PCRs) using different primer sets and PCR product sequencing enabled the detection of ~~a variant called~~ µVar and other ~~related~~ variants in association with high mortality events reported in Europe, Australia and New Zealand (Lynch *et al.*, 2012; Martenot *et al.,* 2011; Peeler *et al.,* 2012; Renault *et al.,* 2012; Segarra *et al.*, 2010). The term microvariant is used to define a single variant presenting all the mutations reported by Segarra *et al.* (2010) in two different virus genome areas. The term OsHV-1 microvariants is used to refer to OsHV-1 microvariant and these related variants.

Although the aetiological agent is represented by all specimens or variants of OsHV-1 (Arzul *et al.*, 2001b; Davison *et al.*, 2005; Martenot *et al.,* 2011; Moss *et al.*, 2007; Renault *et al.,* 2012; Segarra *et al.*, 2010; Shimahara *et al.,* 2012), increased mortality outbreaks recently reported in Europe, Australia and New Zealand among *C. gigas* spat in association with the variant OsHV-1 microvariant or related viral variants suggested differences in terms of virulence among OsHV-1 variants. However, the detection of the ~~variant~~ microvariant or related variants have also been reported in absence of mortality events (Dundon *et al.,* 2011; EFSA, 2010; Shimahara *et al.,* 2012) suggesting that viral infection is influenced by both host and environmental factors.

2.1.2. Survival outside the host

Maximum survival time outside the host is unknown.

Schikorski *et al.* (2011a; 2011b) presented data on detection by real-time PCR of OsHV-1 µVar DNA in seawater following cohabitation experiments. The copy numbers of virus DNA in the water in the first 48 hours after injecting spat with virus reached 1×10^5 ml^{-1}, and reached a maximum of 1×10^6 ml^{-1} following infection of cohabiting oysters. The amount of infectious virus is unknown.

2.1.3. Stability of the agent (effective inactivation methods)

The lack of cell cultures for OsHV-1 has meant that *in-vitro* studies on the stability of the virus with regard to infectivity have not been done. ~~As an alternative, extracted viral DNA was seeded into seawater and 10 pg μl^{-1} was detected for 16, 9 and 1 day at 4, 11 and 20°C respectively, and in a second experiment, 100 pg μl^{-1} was detected after 51 days at each temperature.~~

The longest time for DNA detection in OsHV-1 released from macerated larvae and seeded into seawater was 22 days at 4°C and 12 days at 20°C (Vigneron *et al.*, 2004). However, the relationship between detection of DNA in the PCR and infectivity of the virus is unknown. As a general rule, the survival of many aquatic animal viruses outside the host is greatest at lower temperatures.

2.1.4. Life cycle

Transmission ~~The life cycle~~ is direct from host to host (Le Deuff *et al.*, 1994; Schikorski *et al.*, 2011a; 2011b).

2.2. Host factors

2.2.1. Susceptible host species

~~OsHV-1 has been reported from the Pacific oyster, *C. gigas*, Portuguese oyster, *C. angulata*, suminoe oyster, *C. ariakensis*, European flat oyster, *O. edulis*, Manila clam, *R. philippinarum*, carpet shell clam, *R. decussates*, and scallops, *P. maximus* (Arzul *et al.*, 2001a; 2001b; Renault *et al.*, 2000).~~ Until now, mortality attributable to OsHV-1 microvariants ~~Ostreid herpesvirus 1 µVar~~ (Segarra *et al.,* 2010; Renault *et al.,* 2012) has been ~~mainly~~ reported ~~as affecting~~ in the Pacific oyster, *Crassostrea gigas* and in the Portuguese cupped oyster *Crassostrea angulata* (Batista *et al*., pers. comm.).

2.2.2. Susceptible stages of the host

Although OsHV-1 microvariants can be detected in all oyster stages, mortality due to OsHV-1 microvariants only concern spat and juveniles in a lesser extent.

~~OsHV-1 infection may cause mortality in larvae and juveniles of several bivalve species. The virus can be found in adult bivalves most often in absence of mortality.~~

2.2.3. Species or subpopulation predilection (probability of detection)

Crassostrea gigas and ~~*O. edulis, R. philippinarum*, *R. decussates* and *P. maximus*~~ *C. angulata* are naturally infected by OsHV-1 microvariants. Young stages including ~~larvae,~~ spat and juveniles seem to be more susceptible to the infection. The virus is easier to detect in moribund animals than in healthy ones.

2.2.4. Target organs and infected tissue

The infection-associated lesions in juveniles are mainly observed in connective tissues of all organs in which fibroblastic-like cells exhibit enlarged nuclei with perinuclear chromatin (Arzul *et al*., 2002; Lipart & Renault, 2002; Renault *et al*.; 1995; Schikorski *et al*., 2011a).

2.2.5. Persistent infection with lifelong carriers

Apparently healthy oysters, including adults, have been shown to be PCR-positive for OsHV-1 (Arzul *et al.*, 2002; Moss *et al*., 2007; Sauvage *et al.*, 2009). Pépin *et al.* (2008) showed that DNA copy numbers mg^{-1} tissue were high (up to 10^7) in oysters from populations with abnormal mortalities and low (lowest number detected 10^1) in populations with no abnormal mortalities. Determining the levels of viral DNA in oysters by quantitative PCR (qPCR) might be a summation ~~means~~ to differentiate between mechanical carriage of virus and low level of infection.

As the virus (DNA, protein or particles) has been detected in tissues of adult oysters, including the gonad (Arzul *et al.*, 2002; Lipart & Renault, 2002), adults may be a source of infection for larvae or spat, particularly under stressful conditions, e.g. from high temperature (Le Deuff *et al.*, 1996). However, what is not certain is whether true vertical transmission (transmission within the gametes) occurs or whether transmission is horizontal (Barbosa-Solomieu *et al.*, 2005).

2.2.6. Vectors

No vectors are required: the life cycle is direct (Schikorski *et al*., 2011a; 2011b).

2.2.7. Known or suspected wild aquatic animal carriers

Several bivalve species may act as subclinical and healthy carriers (see Section 2.2.3).

EU comment

The EU is of the opinion that the sentence above is no longer valid, given the deletion of some of the susceptible species in point 2.2.3., and for reasons of consistency should therefore be deleted.

OsHV-1 microvariant DNA has been recently detected in France and in Ireland in blue mussel, *Mytilus edulis,* and in *Donax trunculus* (Renault *et al.*, comm. pers.). However, in these cases, it remains unknown if these bivalve species are susceptible, resistant or may act as vector species.

EU comment

The EU suggests defining the term "vector" in the glossary, in a similar manner as is already done in the Terrestrial Code.

2.3. Disease pattern

2.3.1. Transmission mechanisms

OsHV-1 DNA has been detected by qPCR in the water around diseased Pacific oysters (Sauvage *et al.*, 2009) and the disease induced by the variant OsHV-1 microvariant can be experimentally transmitted horizontally via the water (Schikorski *et al.*, 2011a), which presumably is the main natural mode of OsHV-1 transmission.

The first published report (Le Deuff *et al.*, 1994) described rapid transmission of the virus from an extract of diseased larvae to axenic larvae of *C. gigas*. Inter species transmission from infected axenic larvae of *C. gigas* to axenic larvae of *C. rivularis* and *Ostrea edulis* was demonstrated experimentally (Arzul *et al.*, 2001b). A suspension of OsHV-1 from *R. philippinarum* was shown to infect axenic larvae of *C. gigas*, and a virus suspension from *C. gigas* was shown to infect axenic larvae of *C. angulata* (Arzul *et al.*, 2001b).

Experimental transmission of OsHV-1 µVar has been described by Schikorski *et al.* (2011a; 2011b). The disease can be transmitted to spat at 22°C following intramuscular injection of an extract of naturally infected oysters, and also by cohabiting injected oysters with healthy oysters. Based on qPCR detection, results suggest that the virus may enter the digestive gland and haemolymphatic system, following which the virus was disseminated to other organs.

2.3.2. Prevalence

Reported mortality rates and OsHV-1 microvariants prevalence vary considerably between sites and countries and depend on the age of affected stocks (Lynch *et al.*, 2012; Martenot *et al.*, 2011; Peeler *et al.*, 2012; Renault *et al.*, 2012; Segarra *et al.*, 2010). To better understand the implication of OsHV-1 in *C. gigas* spat mortality outbreaks regularly reported both in the field and in nurseries in France, samples were collected yearly through the French National Network for Surveillance of Mollusc Health between 1997 and 2006 (Garcia *et al.*, 2011). Analyses were carried out by PCR for OsHV-1 detection. Virus DNA was frequently detected in samples collected during mortality events with OsHV-1 detection frequency varying from 9 to 65% depending on the year. Data also demonstrated a particular seasonality and topography of spat oyster mortalities associated with OsHV-1 detection. In the field, mortality outbreaks appeared in summer, preferentially in sheltered environments.

More recently, increased mortality notifications (from 40 to 100%) were reported in 2008-2011 in Europe affecting Pacific oysters. These increased mortalities were associated with the detection of OsHV-1 microvariant or related variants depending of geographical locations (Lynch *et al.*, 2012; Martenot *et al.*, 2011; Peeler *et al.*, 2012; Renault *et al.*, 2012; Segarra *et al.*, 2010).

2.3.3. Geographical distribution

OsHV-1 has been reported from Europe (France, Ireland, Italy, Netherlands, Portugal, Spain, Sweden, United Kingdom), Australia, Brazil, China (People's Rep. of), Korea, Japan, Morocco, Tunisia, Mexico, New Zealand and United States of America. OsHV-1 microvariants have been reported associated with Pacific oyster mass mortalities in Europe, Australia, New Zealand, and Korea, but is known to occur elsewhere in the absence of oyster mortalities.

2.3.4. Mortality and morbidity

Infection by all strains is often lethal for *C. gigas* spat and juveniles. Death usually occurs 1 week after infection, during or shortly after the warmest annual water temperatures (Friedman *et al.*, 2005; Garcia *et al.*, 2011; Renault *et al.*, 1994b).

Infected larvae show a reduction in feeding and swimming activities, and mortality can reach 100% in a few days.

2.3.5. Environmental factors

Mortality outbreaks associated with the detection of OsHV-1 microvariants are more frequent during summer, which might suggest a link between seawater temperature and OsHV-1 microvariants infection. ~~The temperature influence on OsHV-1 detection and virus expression was demonstrated for *C. gigas* larvae (Le Deuff *et al.*, 1996) and strongly suspected for *C. gigas* spat (Burge *et al.*, 2007; Friedman *et al.*, 2005; Renault *et al.*, 1995; Sauvage *et al.*, 2009). A temperature threshold related to enhanced OsHV-1 expression or mortality appears difficult to define precisely. In the literature, according to the site, the temperature threshold was variable: 22°C to 25°C on the west coast of the USA (Friedman *et al.*, 2005; Burge *et al.*, 2007) and 18 to 20°C in France (Samain *et al.*, 2007; Soletchnik *et al.*, 1999).~~ High seawater temperatures appear to be one of the potential factors influencing OsHV-1 infection.

Moreover, stressful conditions particularly rearing techniques seem to favour OsHV-1 infection. In France, during summer, many oyster transfers occur and might also amplify OsHV-1 transmission.

~~Spat mortality outbreaks associated with OsHV-1 detection generally presented a patchy distribution in the field (Garcia *et al.*, 2011). This particular pattern could be partly explained by the nature of the virus. Herpes viruses are enveloped and are assumed to be relatively labile in their environment. Thus, their transmission relies generally on direct contact. These data suggest that when OsHV-1 is excreted by oysters, it would mainly infect nearby oysters. The probable limited dissemination of OsVH-1 in seawater could partly explain the observation of the patchy mortality distribution rather than a uniform distribution as observed in nurseries. In nurseries, oysters are reared at high densities, are very close together and the seawater is often sequentially renewed.~~

2.4. Control and prevention

2.4.1. Vaccination

Not applicable

2.4.2. Chemotherapy

None

2.4.3. Immunostimulation

Not applicable

2.4.4. Resistance breeding

Based on recent data, it has been demonstrated that Pacific cupped oyster families less susceptible to OsHV-1 including ~~the variant~~ OsHV-1 microvariants ~~µVar~~ can be obtained (Degremont, 2011; Sauvage *et al.*, 2009).

2.4.5. Restocking with resistant species

In France, a project of restocking with selected Pacific oysters is ongoing.

Annex 12 (contd)

2.4.6. Blocking agents

None

2.4.7. Disinfection of eggs and larvae

None

2.4.8. General husbandry practices

Biosecurity may be successfully applied in confined and controlled facilities such as hatcheries and nurseries in order to protect the facility and the surrounding environment from the introduction of the virus.

As a herpesvirus, OsHV-1 may be assumed to be fragile outside its hosts. High temperature, chemicals or sunlight (UV) may destroy its lipid-containing envelope, capsid or DNA. However, it has been demonstrated that individual herpesvirus species may have different levels of stability to inactivation treatment. Inorganic salts such as Na_2SO_4 present in seawater may stabilise herpesviruses (Wallis & Melnick, 1965).

In controlled rearing conditions (mollusc hatchery/nursery), OsHV-1 outbreaks may therefore be controlled through quarantine and hygienic measures including virus inactivation through adapted treatments such as ultraviolet irradiation of the recirculating water and water filtration technologies. However, it is necessary to keep in mind that reduction of virus load depends on the initial titre and the virus reduction capacity of the techniques used for inactivation. ~~If there was an initial concentration of 1 million viruses per litre and the inactivation method used allowed inactivation of 100,000 viruses per litre, there would still numerous infective particles in the treated product.~~

Moribund and dead oysters should be removed and destroyed whenever feasible. Equipment used in an infected zone should not be sent and used in a non-affected zone without adequate cleaning and disinfection.

3. Sampling

3.1. Selection of individual specimens

Live or moribund individuals should be sampled.

3.2. Preservation of samples for submission

For histology, the best preservative is Davidson's AFA, but 10% buffered formalin or other standard histology fixatives are also acceptable. For PCR assays, samples must be preserved in 95–100% ethanol or kept frozen (–80°C).

3.3. Pooling of samples

Pooling of ~~small spat is acceptable for PCR/qPCR analyses. However, the effect of pooling samples on PCR/qPCR sensitivity has not been evaluated~~ samples may be acceptable under some circumstances, however, the impact on sensitivity and design prevalence must be considered.

3.4. Best organs or tissues

For histology, ~~a 2 µm thick~~ section~~s~~ through the visceral mass that include digestive gland, gill and mantle are used. For PCR, mantle tissue is best.

3.5. Samples/tissues that are not suitable

Gonad tissues may be not reliable for PCR assays because of the presence of inhibitors.

4. Diagnostic methods

4.1. Field diagnostic methods

4.1.1. Clinical signs

Infection by OsHV-1 microvariants may cause an acute disease. Animals are likely to die within a few days of manifesting clinical signs of the disease. Clinical signs may be dead or gaping bivalves but these are not specific to infection with OsHV-1 microvariants.

4.1.2. Behavioural changes

Infected hosts may be slow to close their valves when disturbed but these behavioural changes are not specific to infection with OsHV-1.

4.2. Clinical methods

4.2.1. Gross pathology

Clinical signs may be dead or gaping bivalves but these clinical signs are not specific to infection with OsHV-1.

4.2.2. Clinical chemistry

None

4.2.3. Microscopic pathology

See Section 4.2.6. Fixed sections

4.2.4. Wet mounts

Not applicable

4.2.5. Smears

Not applicable

4.2.6. Fixed sections

The most consistent features of infection with OsHV-1 are nuclear changes including hypertrophy, nuclear margination and pycnosis. The infection-associated lesions in spat are mainly observed in connective tissues in which fibroblastic-like cells exhibit enlarged nuclei with perinuclear chromatin. Highly condensed nuclei (apoptosis features) were also reported in other cells interpreted as haemocytes. These cellular abnormalities are not associated with massive haemocyte infiltration.

Histological examination of the animal is not sufficient to identify infection with herpesvirus. Whilst Cowdry type A inclusions (eosinophilic intranuclear inclusions with perinuclear chromatin) are typical of many herpesvirus infections they are not a diagnostic feature of herpesvirus infections of oysters (Arzul *et al.*, 2002). Cowdry type A inclusions have never been reported following histological examination of infected Pacific cupped oysters in France (Renault *et al.*, 1994a; 1994b). Moreover, intranuclear inclusion bodies were not observed, although there was other cellular/nuclear pathology, in association with OsHV-1 infections in oysters in Mexico (Vásquez-Yeomans *et al.*, 2010) or USA (California) (Friedman *et al.*, 2005).

4.2.7. Electron microscopy/cytopathology

See Section 4.3.1.1.4.

4.3. Agent detection and identification methods

4.3.1. Direct detection methods

4.3.1.1. Microscopic methods

4.3.1.1.1. Wet mounts

Not applicable

4.3.1.1.2. Smears

Not applicable

4.3.1.1.3. Fixed sections

Samples to be taken: live or moribund oysters.

Technical procedure: Sections of tissue that include mantle, digestive gland, gills and adductor muscle should be fixed for 24 hours in 10% formaldehyde fixatives such as Davidson's AFA or other suitable fixative followed by normal processing for paraffin histology and staining with haematoxylin and eosin. Observations are made at increasing magnifications up to ×400.

Positive controls: These are recommended and are available from the Genetics and Pathology Laboratory, Ifremer, La Tremblade, France. Positive controls are tissue sections from any *OsHV-1* infected mollusc.

Levels of validation:

- *Specificity and sensitivity:* Specificity is very low, and sensitivity is good for moderate- to high-intensity infections, but low for low-intensity infections.
- *Gold standard:* None

Interpretation of results:

- A positive result is the occurrence of cell abnormalities in tissue sections: Fibroblastic-like cells exhibiting enlarged nuclei with perinuclear chromatin. Highly condensed nuclei are also reported in other cells interpreted as haemocytes. These cellular abnormalities are not associated with massive haemocyte infiltration.
- In susceptible host species, within the known range for OsHV-1, a positive result is presumptive evidence of OsHV-1 infection only and should be confirmed by species-specific PCR, *in-situ* hybridisation (ISH) and/or DNA sequencing.

Availability of commercial tests: No commercially available tests

4.3.1.1.4. Electron microscopy/cytopathology

Transmission electron microscopy can be used to confirm the presence of viral particles in infected animals.

Tissue samples (containing connective tissue such as mantle) for examination by electron microscopy should be fixed using 2.5% (v/v) glutaraldehyde in 0.1 M cacodylate buffer and post-fixed in 1% (w/v) osmium tetroxide, washed in 0.1 M cacodylate buffer (3 × 10 minutes), dehydrated in a graded series of ethanol (70%, 1 × 10 minutes; 95%, 2 × 15 minutes; 100%, 3 × 20 minutes), washed in propylene oxide (2 × 15 minutes), pre-infiltrated in 50% propylene oxide/50% Epon resin (1 hour), infiltrated in 100% Epon resin (1 hour) and then embedded in Epon resin.

Virus replication mainly takes place in fibroblastic-like cells throughout connective tissues especially in mantle, labial palps, gills and digestive gland (Renault *et al.*, 1994b; 1995; Schikorski *et al.,* 2011a). Virogenesis begins in the nucleus of infected cells where capsids and nucleocapsids are observed. Viral particles then pass through the nuclear membrane into the cytoplasm and enveloped particles are released at the cell surface. Intranuclear and cytoplasmic capsids present a variety of morphological types including electron lucent capsids, toroidal core-containing capsids, and brick-shaped core-containing capsids.

4.3.1.2. Agent isolation and identification

4.3.1.2.1. Cell culture/artificial media

To date, attempts to culture the virus in both vertebrate and invertebrate cell lines and in primary oyster cell cultures have been unsuccessful.

4.3.1.2.2. Antibody-based antigen detection methods

Specific antibodies have been developed (Arzul *et al.*, 2002). However, they are not currently available for diagnostic purposes.

4.3.1.2.3. Molecular techniques

At present there are a number of different PCR methods available for the detection of OsHV-1. These include both conventional and real-time PCRs (Martenot *et al.,* 2010; Pépin *et al.,* 2008; Renault *et al.,* 2000).

A protocol for quantifying OsHV-1 in Pacific oysters based on a Sybr® Green real-time PCR was first developed (Pepin *et al.,* 2008). Martenot *et al.* (2010) developed an alternative protocol based on TaqMan® chemistry. The quantitation limits were 1000 and 18 UG mg^{-1} of tissues for the Sybr® Green-based method and the TaqMan® method, respectively, and the latter protocol has a detection limit of 6 UG mg^{-1} of tissues. Comparing the two protocols using DNA samples obtained from 210 spat, the kappa index (0.41) indicated a moderate concordance between the protocols, according to the measures of Landis and Koch. All samples that were positive by the reference protocol were also positive by the alternative protocol. Of the 76 samples that were negative by the reference protocol, 49 were positives by the alternative protocol. Although these results may suggest that the alternative protocol can be more sensitive than the reference protocol, formal validation is needed. A protocol based on TaqMan® chemistry is under development and validation for the detection of virus specimens or variants presenting the deletion reported in the microsatellite upstream from the ORF4 area ~~(microsatellite)~~ for OsHV-1 ~~the variant~~ microvariants (Pepin *et al.,* pers. comm.).

A loop-mediated isothermal amplification (LAMP) assay was also developed for OsHV-1 DNA detection (Ren *et al.,* 2010). A set of four primers was designed, based on the sequence of the ATPase subunit of the OsHV-1 DNA-packaging terminase gene. This LAMP technique can be used both in the laboratory and on farms.

Annex 12 (contd)

Samples to be taken: Live or moribund molluscs. Larvae (100–200 mg), spat (100–200 mg) or 2–3 mm^2 tissue pieces are excised aseptically from mantle, placed into 1.5 ml tubes, preserved in 95° alcohol or kept frozen (–80°C). Dissecting utensils should be flamed between samples to prevent cross-contamination.

4.3.1.2.3.1. Conventional PCR assays

Conventional PCR assays have been used successfully to detect OsHV-1 DNA in bivalves and different primer pairs have been designed (see Batista *et al.,* 2007 for a review).

Two pairs of primers (A3/A4 and A5/A6) were designed and used to detect virus DNA in Pacific oyster larvae and spat via nested PCR (Renault *et al.,* 2000). The specificity of these primer pairs was evaluated using DNA from *C. gigas* as well as DNA from vertebrate herpesviruses; 500 fg of virus DNA extracted from purified particles was routinely detected. The one-step PCR assay with the A3/A4 primer pair not only allowed amplification of OsHV-1 DNA but also the detection of a variant of this virus in *C. gigas* and *R. philippinarum* larvae (Arzul *et al.,* 2002).

Other primers were then designed including C2/C6. The combination of primer pairs A3/A4 and A5/A6 allowed less PCR amplification than C2/C6 (21.4% vs 32.4%) when the same larval samples were analysed (Renault & Arzul, 2001). C2/C6 primer pair systematically allowed the detection of 1 fg of purified viral DNA (Renault *et al.,* 2004). A detection limit of 10 fg of purified viral DNA for both primer pairs C13/C5 and Gp3/Gp4 has been reported (Vigneron *et al.,* 2004). As little as 1 pg and 10 pg allowed the C9/C10 and the OsHVDPFor/OsHVDPRev primer pairs, respectively, to detectably amplify a specific product (Webb *et al*., 2007).

EU comment

Primer sequences for C2/C6 are needed, this is important as confirmation of the microvariant is only possible using PCR and sequence analysis using a defined primer set C2/C6. The EU suggests making it clear in the text that this is the only molecular technique that can be used to identify the pathogen as described in the scope.

Although PCR specificity has been assessed for some of the primer pairs used to detect virus DNA (see above), this has not been done for all designed primer pairs. Moreover, the amplification conditions that have been used in PCR assays using different primer pairs were based on the conditions optimised for A3/A4 and A5/A6 (Renault *et al.,* 2000). An experimental procedure scheme used for the detection of OsHV-1 DNA by conventional PCR has been proposed by Bastista *et al.* (2007).

4.3.1.2.3.2. OsHV-1 specific Sybr® Green PCR assay (Pepin et al., 2008)

Fifty mg of larvae/spat/mantle tissue are ground in 50 µl double-distilled water using a disposable piston. The crushed tissues are diluted six-fold and clarified at 10,000 ***g*** for 5 minutes. One hundred µl recovered supernatant are treated using a commercial DNA tissue kit (QIAgen – Qiamp tissue mini kit®) according to the manufacturer's protocol. Final elution of the DNA is performed with 100 µl TE buffer. The DNA is stored at –20°C. Prior to PCR, DNA concentrations can be measured by absorbance at 260 nm. According to total DNA concentration measured in samples, they are diluted in order to obtain 20 ng total DNA per PCR reaction.

Three sets of primers can be used targeting three regions of viral DNA: (ORF4, ORF88 and ORF99). Primer pairs B4/B3 (Arzul *et al.*, 2001b; ORF99 encoding a BIR protein) and C9/C10 (Barbosa-Solomieu *et al.*, 2004; ORF4) were previously designed for single PCR, whereas the Gp4/Gp7 primer pair (ORF88 encoding a class I membrane protein) was assessed for qPCR. The primer pairs B4/B3, C9/C10 and Gp4/Gp7 yield PCR products of 207, 197 and 85 bp, respectively.

B4: 5'-ACT-GGG-ATC-CGA-CTG-ACA-AC-3'

B3: 5'-GTG-GAG-GTG-GCT-GTT-GAA-AT-3'

C9: 5'-GAG-GGA-AAT-TTG-CGA-GAG-AA-3'

C10: 5'-ATC-ACC-GGC-AGA-CGT-AGG-3'

Gp4: 5'-GGC-GTC-CAA-ACT-CGA-TTA-AA-3'

Gp7: 5'-TTA-CAC-CTT-TGC-CGG-TGA-AT-3'

The C9/C10 primer pair yield reliable parameters for qPCR with OsHV-1 DNA, as well as the B3/B4 primer pair, which show closely similar parameters with a slightly lower *E* value (96.3%). The Gp4/Gp7 primer pair is less efficient (*E* = 91.3%) and less sensitive (≥50 copies μl^{-1}). The primer pair C9/C10 appears to be the most sensitive and efficient.

An additional primer pair DPFor/DPRev can be also used producing a197 bp product (ORF100, DNA polymerase).

DPFor: 5'-ATT-GAT-GAT-GTG-GAT-AAT-CTG-TG-3'

DPRev: 5'-GGT-AAA-TAC-CAT-TGG-TCT-TGT-TCC-3'

Targeting different OsHV-1 DNA is important in order to define more precisely viral strains and isolates. Although ORF4 is an interesting candidate to describe diversity because virus polymorphism has been already reported in this area, ORF100 (DNA polymerase) appears to be less polymorphic.

All amplification reactions are performed in a total volume of 25 µl with 96-microwell plates. Each well (25 µl) contains 5 µl extracted DNA dilution (sample) or OsHV-1 ~~genomic~~ DNA (positive control), 12.5 µl Brilliant® SYBR® Green I PCR Master Mix or FullVelocity® Master Mix (Stratagene), 2.5 µl each diluted primer (final concentration 200 nM) and 2.5 µl distilled water. Thermal cycle conditions are: 1 cycle of pre-incubation at 95°C for 10 minutes; 40 cycles of amplification at 95°C for 30 seconds (15 seconds with FullVelocity® Master Mix), 60°C for 45 seconds (30 seconds with FullVelocity® Master Mix) and 72°C for 45 seconds with Brilliant® Master Mix; and melting temperature curve analysis at 95°C for 60 seconds, 60°C for 30 seconds and 95°C for 30 seconds. Real time PCR analysis should be performed in triplicate with 5 µl sample dilutions as DNA template or a viral DNA control.

Absolute quantitation of copies of OsHV-1 DNA (copies μl^{-1}) is carried out by comparing CT (threshold cycle) values obtained with the standard curve, using the Thermocycler software. Each experiment includes a positive DNA control (OsHV-1 genomic DNA for absolute quantitation) and blank controls (NTC, no template control consisting of deionised sterile water). PCR efficiency (*E*) is calculated from standard curves as the percentage of template molecules that is doubled during each cycle ([10 (−1/slope) −1] ×100), with requirements that it fell into the range 95–105% and that the coefficient of determination (*R*2) is >0.98. In order to allow detection of non-specific products, a dissociation protocol (melt curve) takes place after the amplification cycles. The temperature at which SYBR®Green fluorescence is generated by the double-stranded amplicon dissociation is recorded.

Regarding the test's sensitivity, it is considered that it can detect systematically 4 DNA copies μl^{-1}. The dynamic range for the qPCR was estimated from several standard curve assays, and a linear relationship was obtained between input copy number of the viral DNA template and CT value for over 5 log 10 dilutions. It was possible to quantitate OsHV-1 DNA copy numbers at least from 10 to 5×10^6 copies μl^{-1}.

4.3.1.2.3.3. OsHV-1 specific TaqMan® PCR assay (*Martenot* et al., *2010*)

The target was the B region of the OsHV-1 genome, which encodes a putative apoptosis inhibitor (Arzul *et al.,* 2001b). Primer pairs and two TaqMan® probes were designed to detect simultaneously the target gene and an internal control (IC). The IC was a synthesised sequence containing at each end the forward OsHV1BF (5'-GTC-GCA-TCT-TTG-GAT-TTA-ACA-A-3') and reverse B4 (5'-ACT-GGG-ATC-CGA-CTG-ACA-AC-3') primers. The B4 primer used for the TaqMan PCR was the same as that published by Pepin *et al*. (2008).

The amplification of the targeted region and IC was performed by using the OsHV1BF and B4 primers. The B (5'-TGC-CCC-TGT-CAT-CTT-GAG-GTA-TAG-ACA-ATC-3') and the IC (5'-

ATC-GGG-GGG-GGG-GGT-TTT-TTT-TTT-ATC-G-3') probes were labelled at the 5' end with the fluorescent reporter dyes TxR and FAM, respectively, and at the 3' end with an appropriate quencher (BHQI or BHQII).

The reaction mixture contained 12.5 µl premix ExTaq® 2× Takara® (Lonza, Verviers, Belgium), 0.5 µl each primer (20 µM), 0.5 µl TaqMan® probes (10 µM) and 9 µl water. Two µl DNA sample was added to 23 µl reaction mixture. The amplification was performed in two stages under the following conditions: 1 cycle of 95°C for 10 seconds, followed by 40 cycles of amplification at 95°C for 5 seconds, 60°C for 20 seconds. The virus quantitation was carried out by comparison with standard curve values.

4.3.1.2.3.4. OsHV-1 specific in-situ *hybridisation*

The *in-situ* hybridisation (ISH) procedure described here uses a digoxigenin (DIG)-labelled DNA probe to detect OsHV-1 in formalin-fixed, paraffin-embedded tissue (Arzul *et al.*, 2002; Lipart & Renault, 2002). This assay can detect the generic and emergent strains.

Sections of tissue that include mantle, digestive gland, gills and adductor muscle should be fixed for 24 hours in Davidson's AFA or other suitable fixative and processed using standard procedures for histological examination.

~~Seven µm thick~~ Tissue sections on silane-prep™ slides are dewaxed in xylene (2 × 5 minutes), treated in absolute ethanol (2 × 5 minutes) and air dried at room temperature (15 minutes). Sections are then permeabilised with proteinase K (100 µg ml^{-1} in distilled water) for 30 minutes at 37°C in a humid chamber. Proteolysis is stopped by one 3-minute wash in 0.1 M Tris, 0.1 M NaCl buffer (pH 7.5) at room temperature. Sections are dehydrated in 95° ethanol for 1 minute, absolute ethanol for 1 minute and air dried (15 minutes).

A prehybridisation step is carried out with pre-hybridisation buffer (50% formamide, 10% dextran sulfate, 4 × SSC [0.06 M Na_3citrate, 0.6 NaCl, pH 7], 250 µg ml^{-1} yeast tRNA and 10% Denhart) for 30 minutes at 42°C in a humid chamber. The prehybridisation buffer solution is replaced with 100 µl hybridisation buffer solution containing 50 µl digoxigenin-labelled probe (5 ng $µl^{-1}$) and 50 µl hybridisation buffer (50% formamide, 10% dextran sulfate, 4× SSC, 250 µg ml^{-1} yeast tRNA and 10% Denhart). Slides are covered with plastic coverslips (Polylabo, France). DIG-labelled probes are synthesised from OsHV-1 genomic DNA (100 pg per reaction) by incorporation of digoxigenin-11-dUTP (Boehringer Mannheim, Germany) during conventional PCR. The primer pair C1/C6 is used:

C1: 5'- TTC-CCC-TCG-AGG-TAG-CTT-TT -3'

C6: 5'- GTG-CAC-GGC-TTA-CCA-TTT-TT -3'

Target DNA and digoxigenin-labelled probe are denatured at 95°C for 5 minutes and the hybridisation is carried out overnight at 42°C in a humid chamber.

After hybridisation, coverslips were removed carefully and slides were washed for 10 minutes in 1 × SSC (0.2% BSA) at 42°C. Specifically bound probe was detected using a peroxidase-conjugated mouse IgG antibody again digoxigenin (Boehringer Mannheim, Germany) diluted 1:250 in 1 × PBS (1 hour at room temperature). Unbound peroxidase-conjugated antibody was removed by six washes in 1 × PBS (5 minutes). Diaminobenzidine (DAB) tetrahydro-chloride was diluted in 1 × PBS (0.7 mg ml^{-1}). The colour solution was added to tissue sections (500 µl) and incubated at room temperature in the dark for 20 minutes. The reaction was stopped with two 1 × PBS washes. Slides were stained for 20 seconds in Unna Blue (RAL, France) followed by ethanol dehydration and mounted in Eukitt via xylene.

Specific dark brown intra-cellular staining is indicative of the presence of viral DNA.

Thirty Pacific oyster adults have been analysed using three different techniques: PCR, ISH and immunochemistry, in order to detect OsHV-1 in subclinical individuals (Arzul *et al.,* 2002). PCR and ISH allowed detection of oyster herpes virus DNA in 93.3% and 86.6%, respectively, of analysed oysters while polyclonal antibodies allowed detection of viral proteins in 76.6% of analysed adult oysters.

4.3.1.2.4. Agent purification

OsHV-1 can be purified from infected animals using a previously developed technique (Le Deuff & Renault, 1999)

4.3.2. Serological methods

None applicable.

5. Rating of tests against purpose of use

Should perinuclear chromatin be observed by histology, electron microscopy at least should be undertaken to identify any virus-like particles present and demonstrate their location within cells. Viruses observed by EM should be described as e.g. herpesvirus-like until further investigations are done to provide further evidence of the identity of the virus. As different herpesviruses are morphologically similar, a virus should only be described as OsHV-1 if it had been shown to have identity with the latter virus using OsHV-1 specific primers or probes.

For OsHV- 1, the presence of intracellular viral proteins, specific OsHV-1 messenger RNA, non-structural proteins and TEM demonstrating virions within cells constitute evidence for replication, but detection of viral presence by PCR alone does not. As many moribund/dead oysters from populations with abnormal mortalities had high copy numbers of viral DNA, it may be possible in some cases to extrapolate those data to infer that OsHV-1 has replicated in animals (from known or new host species) with such high levels of viral DNA. However, rigorous evaluation and validation is required before those data could be used in that way.

It may be possible to demonstrate viral infectivity by passage to a susceptible host with appropriate control animals (bioassay). Detection of mortality or characteristic changes associated with detection of the virus is an important consideration in the assessment but not conclusive evidence of host susceptibility. The anatomical location of the pathogen is important also to exclude potential passive contamination of the host. This information can be obtained by techniques such as TEM, immunohistochemistry or ISH.

As an example, the methods currently available for targeted surveillance and diagnosis are listed in Table 5.1. The designations used in the Table indicate: a = the method is the recommended method for reasons of availability, utility, and diagnostic specificity and sensitivity; b = the method is a standard method with good diagnostic sensitivity and specificity; c = the method has application in some situations, but cost, accuracy, or other factors severely limits its application; and d = the method is presently not recommended for this purpose.

Table 5.1. Methods for targeted surveillance and diagnosis

Method	Targeted surveillance			Presumptive surveillance			Confirmatory diagnosis		
	Larvae	Juveniles	Adults	Larvae	Juveniles	Adults	Larvae	Juveniles	Adults
Gross signs	d	d	d	d	d	d	d	d	d
Bioassay	d	d	d	d	d	d	c	c	d
Histopathology	d	d	d	d	d	d	d	d	d
Transmission EM	d	d	d	d	d	d	b	b	b
Antibody-based assays	d	d	d	d	d	d	d	d	d
DNA probes – *in situ*	c	c	c	c	c	c	b	b	b
PCR	a	a	a	a	a	a	b	b	b
qPCR	a	a	a	a	a	a	b	b	b
Sequence	d	d	d	d	d	d	a	a	a

EM = electron microscopy; PCR = polymerase chain reaction; qPCR = real-time PCR.

6. Test(s) recommended for targeted surveillance to declare freedom from OsHV-1 infection

~~Not applicable.~~ PCR and real-time PCR are recommended.

7. Corroborative diagnostic criteria

7.1. Definition of suspect case

A suspect case of infection with OsHV-1 microvariants is a case of mortality of susceptible species associated with detection of OsHV-1 by PCR, qPCR, or *in-situ* hybridisation.

7.2. Definition of confirmed case

A confirmed case is defined as a suspect case followed by sequencing of the microsatellite locus upstream the ORF4 (Segarra *et al.,* 2010) leading to sequences consistent with the definition of microvariants.

8. References

ARZUL I., RENAULT T. & LIPART C. (2001a). Experimental herpes-like viral infections in marine bivalves: demonstration of interspecies transmission. *Dis. Aquat. Org*., **46**, 1–6.

ARZUL I., RENAULT T., LIPART C. & DAVISON A.J. (2001b) Evidence for inter species transmission of oyster herpesvirus in marines bivalves. *J. Gen. Virol.*, **82**, 865–870.

ARZUL I., RENAULT T., THÉBAULT A. & GÉRARD A. (2002). Detection of oyster herpesvirus DNA and proteins in asymptomatic *Crassostrea gigas* adults. *Virus Res*., **84**, 151–160.

BARBOSA-SOLOMIEU V., DÉGREMONT L., VÁZQUEZ-JUÁREZ R., ASCENCIO-VALLE F., BOUDRY P. & RENAULT T. (2005). Ostreid herpesvirus 1 (OsHV-1) detection among three successive generations of Pacific oysters (*Crassostrea gigas*). *Virus Res.,* **107**, 47–56.

BARBOSA-SOLOMIEU V., MIOSSEC L., VÁZQUEZ-JUÁREZ R., ASCENCIO-VALLE F. & RENAULT T. (2004). Diagnosis of Ostreid herpesvirus 1 in fixed paraffin-embedded archival samples using PCR and *in situ* hybridisation. *J. Virol. Methods*, **119**, 65–72.

BATISTA F.M., ARZUL I., PEPIN J.F., RUANO F., FREIDMAN C., BOUDRY P. & RENAULT T. (2007) Detection of ostreid herpesvirus-1 DNA in bivalve molluscs: a critical review. *J. Virol. Methods*, **139** (1), 1–11.

~~BURGE C.A., JUDAH L.R., CONQUEST L.L., GRIFFIN F.J., CHENEY D.P., SUHRBIER A., VADOPALAS B., OLIN P.G., RENAULT T. & FRIEDMAN C.S. (2007). Summer seed mortality of the Pacific oyster, *Crassostrea gigas* Thunberg grown in Tomales Bay, California, USA: The influence of oyster stock, planting time, pathogens, and environmental stressors. *J. Shellfish Res.,* **26**, 163–172.~~

DAVISON A.J., EBERLE R., EHLERS B., HAYWARD G.S., MCGEOCH D.J., MINSON A.C., PELLETT P.E., ROIZMAN B., STUDDERT M.J. & THIRY E. (2009). The order Herpesvirales. *Arch. Virol.*, **154**, 171–177.

DAVISON A.J., TRUS B.L., CHENG N., STEVEN A.C., WATSON M.S., CUNNINGHAM C., LE DEUFF R.M. & RENAULT T. (2005). A novel class of herpesvirus with bivalve hosts. *J. Gen. Virol.*, **86**, 41–53.

DEGREMONT L. (2011). Evidence of herpesvirus (OsHV-1) resistance in juvenile *Crassostrea gigas* selected for high resistance to the summer mortality phenomenon. *Aquaculture*, **317** (1–4), 94–98.

DUNDON W.G., ARZUL I., OMNES E,. ROBERT M., MAGNABOSCO C., ZAMBON M., GENNARI L., TOFFAN A., TERREGINO C., CAPUA I., & ARCANGELI G. (2011). Detection of Type 1 Ostreid Herpes variant (OsHV-1 mu var) with no associated mortality in French-origin Pacific cupped oyster *Crassostrea gigas* farmed in Italy. *Aquaculture,* **314**, (1–4), 49–52.

EUROPEAN FOOD SAFETY AUTHORITY (EFSA) (2010). Scientific opinion on the increased mortality events in Pacific oysters, *Crassostrea gigas*. *EFSA J.*, **8**, 1–59.

FRIEDMAN C.S., ESTES R.M., STOKES N.A., BURGE C.A., HARGOVE J.S., BARBER B.J., ELSTON R.A., BURRESON E.M. & REECE K.S. (2005). Herpes virus in juvenile Pacific oysters *Crassostrea gigas* from Tomales Bay, California, coincides with summer mortality episodes. *Dis. Aquat. Org.*, **63** (1), 33–41.

GARCIA C., THÉBAULT A., DÉGREMONT L., ARZUL I., MIOSSEC L., ROBERT M., CHOLLET B., FRANÇOIS C., JOLY J.-P., FERRAND S., KERDUDOU N. & RENAULT T. (2011). OsHV-1 detection and relationship with *C. gigas* spat mortality in France between 1998 and 2006. *Vet. Res*, **42**, 73–84.

LE DEUFF R.M., NICOLAS J.L., RENAULT T. & COCHENNEC N. (1994) Experimental transmission of a herpes-like virus to axenic larvae of Pacific oyster, *Crassostrea gigas*. *Bull. Eur. Assoc. Fish Pathol.,* **14**, 69–72.

LE DEUFF R.-M. & RENAULT T. (1999). Purification and partial genome characterization of a herpes-like virus infecting the Japanese oyster, *Crassostrea gigas*. *J. Gen. Virol.*, **80**, 1317–1322.

LE DEUFF R.-M., RENAULT T. & GERARD A. (1996). Effects of temperature on herpes-like virus detection among hatchery-reared larval Pacific oyster *Crassostrea gigas*, *Dis. Aquat. Org.*, **24**, 149–157.

LIPART C. & RENAULT T. (2002). Herpes-like virus detection in *Crassostrea gigas* spat using DIG-labelled probes. *J. Virol. Methods*, **101**, 1–10.

LYNCH S.A., CARLSON J., REILLY A.O., COTTER E. & CULLOTY S.C. (2012). A previously undescribed ostreid herpes virus 1 (OsHV-1) genotype detected in the Pacific oyster, *Crassostrea gigas*, in Ireland. *Parasitol.*, **139**, 1526-1532.

MARTENOT C., ODEN E., TRAVAILLE E., MALAS J.P. & HOUSSIN M. (2010). Comparison of two real-time PCR methods for detection of ostreid herpesvirus 1 in the Pacific oyster *Crassostrea gigas*. *J. Virol. Methods*, **70** (1–2), 86–99.

MARTENOT C., ODEN E., TRAVAILLÉ E., MALAS J.P. & HOUSSIN M. (2011). Detection of different variants of Ostreid Herpesvirus 1 in the Pacific oyster *Crassostrea gigas*. *Virus Res.*, **160**, 25-31.

MOSS J.A., BURRESON E.M., CORDES J.F., DUNGAN C.F., BROWN G.D., WANG A., WU X. & REECE K.S. (2007). Pathogens in *Crassostrea ariakensis* and other Asian oyster species: implications for non-native oyster introduction to Chesapeake Bay. *Dis. Aquat. Org*., **77**, 207–223.

PEELER J.E., REESE R.A., CHESLETT D.L., GEOGHEGAN F., POWER A. & TRUSH M.A. (2012). Investigation of mortality in Pacific oysters associated with Ostreid herpesvirus-1 µVar in the Republic of Ireland in 2009. *Preventive Vet. Med.*, **105**, 136-143.

PÉPIN J. F., RIOU A. & RENAULT T. (2008). Rapid and sensitive detection of ostreid herpesvirus 1 in oyster samples by real-time PCR. *J. Virol. Methods*, **149**, 269–276.

REN W., RENAULT T., CAI Y. & WANG C. (2010) Development of a loop-mediated isothermal amplification assay for rapid and sensitive detection of ostreid herpesvirus 1 DNA. *J. Virol. Methods*, **170**, 30–36.

Annex 12 (contd)

RENAULT T. & ARZUL I. (2001). Herpes-like virus infections in hatchery-reared bivalve larvae in Europe: specific viral DNA detection by PCR. *J. Fish Dis*., **24** (3), 161.

RENAULT T., ARZUL I. & LIPART C. 2004. Development and use of an internal standard for oyster herpesvirus 1 detection by PCR. *J. Virol. Method*, **121**, 17–23.

RENAULT T., COCHENNEC N., LE DEUFF R.M. & CHOLLET B. (1994a) Herpes-like virus infecting Japanese oyster (*Crassostrea gigas*) spat. *Bull. Eur. Assoc. Fish Pathol.*, **14**, 64–66.

RENAULT T., LE DEUFF R.M., COCHENNEC N., CHOLLET B. & MAFFART P. (1995). Herpes-like viruses associated with high mortality levels in larvae and spat of Pacific oysters, *Crassostrea gigas*: A comparative study, the thermal effects on virus detection in hatchery-reared larvae, reproduction of the disease in axenic larvae. *Vet. Res*., **26**, 539–543.

RENAULT T., LE DEUFF R.-M., COCHENNEC N. & MAFFART P. (1994b). Herpesviruses associated with mortalities among Pacific oyster, *Crassostrea gigas*, in France – comparative study. *Revue de Médecine Vétérinaire*, **145**, 735–742.

RENAULT T., LE DEUFF R.-M., LIPART C. & DELSERT C. (2000). Development of a PCR procedure for the detection of a herpes-like virus infecting oysters in France. *J. Virol. Methods*, **88**, 41–50.

RENAULT T., MOREAU P., FAURY N. PEPIN J.-F., SEGARRA A. & WEBB S. (2012). Analysis of Clinical Ostreid Herpesvirus 1 (*Malacoherpesviridae*) Specimens by Sequencing Amplified Fragments from Three Virus Genome Areas. *J. Virol.,* **86**(10), 5942-5947.

~~ROQUE A., CARRASCO N., ANDREE K.B., LACUESTA B., ELANDALOUSSI L. GAIRIN I., RODGERS C.J. & FURONES M.D. (2012). First report of OsHV-1 microvar in Pacific oyster (*Crassostrea gigas*) cultured in Spain. *Aquaculture*, **324–325**, 303–306.~~

~~SAMAIN J.F., DÉGREMONT L., SOLETCHNIK P., HAURE J., BÉDIER E., ROPERT M., MOAL J., HUVET A., BACCA H., VAN WORMHOUDT A., DELAPORTE M., COSTIL K., POUVREAU S., LAMBERT C., BOULO V., SOUDANT P., NICOLAS J.L., LE ROUX F., RENAULT T., GAGNAIRE B., GERTET F.,BOUTET I., BURGEOT T. & BOUDRY P. (2007). Genetically based resistance to summer mortality in the Pacific oyster (*Crassostrea gigas*) and its relationship with physiological, immunological characteristics and infection processes. *Aquaculture*, **268** (1–4), 227–243.~~

SAUVAGE C., PÉPIN J.F., LAPÈGUE S., BOUDRY P. & RENAULT T. (2009). Ostreid herpes virus 1 infection in families of the Pacific oyster, *Crassostrea gigas*, during a summer mortality outbreak: difference in viral DNA detection and quantification using real-time PCR. *Virus Res.,* **142**, 181–187.

SCHIKORSKI D., FAURY N., PEPIN J.F., SAULNIER D., TOURBIEZ D. & RENAULT T. (2011a). Experimental ostreid herpesvirus 1 infection of the Pacific oyster *Crassostrea gigas*: kinetics of virus DNA detection by q-PCR in seawater and in oyster samples. *Virus Res.,* **155** (1), 28–34.

SCHIKORSKI D., RENAULT T., SAULNIER D., FAURY N., MOREAU P. & PEPIN J.F. (2011b). Experimental infection of Pacific oyster *Crassostrea gigas* spat by ostreid herpesvirus 1: demonstration of oyster spat susceptibility. *Vet. Res.,* **42**, 1–13.

SEGARRA A., PEPIN J.F., ARZUL I., MORGA B., FAURY N. & RENAULT T. (2010). Detection and description of a particular *Ostreid herpesvirus 1* genotype associated with massive mortality outbreaks of Pacific oysters, *Crassostrea gigas,* in France in 2008. *Virus Res.,* **153**, 92–95.

SHIMATA Y., KURITA J., KIRYU I., NISHIOKA T., YUASA K., KAWANA M., KAMAISHI T. & OSEKO N. (2012). Surveillance of Type 1 Ostreid Herpesvirus (OsHV-1) variants in Japan. *Fish Pathol.*, **47** (4), 129–136.

~~SOLETCHNIK P., LE MOINE O., FAURY N., RAZET D., GEAIRON P. & GOULLETQUER P. (1999). Mortalité de l'huître *Crassostrea gigas* dans le bassin de Marennes-Oléon : étude de la variabilité spatiale de son environnement et de sa biologie par un système d'informations géographiques (SIG), *Aquatic Living Resources*, **12**, 131–143.~~

VÁSQUEZ-YEOMANS R., GARCÍA-ORTEGA M. & CÁCERES-MARTÍNEZ J. (2010). Gill erosion and herpesvirus in *Crassostrea gigas* cultured in Baja California, Mexico. *Dis. Aquat. Org.,* **89**, 137–144.

VIGNERON V., SOLLIEC G., MONTANIÉ H. & RENAULT T. (2004). Detection of ostreid herpes virus 1 (OsHV-1) DNA in seawater by PCR: influence of water parameters in bioassays. *Dis. Aquat. Org.*, **62**, 35–44.

WALLIS C. & MELNICK J. (1965). Thermostabilization and thermosensitization of herpesvirus. *J. Bacteriol*., **90**, 1632–1637.

WEBB S.C., FIDLER A. & RENAULT T. (2007). Primers for PCR-based detection of ostreid herpes virus-1 (OsHV-1): Application in a survey of New Zealand molluscs. *Aquaculture*, **272**, 126–139.

*
* *

Annex 13

AQUATIC ANIMALS COMMISSION WORK PLAN 2013–2014

***Aquatic Code*:**

Task	Oct 2013	February 2014	May GS 2014	Sept 2014
OsHV-1 µvar - listed as an emerging disease		Review status as a listed emerging disease		
Chapter 10.X. Infection with salmonid alphavirus	AAC developed new *Code* chapter and circulated for Member comments	Review Member comments	Propose for adoption	
Chapter 6.1. Chapter on control of hazards in feed	AAC agreed on the structure of a revised chapter. AAC to develop revised chapter	Review draft chapter (developed by AAC) and circulate for Member comments		Review Member comments
Chapter 6.X. Risk analysis for antimicrobial resistance in aquaculture (new)	Request report from AHG re progress	Review AHG report		
Chapter X.X. Criteria for listing susceptible species (new)	Review Member comments and circulate revised text for Member comments	Review Member comments	Propose for adoption	
Chapter on Evaluation of AAHS (new)				Consider the development of a new chapter
Revision of Section 4 to improve guidance on the control of disease	Develop Concept Note for revision of this section	Review new introductory chapter for this section (to be developed by AAC)		
Article 1.2.3. Criteria for listing a disease and an emerging disease	AAC proposed deletion and circulated for Member comments	Review Member comments	Propose for adoption	
Chapter 1.1.	AAC revised text and circulated for Member comments	Review Member comments	Propose for adoption	
Glossary	AAC revised definitions for emerging disease, susceptible species and veterinarian. Circulated for Member comments	Review Member comments	Propose for adoption	

Annex 13 (contd)

Aquatic Manual:

Manual Tasks	Oct. 2013	Feb 2014	May GS 2014	Sept 2014
Chapter 2.3.5. Infection with ISAV	AAC revised chapter and circulated for comments	Review Member comments	Propose for adoption	
Chapter 2.4.9. OsHV-1 µvar	AAC revised chapter and circulated for comments	Review Member comments	Propose for adoption	
Chapter 1.1.3. Disinfection	**November 2013:** Circulate revised text for Member comments	Review Member comments	Propose for adoption	
Chapter X.X.X. Infection with salmonid alphavirus	**November 2013:** Circulate new chapter for Member comments	Review Member comments	Propose for adoption	

Other items:

Tasks	Oct. 2013	Feb. 2014	late 2014	early 2015
OIE Global Aquatic Animal Health Conference (January 2015, TBC)	Establish Scientific Committee. Develop the programme	Finalise programme		Conference
OIE Ref Lab Conference (7–9 October 2014)	AAC to provide input into the programme and Scientific Committee		Conference (7-9 October, 2014)	

CHAPTER 2.3.X.

INFECTION WITH SALMONID ALPHAVIRUS

EU comment

The EU commends the OIE and in general supports the adoption of this new chapter.

In Section 3.4, it is suggested to include the reference Graham *et al*. (2010). J. Fish Dis. 33, 123-135. This paper shows that SAV was detected in the serum by real-time RT-PCR two weeks before any of the other standard tests were positive and is recommended for farms as an early warning screening test.

In Section 2.2.7, SAV RNA has also been detected from dab and plaice off the Irish coast by real-time RT-PCR. Samples were only collected for molecular analysis and all belonged to subtype 1, the dominant subtype in Ireland. McCleary S.J., Giltrap M., Henshilwood K., Ruane N.M. (Year). Detection of salmonid alphavirus RNA in Celtic and Irish Sea flatfish. Submitted to Dis. Aquat. Org. (June 2013).

Further comments are inserted in the text below.

1. Scope

For the purpose of this chapter, infection with salmonid alphavirus (SAV) means infection with any subtype of SAV of the genus *Alphavirus* of the family *Togaviridae.*

Infection with SAV may cause pancreas disease (PD) or sleeping disease (SD) in Atlantic salmon (*Salmo salar* L.), rainbow trout (*Oncorhynchus mykiss* [Walbaum]) and brown trout (*Salmo trutta* L.) (Boucher *et al.,* 1995; McLoughlin & Graham, 2007). The virus is horizontally transmitted, and the main reservoirs of SAV are clinically diseased or covertly infected fish (Viljugrein *et al.,* 2009). The disease is a generalised and lethal condition characterised microscopically by necrosis and loss of exocrine pancreatic tissue, and severe heart and skeletal muscle changes. The mortality varies significantly, from negligible to over 50% in severe cases of PD and up to 15% of surviving fish will develop into long, slender fish ('runts') (McLoughlin & Graham, 2007).

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent, agent strains

SAV is an enveloped, single-stranded, positive-sense RNA virus, approximately 55–65 nm in diameter, with a genome of ~12 kb. The genome codes for eight proteins: four capsid glycoproteins (E1, E2, E3 and 6K) and four nonstructural proteins (nsP1–4). SAV is considered to belong to the genus *Alphavirus* of the family *Togaviridae*. This is based on nucleotide sequence studies of SAV isolates, and is also supported by biological properties of the virus, including cross-infection and neutralisation trials. In addition, four conserved nucleotide sequence elements (CSEs) and a conserved motif (GDD), characteristic of alphaviruses, are present in the SAV genome (McLoughlin & Graham, 2007).

SAV has been divided into six subtypes (SAV1–SAV6) based solely on nucleic acid sequence differences (Fringuelli *et al.,* 2008). The level of antigenic variation among subtypes is considered low as monoclonal antibodies (MAbs) raised against a specific SAV subtype are likely to cross react with other SAV isolates (Graham *et al.,* 2013a; Jewhurst *et al.,* 2004) The genotype groups are presented in the table below (abbreviations: SW= sea water, FW = fresh water):

SAV subtype	Host and environment	Country
SAV 1 (PD)	Atlantic salmon (SW) Rainbow trout (FW)	Ireland, Scotland (UK)

SAV 2 FW (SD) SAV 2 Marine (PD)	Rainbow trout (FW) Atlantic salmon (SW)	France, Germany, Italy, Spain, Switzerland, UK (England, Scotland), Norway
SAV 3 (PD)	Rainbow trout (SW) Atlantic salmon (SW)	Norway only
SAV 4 (PD)	Atlantic salmon (SW)	Ireland, Scotland (UK)
SAV 5 (PD)	Atlantic salmon (SW)	Scotland (UK)
SAV 6 (PD)	Atlantic salmon (SW)	Ireland only

2.1.2. Survival outside the host

Laboratory tests have shown that virus survival is inversely related to temperature, and is reduced by the presence of organic matter. In the presence of organic matter, markedly longer survival times are observed in sea water compared with fresh water. (Graham *et al.,* 2007c). SAV has been detected in fat leaking from dead PD-diseased fish, indicating that this may be a route for transmission. Fat droplets may accumulate at the sea water surface, contributing to long distance spread (Stene *et al.,* submitted).

The half-life of SAV in serum has been found to be inversely related to temperature, emphasising the need for rapid shipment of samples at 4°C to laboratories for virus isolation. For long-term conservation of SAV-positive samples and cultured virus, storage at –80°C is recommended (Graham *et al.,* 2007c).

2.1.3. Stability of the agent

SAV is rapidly inactivated in the presence of high levels of organic matter at 60°C, at pH 7.2, and at pH 4 and pH 12 at 4°C, suggesting that composting, ensiling and alkaline hydrolysis would all be effective at inactivating virus in fish waste (Graham *et al.,* 2007a).

2.1.4. Life cycle

Probable infection routes are through the gills or via the intestine. In the acute stages of the disease, large amounts of SAV can be detected and live virus can be isolated from the heart, kidney, blood and several other organs, but the actual target cells for the virus has not yet been identified.

Viraemia precedes both the onset of histological changes and clinical signs (McLoughlin & Graham, 2007). The route of shedding may be through natural excretions/secretions, supported by the detection of SAV by reverse transcriptase polymerase chain reaction (RT-PCR) in the faeces and mucus of experimentally infected Atlantic salmon. These matrices may therefore play a role in the horizontal transmission of SAV through water (Graham *et al.,* 2012). Virus has been detected in water 4–13 days after infection, indicating that virus shedding coincides with the viraemic stage (Andersen *et al.,* 2010). An incubation period of 7–10 days at sea water temperatures of 12–15°C has been estimated based on analysis of antibody production in intraperitoneally infected fish and cohabitants in an experimental trial (McLoughlin & Graham, 2007). Several studies have shown that SAV RNA can be detected in fish for an extended period post-infection (Jansen *et al.,* 2010a; McLoughlin & Graham, 2007). Subclinical infection has been reported, suggesting that the severity of an outbreak may be influenced by several environmental factors (McLoughlin & Graham, 2007), and recent data show that seasonal increase in water temperature may trigger PD outbreaks in SAV-infected farms (Stene *et al.,* 2013).

2.2. Host factors

2.2.1. Susceptible host species

Disease outbreaks and infection experiments have shown that Atlantic salmon (*Salmo salar* L.), rainbow trout (*Oncorhynchus mykiss* [Walbaum]) and brown trout (*Salmo trutta* L.) are susceptible (Boucher *et al.,* 1995; McLoughlin & Graham, 2007).

EU comment

The EU queries the OIE whether this section specifies farmed fish only, as the text in section 2.2.7 indicates a question over wild salmonids as carriers.

2.2.2. Susceptible stages of the host

All life stages should be considered as susceptible to SAV.

Farmed rainbow trout in fresh water are affected at all stages of production (Kerbart Boscher *et al.,* 2006). Experience from Norway show that farmed rainbow trout and Atlantic salmon are susceptible at all stages in sea water, probably reflecting a sea water reservoir of SAV. Experimental infection has also shown susceptibility of Atlantic salmon parr in fresh water (McVicar, 1990).

2.2.3. Species or subpopulation predilection (probability of detection)

There is no known species or subpopulation predilection.

2.2.4. Target organs and infected tissue

Infection with SAV is a systemic disease with an early viraemic phase. After infection, SAV has been detected in all organs that have been examined: brain, gill, pseudobranch, heart, pancreas, kidney and skeletal muscle (Andersen *et al.,* 2007; McLoughlin & Graham, 2007) as well as in mucus and faeces (Graham *et al.,* 2012).

2.2.5. Persistent infection with lifelong carriers

SAV has been detected in surviving fish 6 months after experimental infection (Andersen *et al.,* 2007). At the farm level, an infected population will harbour SAV until slaughter (Jansen *et al.,* 2010a; 2010b). On an individual level, however, lifelong persistent infection has not been documented.

2.2.6. Vectors

SAV has been detected in salmon lice (*Lepeophtheirus salmonis*) collected during acute PD outbreaks in Atlantic salmon, but transfer to susceptible fish species has not been studied (Petterson *et al.,* 2009). Vectors are not needed for transmission of SAV.

2.2.7. Suspected aquatic animal carriers

In surveys of wild marine fish, SAV RNA has been detected in the flatfish species common dab (*Limanda limanda*), long rough dab (*Hippoglossoides platessoides*) and plaice (*Pleuronectes platessa*) (Snow *et al.,* 2010). The importance of wild marine and anadromous fish, as well as fresh water salmonids, as virus carriers needs to be clarified.

2.3. Disease pattern

2.3.1. Transmission mechanisms

Transmission of SAV occurs horizontally. This is supported by phylogenetic studies, successful transmission among fish in cohabitant studies, proven transmission between farming sites and studies on survival of SAV in sea water and the spread via water currents (Graham *et al.,* 2007b; 2011; Jansen *et al.,* 2010a; Kristoffersen *et al.,* 2009; Viljugrein *et al.,* 2009).

Long-distance transmission and thus introduction of SAV in a previously uninfected area is most likely assigned to movement of infected live fish (Kristoffersen *et al.,* 2009; Rodger & Mitchell, 2007). Once SAV has been introduced into an area, shared ownership, close proximity and water currents are factors involved in local transmission (Aldrin *et al.,* 2010; Kristoffersen *et al.,* 2009; Viljugrein *et al.,* 2009). Risk factors for outbreaks of PD on a farming site include a previous history of PD, high feeding rate, high sea lice burden, the use of autumn smolts and previous infection with infectious pancreas necrosis (IPN) (Bang Jensen *et al.,* 2012; Kristoffersen *et al.,* 2009; Rodger & Mitchell, 2007).

Vertical transmission of SAV has been suggested (Bratland & Nylund, 2009), but the evidence is not convincing (Kongtorp *et al.,* 2010; McLoughlin & Graham, 2007) The Norwegian Scientific Committee for Food Safety has recently carried out a risk assessment on brood fish surveillance and vertical transmission of infection, concluding that the risk of vertical transmission of SAV is insignificant.

2.3.2. Prevalence

The prevalence of infected fish within an SAV-infected fish farm is high. Exact estimates vary with the diagnostic method used, but prevalence of 70–100% has been reported from field samples of Atlantic salmon farming sites (Graham *et al.,* 2005; Stene *et al.,* 2013; Taksdal *et al.,* 2007). A single study indicates that the prevalence amongst farmed rainbow trout in sea water may be less, but this needs to be confirmed by other studies (Taksdal *et al.,* 2007).

Prevalence in wild fish is largely unknown. SAV RNA has been detected in some flatfish species in sea water in Scotland (Snow *et al.,* 2010). A serological survey of wild salmonids in fresh water river systems in Northern Ireland did not detect virus neutralisation antibodies against SAV in any of 188 sera tested, whereas the majority of sera from farmed salmon in sea water in the same area tested positive (Graham *et al.,* 2003).

2.3.3. Geographical distribution

Infection with SAV is known to be present in farmed salmoid fish in Croatia, France, Germany, Ireland, Italy, Norway, Spain, Switzerland and the United Kingdom (England, Scotland and Northern Ireland).

EU comment

The EU asks the OIE to include Poland as a country with laboratory confirmed presence of salmonid alphavirus, both in the point above and in the table in section 2.1.1.

Indeed, in July 2011, the Department of Fish Diseases of NVRI received two samples of rainbow trout fry from the two trout farms in the northern Poland with suspected viral infection. In an attempt to identify viral species, several diagnostics methods targeting various fish viruses that usually occur in Poland were used. Aiming to isolate and identify the pathogen, monolayers of RTG-2 and CHSE-214 cells were plated and inoculated with tissue homogenates. The cythopathic effect in the first as well in the second passage was observed. To identify the pathogen RT-PCR and sequencing were applied. Analysis of the sequencing data (a conserved region of the SDV E2 gene) indicated that the Polish strain belongs to SAV subtype 2. This was the first laboratory confirmation of SAV infection in Polish aquaculture. Since that time 6 other cases were confirmed.

2.3.4. Mortality and morbidity

Mortality rates due to infection with SAV may vary with subtype, season, year, use of biosecurity measures and species of fish (Bang Jensen *et al.,* 2012; Graham *et al.,* 2011; Rodger & Mitchell, 2007; Stormoen *et al.,* 2013). The cumulative mortality at the farm level ranges from close to zero to over 50% in severe cases for PD and from negligible to over 22% for SD (Bang Jensen *et al.,* 2012; Graham *et al.,* 2003, Rodger & Mitchell, 2007; Ruane *et al.,* 2008; Stene *et al.,* 2013).

Duration of PD outbreaks, defined as the period with increased mortality, varies from 1 to 32 weeks (Jansen *et al.* 2010a; Jansen *et al.,* submitted; Ruane *et al.,* 2008).

2.3.5. Environmental factors

Clinical outbreaks of PD more often occur in the spring and summer months when temperatures are high (McLoughlin & Graham, 2007; Rodger & Mitchell, 2007), indicating an influence of temperature on disease development. Stressing the fish by movement, crowding or treatment may initiate disease outbreaks on infected farms.

2.4. Control and prevention

2.4.1. Vaccination

At present, one vaccine is available commercially. This vaccine was introduced in 2007 and is widely used in Atlantic salmon farms in PD-endemic areas in Norway, Ireland and Scotland. This vaccine is based on inactivated PD virus suptype 1, and claims a reduction in mortality of at least 50% in comparisons of vaccinated fish against unvaccinated fish at the same farm. The vaccine does not seem to offer complete protection, but a field evaluation carried out in Norway demonstrated that the mortality in farms with vaccinated fish is comparable with mortality in farms without PD. Furthermore, a small reduction in the number of outbreaks was seen (Bang Jensen *et al.,* 2012).

A vaccine based on inactivated PD virus of another subtype is under development. Furthermore, a DNA-based vaccine is showing promising results. To date, only North America has allowed the use of DNA-based vaccines for control of fish diseases; it is not certain whether this vaccine will be licensed for use in other markets.

2.4.2. Chemotherapy

No chemotherapy is available.

2.4.3. Immunostimulation

No immunostimulation is available.

2.4.4. Resistance breeding

Differences in susceptibility among different family groups of Atlantic salmon have been observed in challenge experiments and in the field, indicating the potential for resistance breeding. Both in Ireland and Norway, efforts are being made to breed fish that are more resistant to PD (McLoughlin & Graham, 2007).

EU comment

The EU queries the OIE about QTL ova. "QTL selection based stocks for IPN/PD have shown promising results in field trials with initial challenges with PD showing a similar level of protection as against IPN" (Atlantic QTL-innOva IPN/PD).

2.4.5. Restocking with resistant species

Not relevant.

2.4.6. Blocking agents

Not relevant.

2.4.7. Disinfection of eggs and larvae

Traditional disinfection methods are considered efficient against SAV (Graham *et al.,* 2007a)

2.4.8. General husbandry practices

To avoid infection with SAV, general good hygiene practices should be applied: use of appropriate sites for farming, segregation of generations, stocking with good quality fish, regular cleaning of tanks and pens, controlling parasites and other pathogens as well as careful handling of fish. Once a site has been infected, mortality may be reduced by imposing a general stop on handling of the fish as well as a general stop on feeding the fish.

EU comment

The EU suggests including mortality removal in the paragraph above.

3. Sampling

3.1. Selection of individual specimens

All production units (ponds, tanks, net-cages, etc.) should be inspected for the presence of dead, weak or abnormally behaving fish. Extremely weak ('sleeping') fish may be found at the bottom of a tank or in the net-cages. If the number of clinically diseased fish is low, samples from long, thin fish ('runts') may be added (Jansen *et al.,* 2010b).

3.2. Preservation of samples for submission

Histology and immunohistochemistry:	Fixation in neutral phosphate-buffered 10% formalin
Molecular biology (RT-PCR and sequencing):	Appropriate medium for preservation of RNA
Cell culture:	Virus transport medium
Serology:	Blood plasma or serum

3.3. Pooling of samples

Pooling of samples is not recommended for confirmation of SAV as it is usually of interest to compare results from the various examinations of individual fish. For surveillance purposes, pooling of samples for virological examination (PCR or cell culture) may be accepted, but will decrease the sensitivity of the test.

3.4. Best organs or tissues

Heart and mid-kidney are the recommended organs for detection of SAV either by molecular biological methods or by cell culture as these organs contain SAV both during the initial viraemic phase and later.

During the initial viraemic phase, serum samples are also suitable for detection of SAV either by molecular biological methods or by cell culture. From approximately 3 weeks after SAV infection, blood serum or plasma is suitable for a virus neutralisation test that identifies neutralising antibodies against SAV in fish exposed to SAV (Graham *et al.,* 2003).

Tissues for histological examinations should include gill, heart, pyloric caeca with attached pancreatic tissue, liver, kidney, spleen and skeletal muscle containing both red (aerobe) and white (anaerobe) muscle. The latter is most easily found close to the side line of the fish.

EU comment

The EU suggests replacing the second sentence in the paragraph above by the following:

"Skin with associated skeletal muscle sample should be taken at the lateral line level and deep enough to include both red and white muscle".

Reason to mention "skin with.." is to prevent implying that the skeletal muscle sample is taken without it, which for histological assessment would results in sample damage and potentially, missing/losing the red muscle layer.

4. Diagnostic methods

4.1. Field diagnostic methods

4.1.1. Clinical signs

A sudden drop in appetite may be observed 1–2 weeks before the detection of enhanced mortality. Clinically diseased fish may be observed swimming slowly at the water surface. In some cases, extremely weak (“sleeping”) fish can be found at the bottom of tanks or in net-cages. An increased number of faecal casts may also be observed in the water. However, it is important to notice that clinical signs are not pathognomonic and that careful observation and examinations of any dead, weak or abnormally behaving fish is necessary.

Initially, nutritional status is usually normal, but in the months after an outbreak or in the later stages of disease, long slender fish (‘runts’) with low condition are typically observed. The development of long, slender fish can be caused by factors other than SAV.

4.2. Clinical methods

4.2.1. Gross pathology

Yellow mucoid gut contents are a usual post-mortem finding, as is typically seen in fish that are not eating. Occasionally signs of circulatory disturbances, such as petechial haemorrhages, small ascites or reddening of the pancreatic region between the pyloric caeca, may be seen. Some diseased fish may show pale hearts or heart ruptures. It is important to notice that post-mortem findings are not pathognomonic.

4.2.2. Clinical chemistry

Not documented for diagnostic use.

4.2.3. Microscopic pathology

The changes most commonly found in clinically diseased fish are severe loss of exocrine pancreatic tissue, cardiomyocytic necrosis and inflammation, red (aerobe) skeletal muscle inflammation and white (anaerobe) skeletal muscle degeneration or inflammation. A less frequent but supporting finding is the detection of cells with many cytoplasmic eosinophilic granules along kidney sinusoids.

As the disease progresses, the development of these changes is not simultaneous in all organs: In a very short, early phase, the only lesion present can be necrosis of exocrine pancreatic tissue. Shortly

thereafter, heart muscle cell degeneration and necrosis develops before the inflammation response in the heart becomes more pronounced. The pancreatic necrotic debris will seemingly disappear and the typical picture of severe loss of exocrine pancreatic tissue will soon appear simultaneously with the increasing inflammation in the heart. Somewhat later, skeletal muscle degeneration, inflammation and fibrosis develops (Christie *et al.,* 2007; Kerbart Boscher *et al.,* 2006; McLoughlin & Graham, 2007; Taksdal *et al.,* 2007).

EU comment

The EU suggests replacing the second sentence in the paragraph above by the following:

"In a very short, early phase, the only lesion present can be necrosis of exocrine pancreatic tissue and a variable inflammatory reaction in the peripancreatic fat".

Furthermore, please add the following sentence at the end of the paragraph:

"In a proportion of fish, severe fibrosis of the peri-acinar tissue may occur and in this case the pancreas does not recover (runts)"

4.2.4. Wet mounts

Not relevant.

4.2.5. Smears

Not relevant.

4.2.6. Fixed sections, immunohistochemistry

The single immunohistochemical method published (Taksdal *et al.,* 2007) is only recommended for samples from fish with acute necrosis of exocrine pancreatic tissue.

4.2.6.1. Preparation of tissue sections

The tissues are fixed in neutral phosphate-buffered 10% formalin for at least 1 day, dehydrated in graded ethanol, cleared in xylene and embedded in paraffin, according to standard protocols. Approximately 3 µm thick sections (for immunohistochemistry sampled on poly-L-lysine-coated slides) are heated at 56–58°C (maximum 60°C) for 20 minutes, dewaxed in xylene, rehydrated through graded ethanol, and stained with haematoxylin and eosin for histopathology and immunohistochemistry as described below.

4.2.6.2. Staining procedure for immunohistochemistry

All incubations are carried out at room temperature and all washing steps are done with Tris-buffered saline (TBS).

i) Nonspecific antibody binding sites are first blocked in 5% bovine serum albumin (BSA) in TBS for 20 minutes. The solution is then poured off without washing.

ii) Sections are incubated with primary antibody (monoclonal mouse antibody 4H1 against E1 SAV glycoprotein [Todd *et al.,* 2001]), diluted 1/3000 in 2.5% BSA in TBS and then incubated overnight, followed by two wash out baths lasting a minimum of 5 minutes.

iii) Sections are incubated with secondary antibody (biotinylated rabbit anti-mouse) diluted 1/300 for 30 minutes, followed by wash out baths as in step ii above.

iv) Sections are incubated with streptavidine with alkaline phosphatase 1/500 for 30 minutes followed by wash out baths as in step ii above.

v) For detection of bound antibodies, sections are incubated with Fast Red[1] (1 mg ml^{-1}) and Naphthol AS-MX phosphate (0.2 mg ml^{-1}) with 1 mM Levamisole in 0.1 M TBS (pH 8.2) and allowed to develop for 20 minutes followed by one wash in tap water before counterstaining with Mayer's haematoxylin and mounting in aqueous mounting medium.

1 Reference to specific commercial products as examples does not imply their endorsement by the OIE. This applies to all commercial products referred to in this *Aquatic Manual*.

SAV-positive and SAV-negative tissue sections are included as controls in every setup (Taksdal *et al.,* 2007)

4.2.7. Electron microscopy/cytopathology

Not relevant for diagnostic use.

4.2.8. Differential diagnoses

Tissues that are changed by SD and PD are also changed by heart and skeletal muscle inflammation (HSMI), cardiomyopathy syndrome (CMS) and IPN. However, if all the main organs are examined by histopathology, the pattern of affected organs will usually appear different:

	PD	HSMI	CMS	IPN
Heart*	+	+	+	–
Pancreas	+	–	–	+
Skeletal muscle	+	+	–	–

*Heart changes in CMS affects mainly the inner spongy layer of the ventricle and the atrium, whereas in PD and HSMI, the compact layer of the ventricle is more severely affected. Although these three diseases induce epicarditis, HSMI causes the most severely inflamed epicardium.

The early acute stage of PD and SD when only necrosis of exocrine pancreas has developed, can, when examined by histopathology only, be mistaken for IPN caused by IPN virus (IPNV).

Virological and serological examinations combined with histopathological examination of 5–10 clinically diseased fish will usually clarify the situation. HSMI and CMS have only been detected in Atlantic salmon.

4.3. Agent detection and identification methods

4.3.1. Direct detection methods

4.3.1.2. Agent isolation and identification

4.3.1.2.1. Cell culture

CHSE-214 are commonly used for primary SAV isolation, but other susceptible cell lines such as BF-2, FHM, SHK-1, EPC, CHH-1 or others, may be used. Variation in cell line susceptibility among different SAV field isolates has been reported (Graham *et al.,* 2008; Herath *et al.,* 2009), and it is therefore recommended that several cell lines are tested for initial cell culture isolation of SAV in a new laboratory or for a new virus strain.

The CHSE-214 cells are grown at 20°C in Eagle's minimal essential medium (EMEM) with non-essential amino acids and 0.01 M HEPES (N-2-hydroxyethyl-piperazine-N-2-ethanesulfonic acid) buffer, or Leibovitz's L-15 cell culture medium, both supplemented with fetal bovine serum (FBS) (5% or 10%) and L-glutamine (4 mM).

For virus isolation, cells are grown in tissue culture flasks or multi-well cell culture plates. SAV-positive controls may be inoculated in parallel with the tissue samples as a test for cell susceptibility to SAV (this should be performed in a separate location from that of the test samples).

i) Inoculation of cell monolayers

Prepare a 2% suspension of tissue homogenate or a 10% suspension of serum using L-15 medium or EMEM without serum or other medium with documented suitability. Remove growth medium from actively growing monolayers (1- to 2-day-old cultures or cultures of 70–80% confluency) grown in tissue culture flasks or multi-well cell culture plates (see above). Inoculate monolayers with a low volume of the 2% tissue homogenate or 10% serum dilution (for 25 cm^2 flasks: 1.5 ml). Adjust volume to the respective surface area in use. Allow 2–3 hours' incubation at 15°C followed by removal of the inoculum, and addition of fresh L-15 or EMEM medium supplemented with 2–5% fetal calf serum (for 25 cm^2 flasks: 5 ml).

When fish samples come from production sites where IPNV is regarded as endemic, the tissue homogenate supernatant should be incubated (for a minimum of 1 hour at 15°C) with a pool of antisera to the indigenous serotypes of IPNV prior to inoculation.

ii) Monitoring incubation

Inoculated cell cultures (kept at 15°C) are examined at regular intervals (at least every 7 days) for the occurrence of cytopathic effect (CPE). Typical CPE due to SAV appears as plaques of pyknotic, vacuolated cells. However, Norwegian SAV field isolates (both SAV3 and marine SAV2) usually do not produce CPE in low passages, and this is also reported for other SAV subtypes. If no CPE has developed after 14 days, subculture to fresh cell cultures.

iii) Subcultivation procedure

14 days (or earlier when obvious CPE appears) after inoculation, the cultures are freeze–thawed at –80°C (the procedure can be repeated 1–2 times) to release virus from the infected cells.

Following centrifugation at 3000 ***g*** for 5 minutes, the supernatants are inoculated into fresh cell cultures as described for the primary inoculation: remove growth medium, inoculate monolayers with a small volume of diluted supernatant (1/5 and higher dilutions) for 2–3 hours before addition of fresh medium.

Inoculated cell cultures are incubated for at least 14 days and examined at regular intervals, as described for the primary inoculation. At the end of the incubation period, or earlier if obvious CPE appears, the medium is collected for virus identification, as described below. Cell cultures should always be examined for the presence of SAV by immunofluorescence (indirect fluorescent antibody test [IFAT]), as virus replication may occur without development of apparent CPE.

iv) Antibody-based verification of SAV growth in cell culture

All incubations below are carried out at room temperature unless otherwise stated.

a) Prepare monolayers of cells in appropriate tissue culture plates (e.g. 96-well plates), or on cover-slips, depending on the type of microscope available (an inverted microscope equipped with UV light is necessary for monolayers grown on tissue culture plates). The necessary monolayers for negative and positive controls must be included.

b) Inoculate the monolayers with the virus suspensions to be identified in tenfold dilutions, two monolayers for each dilution. Add positive virus control in dilutions known to give a good staining reaction. Incubate inoculated cell cultures at 15°C for 9–11 days.

c) Fix in 80% acetone for 20 minutes after removing cell culture medium and rinsing once with 80% acetone. Remove the fixative and air dry for 1 hour. If necessary, the fixed cell cultures may be stored dry for 14 days at 4°C until staining.

d) Incubate the cell monolayers with anti-SAV MAb in an appropriate dilution in phosphate-buffered saline (PBS) for 1 hour and rinse three times with PBS with 0.05% Tween 20.

e) Incubate with fluoescein isothiocyanate (FITC)-conjugated anti-mouse immunoglobulin for 1 hour (or if the primary Ab is polyclonal from rabbits, use FITC-conjugated antibody against rabbit immunoglobulin), according to the instructions of the supplier. To increase the sensitivity of the test, FITC-conjugated anti-mouse Ig may be replaced with biotin-labelled anti-mouse Ig and FITC-labelled streptavidin with rinsing as in step d in between the steps. The nuclei can be stained with propidiumiodid (100 µg ml^{-1} in sterile distilled water). Add PBS (without Tween 20) and examine under UV light. To avoid fading, the stained plates should be kept in the dark until examination. For long periods of storage (more than 2–3 weeks) a solution of 1,4-diazabicyclooctane (DABCO 2.5% in PBS, pH 8.2) or similar reagent may be added as an anti-fade solution.

4.3.1.2.3. Reverse-transcription polymerase chain reaction (RT-PCR) and sequencing

The primers described below for real-time RT-PCR and RT-PCR with sequencing will detect all known subtypes of SAV.

RT-PCR may be used for detection of SAV from total RNA (or total nucleic acid) extracted from recommended organs or tissues (see Section 3.4). Real-time RT-PCR for the detection of SAV is recommended as it increases the specificity and also the sensitivity of the test.

For genotyping, RT-PCR with subsequent sequencing of fragments from the E2 and nsP3 genes is recommended.

The primers and probe for real-time RT-PCR from the nsP1 gene, as well as primers for genotyping, are listed below. The E2-primers may also be used for conventional RT-PCR detection of SAV, if necessary.

RT-PCR: Primer and probe sequences	Named	Genomic segment	Product size	Reference
QnsP1F: 5'-CCG-GCC-CTG-AAC-CAG-TT-3' QnsP1R: 5'-GTA-GCC-AAG-TGG-GAG-AAA-GCT-3' QnsP1probe: 5'FAM-CTG-GCC-ACC-ACT-TCG-A-MGB3'	forward primer reverse primer Taqman®probe	QnsP1	107 nt	Hodneland *et al*., 2006
E2F: 5'-CCG-TTG-CGG-CCA-CAC-TGG-ATG-3' E2R: 5'-CCT-CAT-AGG-TGA-TCG-ACG-GCA-G-3'	forward primer reverse primer	E2	516 nt	Fringuelli *et a*l., 2008
nsP3F: 5'-CGC-AGT-CCA-GCG-TCA-CCT-CAT-C-3' nsP3R: 5'-TCA-CGT-TGC-CCT-CTG-CGC-CG-3'	forward primer reverse primer	nsP3	490 nt	Fringuelli *et al.,* 2008

4.3.2. Serological methods

4.3.2.1 Immunoperoxidase-based virus neutralisation assay (Graham *et al.,* 2003)

Experimental studies have shown that neutralising antibodies can first be detected 10–16 days post-infection (Graham *et al.,* 2003), and virus neutralisation (VN) assays can be used as a diagnostic tool for the detection of SAV antibodies. VN assays are based on the presence or absence of detectable virus growth in cultured cells following incubation with serum that may contain neutralising antibodies. In addition, the assay allows detection of virus in serum or plasma, if present.

CHSE-214 cells are grown as described in Section 4.3.1.2.1 Cell culture. A suspension of trypsinised cells, diluted 1/3 in growth medium (10% FBS) is prepared for the VN assay.

i) 1/20 and 1/40 dilutions of each test serum are prepared in maintenance medium (2% FBS), and transferred to two duplicate wells (15 µl per well) on a flat-bottomed tissue culture grade microtitre plate. An equal volume of virus (100 $TCID_{50}$ [median tissue culture infective dose]) is added and are the plate is incubated for 2 hours at room temperature.

ii) 70 µl of maintenance medium, and 50 µl of the CHSE-214 cell suspension is added to each well, and the plates are incubated for 3 days at 15°C.

iii) The cell monolayer is then fixed and stained as described in Section 4.3.1.2.1, step iv Antibody-based verification of SAV growth in cell culture, or using the following procedure: monolayers of CHSE-214 cells are fixed for 30 minutes at room temperature in 10% neutral buffered formalin. Following two washes with 0.01 M PBS, an MAb against SAV is added to the monolayers in an appropriate dilution. Bound MAb is visualised using a labelled streptavidin–biotin system according to the manufacturer's instructions.

iv) VN titres (ND_{50}) are then calculated according to the method of Karber (1931), with titres ≥ 1:20 being considered positive. Both serum controls (without virus added) and a virus control (without serum added) must always be included in the assay, to ensure valid results.

5. Rating of tests against purpose of use

As an example, the methods currently available for targeted surveillance and diagnosis of infection with SAV are listed in Table 5.1. The designations used in the Table indicate: a = the method is the recommended method for reasons of availability, utility, and diagnostic specificity and sensitivity; b = the method is a standard method with good diagnostic sensitivity and specificity; c = the method has application in some situations, but cost, accuracy, or other factors severely limits its application; and d = the method is presently not recommended for this purpose. These are somewhat subjective as suitability involves issues of reliability, sensitivity, specificity and utility. Although not all of the tests listed as category a or b have undergone formal standardisation and validation, their routine nature and the fact that they have been used widely without dubious results, makes them acceptable.

Table 5.1. *Methods for targeted surveillance and diagnosis*

Method	Targeted surveillance			Presumptive diagnosis	Confirmatory diagnosis
	Fry	Juveniles	Adults		
Gross signs	d	d	d	c	d
Histopathology	c	c	c	a	a
Immunohistochemistry	d	d	d	b	b
Isolation in cell culture	d	d	d	c	c
Virus neutralisation assay	d	c	c	a	b
Real-time RT-PCR	b	a	a	a	a
RT-PCR with sequencing	d	d	d	d	a

RT-PCR = Reverse transcriptase polymerase chain reaction.

EU comment

The EU believes that there is a lack of published data regarding the presumed high diagnostic sensitivity and specificity of any tests including real-time PCR (as indicated by the "a" assigned to this test in Table 5.1). The EU would like to draw the OIE's attention to a draft proceeding paper to be published soon in Preventative Veterinary Medicine which suggests that the sensitivity of real-time RT-PCR may be low (39% in this study). While this may be a consequence of the use of kidney tissue only there are to date no other published diagnostic sensitivity for any RT-PCR tests sufficient to support this high confidence of the test. This categorisation appears to be based on laboratory analytical sensitivities rather than diagnostic sensitivity.

6. Test(s) recommended for targeted surveillance to declare freedom from infection with SAV

The recommended test to be used in surveillance of susceptible fish populations for declaration of freedom fom SAV is real-time RT-PCR as described in Section 4.3.1.2.2 in this chapter.

7. Corroborative diagnostic criteria

7.1. Definition of suspect case

A suspected case of infection with SAV is defined as:

i) Detection of SAV

or

ii) Detection of antibodies against SAV

or

iii) Detection of histopathological changes consistent with the disease

If epidemiological information of infectious contact with suspected or confirmed case(s) appear.

7.2. Definition of confirmed case

Detection of SAV or detection of antibodies against SAV and detection of histopathological changes consistent with the disease.

8. References

ALDRIN M., STORVIK B., FRIGESSI A., VILJUGREIN H. & JANSEN P.A. (2010). A stochastic model for the assessment of the transmission pathways of heart and skeleton muscle inflammation, pancreas disease and infectious salmon anaemia in marine fish farms in Norway. *Prev. Vet. Med.*, **93**, 51–61.

ANDERSEN L., BRATLAND A., HODNELAND K. & NYLUND A. (2007). Tissue tropism of salmonid aplphaviruses (subtypes SAV1 and SAV3) in experimentally challenged Atlantic salmon (*Salmon salar* L.). *Arch. Virol.,* **152**, 1871–1883.

ANDERSEN L., HODNELAND H. & NYLUND A. (2010). No influence of oxygen levels on pathogenesis and virus shedding in Salmonid alphavirus (SAV)-challenged Atlantic salmon (*Salmon salar* L.). *Virol. J*., **7**, 198.

BANG JENSEN B., KRISTOFFERSEN A.B., MYR C. & BRUN E. (2012). Cohort study of effect of vaccination on pancreas disease in Norwegian salmon aquaculture. *Dis. Aquat. Org.*, **102**, 23–31.

BOUCHER P., RAYNARD R.S., HOUGHTON G. & BAUDIN LAURENCIN F. (1995). Comparative experimental transmission of pancreas disease in Atlantic salmon, rainbow trout and brown trout. *Dis. Aquat. Org.*, **22**, 19–24.

BRATLAND A. & NYLUND A. (2009). Studies on the possibility of vertical transmission of Norwegian salmonid Alphavirus in production of Atlantic salmon in Norway. *J. Aquat. Anim. Health*, **21**, 73–78.

CHRISTIE K.E., GRAHAM D.A., MCLOUGHLIN M. F., VILLOING S., TODD D. & KNAPPSKOG D. (2007). Experimental infection of Atlantic salmon *Salmo salar* pre-smolts by i.p. injection of new Irish and Norwegian salmonid alphavirus (SAV) isolates: a comparative study. *Dis. Aquat. Org.*, **75**, 13–22.

FRINGUELLY E., ROWLEY H.M., WILSON J.C., HUNTER R., RODGER H. & GRAHAM D.A. (2008). Phylogenetic analyses and molecular epidemiology of European salmonid alphaviruses (SAV) based on partial E2 and nsP3 gene nucleotide sequences. *J. Fish Dis*., **31**, 811–823.

EU comment

The correct spelling of the first author's name is as follows: "Fringuelli".

GRAHAM D.A., BROWN A., SAVAGE P. & FROST P. (2012). Detection of salmon pancreas disease in the faeces and mucus of Atlantic salmon *Salmon salar* by real-time RT-PCR and cell culture following experimental challenge. *J. Fish Dis*., **35**, 949–951.

GRAHAM D.A., CHERRY K., WILSON C.J. & ROWLEY H.M. (2007a). Susceptibility of salmonid alphavirus to a range of chemical disinfectants. *J. Fish Dis*., **30**, 269–277.

GRAHAM D.A., FROST P., MCLAUGHLIN K., ROWLEY H.M., GABESTAD I., GORDON A. & MCLOUGHLIN M.F. (2011). A comparative study of marine salmonid alphavirus subtypes 1–6 using an experimental cohabitation challenge model. *J. Fish Dis*., **34**, 273–286.

GRAHAM D.A., JEWHURST H.L., MCLOUGHLIN M.F., BRANSON E.J., MCKENZIE K., ROWLEY H.M. & TODD D. (2007b). A prospective longitudinal serological, virological and histopathological study of an outbreak of sleeping disease in farmed rainbow trout (*Onchorrhynchus mykiss*). *Dis. Aquat. Org.*, **74**, 191–197.

GRAHAM D.A., JEWHURST V.A., ROWLEY H.M., MCLOUGHLIN M.F.,RODGER H. & TODD D. (2005). Longitudinal serological syrveys of Atlantic salmon, *Salmo salar* L., using a rapid immunoperoxidase-based neutralization assay for salmonid alphavirus. *J. Fish Dis*., **28**, 373–379.

GRAHAM D.A., JEWHURST V.A., ROWLEY H.M., MCLOUGHLIN M.F. & TODD D. (2003). A rapid immunoperoxidase-basd neutralization assay for salmonid alphavirus used for a serological survey in Northern Ireland. *J. Fish Dis*., **26**, 407–413.

GRAHAM D.A., ROWLEY H.M. & FROST P. (2013b). Cross-neutralization studies with salmonid alphavirus subtype 1–6 strains: results with sera from experimental studies and natural infections. *J. Fish Dis*., published online doi: 10.1111/jfd.12167

GRAHAM D.A., STAPLES V., WILSON C.J., JEWHURST H., CHERRY K., GORDON A. & ROWLEY H.M. (2007c). Biophysical properties of salmonid alphaviruses: influences of temperature and pH on virus survival. *J. Fish Dis*., **30**, 533–543.

GRAHAM D.A., WILSON C., JEWHURST H. & ROWLEY H. (2008). Cultural characteristics of salmonid alphaviruses – influences of cell line and temperature. *J. Fish Dis*., **31**, 859–868.

HERATH T., COSTA J., THOMPSON K., ADAMS A. & RICHARDS R. (2009). Alternative cell line for the isolation of salmonid alphavirus-1. *Icelandic Agricultural Sci.,* **22**, 19–27.

HODNELAND K. & ENDRESEN C. (2006). Sensitive and specific detection of salmonid alphavirus using real-time PCR (TaqMan). *Journal of Virological Methods* **131**, 184–192.

JANSEN M.D., BANG JENSEN B. & BRUN E. (YEAR). Clinical manifestations of pancreas disease (PD) outbreaks in Norwegian marine salmon farming – variations due to salmonid alphavirus (SAV) subtype. Submitted to *J. Fish Dis*. November 2013.

JANSEN M.D., TAKSDAL T., WASMUTH M.A., GJERSET B., BRUN E., OLSEN A.B., BRECK O. & SANDBERG M. (2010a). Salmonid alphavirus (SAV) and pancreas disease (PD) in Atlantic salmon (*Salmo salar* L.) in freshwater and seawater sites in Norway from 2006 to 2008. *J. Fish Dis*., **33**, 391–402.

JANSEN M.D., WASWUTH M.A., OLSEN A.B., GJERSET B., MODAHL I., BRECK O., HALDORSEN R.N., HJELMELAND R., TAKSDAL T. (2010b). Pancreas disease (PD) in sea-reared Atlantic salmon, *Salmon salar* L., in Norway; a prospective, longitudinal study of disease development and agreement between diagnostic test results. *J. Fish Dis*., **33**, 723–736.

JEWHURST V.A., TODD D., ROWLEY H.M., WALKER I.W., WESTON J.H. MCLOUGHLIN M.F & GRAHAM D.A. (2004). Detection and antigenic characterization of salmonid alphavirus isolates fram sera obtained from farmed Atlantic salmon , *salmo salar* L., and farmed rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J. Fish Dis*., **27**, 143–149.

KERBART BOSCHER S., MCLOUGHLIN M., LE VEN A., CABON J., BAUD M. & CASTRIC J. (2006). Experimental transmission of sleeping disease in one-year-old rainbow trout *Onchorhynchus mykiss* (Walbaum), induced by sleeping disease virus. *J. Fish Dis*., **29**, 263–273.

KONGTORP R.T., STENE A., ANDREASSEN P.A., ASPEHAUG V., GRAHAM D.A., LYNGSTAD T.M., OLSEN A.B., OLSEN R.S., SANDBERG M., SANTI N., WALLACE C. & BRECK O. (2010). Lack of evidence for vertical transmission of SAV 3 using gametes of Atlantic salmon, *salmo salar* L., exposed by natural and experimental routes. *J. Fish Dis*., **33**, 879–888.

KRISTOFFERSEN A.B., VILJUGREIN H., KONGTORP R.T., BRUN E. & JANSEN P.A. (2009). Risk factors for pancreas disease (PD) outbreaks in farmed Atlantic salmon and rainbow trout in Norway during 2003–2007. *Prev. Vet. Med.*, **90**, 127–136.

MCLOUGHLIN M.F. & GRAHAM D.A. (2007). Alphavirus infections in salmonids- a review. *J. Fish Dis*., **30**, 511–531.

MCVICAR A.H. (1990). Infection as a primary cause of pancreas disease in farmed Atlantic salmon. *Bull. Eur. Assoc. Fish Pathol*., **10** (3), 84–87.

PETTERSON E., SANDBERG M. & SANTI N. (2009). Salmonid alphavirus associated with *Lepeoptheirus salmonis* (Copepoda: Caligidae) from Atlantic salmon, *Salmo salar* L. *J. Fish Dis*., **30**, 511–531.

RODGER H. & MITCHELL S. (2007). Epidemiological observations of pancreas disease of farmed Atlantic salmon, *Salmo salar* L., in Ireland. *J. Fish Dis*., **32**, 477–479.

RUANE N., GRAHAM D. & RODGER H. (2008). Pancreas disease in farmed salmon – health management and investigations at Irish farm sites 2005–2008. Marine environments and health series, No. 34, Marine Institute. Available at http://oar.marine.ie/handle/10793/267

EU comment

The correct title of the publication is as follows: "Marine Environment and Health Series".

SNOW M., BLACK I., MCINTOSH R., BARETTO E., WALLACE I.S. & BRUNO D.W. (2010). Detection of salmonid alphavirus RNA in wild marine fish: implications for the origin of salmon pancreas disease in aquaculture. *Dis. Aquat. Org.*, **91**, 177–188.

STENE A., BANG JENSEN B., KNUTSEN Ø., OLSEN A. & VILJUGREIN H. (2013). Seasonal increase in sea temperature triggers pancreas disease in Norwegian salmon farms. *J. Fish Dis*., published online, DOI: 10.1111/jfd.12165

STENE A., HELLEBØ A., VILJUGREIN H., SOLEVÅG S.E. & ASPEHAUG V. (YEAR). First detection of salmonid alphavirus in liquid fat fractions leaking from dead PD infected salmon. Manuscript submitted to *J. Fish Dis*., November 2013.

STORMOEN M., KRISTOFFERSEN A.B. & JANSEN P.A. (2013). Mortality related to pancreas disease in Norwegian farmed salmonid fish, *Salmo salar* L. and *Oncorhynchus mykiss* (Walbaum). *J. Fish Dis*., **36**, 639–645.

TAKSDAL T., OLSEN A.B., BJERKAAS I., HJORTAAS M.J.DANNEVIG B.H., GRAHAM D.A. & MCLOUGHLIN M.F. (2007). Pancreas disease in farmed Atlantic salmon, *Salmo salar* L., and rainbow trout, *Oncorhynchus mykiss* (Walbaum), in Norway. *J. Fish Dis*., **30**, 545–558.

TODD D., JEWHURST V.A., WELSH M.D., BORGHMANS B.J., WESTON J.H., ROWLEY H.M., MACKIE D.P. & MCLOUGHLIN M.F. (2001). Production and characterisation of monoclonal antibodies to salmon pancreas disease virus. *Dis. Aquat. Org.*, **46**, 101–108.

VILJUGREIN H., STAALSTRØM A., MOLVÆR J., URKE H.A. & JANSEN P.A. (2009). Integration of hydrodynamics into a statistical mode on the spread of pancreas disease (PD) in salmon farming. *Dis. Aquat. Org.*, **88**, 35–44.

WESTON J., VILLOING S., BRÉMONT M., CASTRIC J., PFEFFER M., JEWHURST V., MCLOUGHLIN M., RØDSETH O., CHRISTIE K.E., KOUMANS J. & TODD D. (2002). Comparison of two aquatic alphaviruses, salmon pancreas disease virus and sleeping disease virus, by using genome sequence analysis, monoclonal reactivity, and cross-infection. *J. Virol.,* **76**, 6155–6163.

*

* *