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**REPORT OF THE MEETING OF THE OIE  
AQUATIC ANIMAL HEALTH STANDARDS COMMISSION  
Paris, 25 September–2 October 2019**

**EU comment**

**The EU would like to commend the OIE Aquatic Animal Health Standards Commission for its work and for having taken into consideration EU comments on the Aquatic Code and Manual submitted previously.**

**A number of general comments on this report of the September 2019 meeting of the Aquatic Animals Commission are inserted in the text below, while specific comments are inserted in the text of the respective annexes.**

**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 ad hoc groups for future work on the Aquatic Code and Manual.**

The OIE Aquatic Animal Health Standards Commission (Aquatic Animals Commission) met at OIE Headquarters in Paris from 25 September to 2 October 2019. The list of participants is presented as [Annex 1](#).

The Aquatic Animals Commission thanked the following OIE Members for providing written comments on draft texts for the OIE *Aquatic Animal Health Code* (hereinafter referred to as the *Aquatic Code*) and OIE *Manual of Diagnostic Tests for Aquatic Animals* (hereinafter referred to as the *Aquatic Manual*) circulated after the Commission's February 2019 meeting: Australia, Canada, Chinese Taipei, China (People's Rep. of), Japan, New Zealand, Norway, Singapore, Switzerland, Thailand, the United States of America (the USA) and the Member States of the European Union (the EU).

The Commission reviewed Member comments and amended relevant chapters of the *Aquatic Code* and the *Aquatic Manual* where appropriate. The amendments are shown in the usual manner by 'double underline' and '~~strike through~~' and are presented in the Annexes to this report. In Annexes, amendments proposed at this meeting are highlighted with a coloured background in order to distinguish them from those proposed previously.

The Commission considered all Member comments that were submitted on time and supported by a rationale. However, the Commission was not able to draft a detailed explanation of the reasons for accepting or not each of the proposals received and focused its explanations on the most significant issues.

The Commission encourages Members to refer to previous reports when preparing comments on longstanding issues. It also draws the attention of Members to the reports of *ad hoc* Groups, which include important information, and encourages Members to review these reports together with the report of the Commission, where relevant. These reports are readily available on the [OIE Website](#).

The table below summarises the texts as presented in the Annexes. Members should note that texts in [Annexes 3 to 13](#) are presented for Member comments and [Annexes 14 to 17](#) are presented for Members' information.

Comments on [Annexes 3 to 13](#) of this report must reach OIE Headquarters by the 13 January 2020 to be considered at the February 2020 meeting of the Commission.

All comments should be sent to the OIE Standards Department at: [standards.dept@oie.int](mailto:standards.dept@oie.int).

Comments should be submitted as Word files rather than pdf files because pdf files are difficult to incorporate into the Commission's working documents.

Comments should be submitted as specific proposed text changes, supported by a structured rationale or by published scientific references. Proposed deletions should be indicated in '~~strikethrough~~' and proposed additions with 'double underline'. Members should not use the automatic 'track-changes' function provided by Word processing software as such changes are lost in the process of collating Members' submissions into the Commission's working documents. Members are also requested not to reproduce the full text of a chapter as this makes it easy to miss comments while preparing the working documents.

The Commission strongly encourages Members to participate in the development of the OIE's international standards by submitting comments on this report and participate in the process of adoption at the General Session.

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### 1. WELCOME FROM THE DEPUTY DIRECTOR GENERAL

Dr Matthew Stone, OIE Deputy Director General for International Standards and Science, welcomed the members of the Aquatic Animals Commission and thanked them, their institutions and their governments for making their expertise and time available to support the OIE's work.

Dr Stone provided the Commission with a brief overview of the development of the draft 7<sup>th</sup> OIE Strategic Plan, noting its focus on scientific expertise and the use of multidisciplinary evidence in standard setting and capacity building; ensuring the OIE is a good partner, and targets collaborations for impact; improving monitoring and evaluation to demonstrate performance across OIE's strategies, programmes and projects; and the development of internal data management, stewardship and governance practices that support the ongoing digital transformation of the OIE. He also provided a brief update on the culmination of the design phase of the OIE Observatory project; the OIE-WAHIS development project; and the ongoing work on the OIE Reference Centre system.

Dr Stone noted that the OIE's continuous improvement approach to ensuring good coordination across all the Specialist Commissions through the internal mechanism of the Common Secretariat is maturing and realising clear benefits. The recent focus had been on identifying and supporting discussions between Commissions on common issues. He finished his opening remarks by reassuring members that the OIE's performance management system for Specialist Commissions was providing very useful feedback, and all parties now appreciated the process was important to optimise the performance of the elected Commissions and their partnership with the OIE Secretariat.

### 2. MEETING WITH THE DIRECTOR GENERAL

Dr Monique Eloit, OIE Director General, met with the Aquatic Animals Commission and acknowledged the significant work being undertaken by this Commission. Dr Eloit thanked the members and the Secretariat for their work and highlighted the Commission's active participation in the OIE Global Conference on Aquatic Animal Health held in Chile in April 2019 that resulted in a very successful conference.

### 3. ADOPTION OF THE AGENDA

The provisional agenda circulated prior to the meeting was discussed, updated, and agreed. The adopted agenda of the meeting is presented as [Annex 2](#).

#### 4. ONE YEAR PROJECT ON ANTIMICROBIAL RESISTANCE (AMR) IN AQUACULTURE

Dr Dante Matéo who recently joined the OIE Department of Antimicrobial Resistance & Veterinary Products met with the Commission to inform them that he will spend the next year developing a work plan on how the OIE could better address the prudent use of antimicrobial agents and issues of antimicrobial resistance in aquaculture. This project was initiated following the global conference in Chile 2019 and is funded by the Norwegian Agency for Development and Cooperation (NORAD). He explained that the plan will ensure that the OIE has up to date and relevant tools to support Members in this area. The work plan, and notably targeted strategies and training events will, be aligned with the OIE Strategy on Antimicrobial Resistance and the Prudent Use of Antimicrobials. The Commission offered to provide support and advice and requested that the work plan be achievable and realistic considering the impacts and feasibility of any proposed measures.

Dr Dante Matéo may be contacted at [d.mateo@oie.int](mailto:d.mateo@oie.int) for any further information.

#### 5. WORK PLAN OF THE AQUATIC ANIMAL HEALTH STANDARDS COMMISSION

The Commission reviewed and updated its work plan taking into account previous decisions, Members' comments, and recommendations from the OIE Global Aquatic Animal Health Conference held in April 2019.

The Commission proposed the addition of two new items: 1. 'development of a new standard for ornamental aquatic animals' and 2. 'development of a new standard to address trade in genetic products', noting that these are well-defined, longstanding issues with significant trade impacts. The Commission also highlighted that both issues were included in the recommendations from the OIE Global Aquatic Conference on Aquatic Animal Health.

The Commission noted that the development of an OIE Strategy on Aquatic Animal Health would likely have implications for its workplan that would need to be considered at a later time.

The revised work plan is presented as [Annex 17](#) for Member comment.

#### EU comment

**The EU supports the proposed work plan. We have included comments on what we consider future priorities.**

#### 6. TEXTS PRESENTED FOR MEMBER COMMENTS

The Commission considered all comments and made amendments to draft texts to improve clarity and readability, where relevant. Where amendments were of an editorial nature, no explanatory text has been provided in this report. In addition, the Commission did not consider comments where a rationale had not been provided or that were difficult to interpret. The Commission wished to note that not all alternative drafting proposed by Members to improve clarity were accepted; in these cases, it considered the existing text and not improved by the proposed changes.

#### OIE AQUATIC ANIMAL HEALTH CODE

##### 6.1. New draft chapter on Biosecurity for Aquaculture Establishments (Chapter 4.X.)

Comments were received from Australia, Canada, China (People's Rep. of), Japan, New Zealand, Norway, USA and the EU.

The new draft chapter on Biosecurity for Aquaculture Establishments (Chapter 4.X.) was circulated for the second time for Member comments in the Aquatic Animals Commission's February 2019 report. This is the second new chapter to be developed for Section 4 Disease Prevention and Control of the *Aquatic Code* following the adoption of Chapter 4.3. Disinfection of aquaculture establishments and equipment in 2017.

##### General Comments

The Commission agreed with the proposal to change 'risk' to 'likelihood' where appropriate throughout the chapter to reflect the intended meaning. Several editorial amendments throughout the chapter for improved readability were also made.

##### Article 4.X.1. Purpose

The Commission considered a comment to move away from a species-specific approach towards a pathway management approach. The Commission noted that the chapter currently does adopt a pathway approach to biosecurity, combined with risk assessments for identified hazards. Hazards can be single pathogens or groups of pathogens. This approach was considered to be appropriate for the development of a biosecurity plan.

UNOFFICIAL VERSION

### **Article 4.X.3. Introduction**

In paragraph 1, the Commission agreed with a comment to move the last sentence regarding the importance of biosecurity at the country, zone or compartment level earlier in the paragraph to clarify the context in which biosecurity at the establishment level operated.

In paragraph 2, the Commission agreed with a comment to change ‘aquaculture staff’ to ‘aquaculture establishment staff’. This addition clarified and emphasised that individuals who are developing and implementing the biosecurity plan for an establishment are not governmental employees, but rather staff working at that specific establishment.

In paragraph 2, the Commission did not agree with a comment that ‘veterinary paraprofessionals’ should be included in the text and that the term ‘Veterinary professionals’ should be included in the Glossary. The Commission noted that the responsibilities of veterinary paraprofessionals are currently incorporated within the Glossary definition for ‘Aquatic Animal Health Professionals’.

In paragraph 3, the Commission agreed that improved aquatic animal welfare is also an outcome achieved through implementation of effective biosecurity and included a reference to welfare to address this point.

In paragraph 3, the Commission agreed with a comment to replace ‘treatments’ with ‘veterinary medicinal products (including antimicrobial agents)’ agreeing that this was more precise.

### **Article 4.X.4. General principles**

The Commission agreed with a comment to delete point 1 noting that it was already addressed in other points.

The Commission also agreed to add a new point 5 regarding appropriate documentation and record keeping.

### **Article 4.X.5bis Area management**

The Commission accepted a comment to develop a new article on ‘Area Management’ to provide recommendations for situations where there are epidemiological links between aquaculture establishments and biosecurity measures cannot be effectively applied by a single establishment. In these circumstances, a consistent set of biosecurity measures should be applied by all of the aquaculture establishments considered to be epidemiologically linked.

### **Article 4.X.6. Transmission pathways and mitigation measures**

The Commission amended the title from ‘Transmission pathways, and associated risks and mitigation measures’ to ‘Transmission pathways and mitigation measures’ to more accurately reflect the content of this article.

For point 1 regarding transmission risks associated with aquatic animals, the Commission agreed with a comment to add a new point (i) providing guidance on fallowing of aquaculture establishments.

The Commission reminded Members that as agreed in the planned restructure of Section 4, Chapter 4.6. Fallowing in aquaculture, will be deleted from the *Aquatic Code* once the new chapter on Biosecurity for aquaculture establishments is adopted. The Commission also noted that any relevant information in Chapter 4.6. not included in the new biosecurity chapter will be considered for inclusion in the new chapter on disease outbreak management (see [Annex 17](#) work plan).

For point 3 regarding transmission risks associated with water, the Commission agreed with a comment to add factors such as distance and water currents to point (c). The Commission also accepted to include the risks of flooding and defective infrastructure addressed in a new point (d).

For point 4 regarding transmission risks associated with feed, the Commission agreed with a comment to highlight the risks associated with inappropriate storage and management of feed on site. Instead of adding a new point, point (c) was amended to include measures to mitigate the risk. The Commission did not agree with a comment to include disinfection of feed, as it considered this was addressed in point (a), which covers processing to inactivate pathogenic agents.

For point 5 regarding transmission risks associated with fomites, the Commission agreed with a comment to improve clarity and revised the text accordingly. The Commission also included a new point (d) to ensure that the text included concepts for both movements of fomites between aquaculture establishments and for movements between production units within an aquaculture establishment.

For point 6 regarding transmission risks associated with vectors, the Commission amended the text to improve clarity and provide further guidance on possible mitigation measures, including control of access by personnel.

#### **Article 4.X.7. Risk Analysis**

The Commission did not agree with a comment requesting that the matrix be included in Chapter 2.1. Import risk analysis, noting that the matrix was developed specifically for this chapter and that interpretation of risk estimates are specific for biosecurity for aquaculture establishments and are, therefore, only relevant to Chapter 4.X. In addition, the Commission confirmed that the principles of risk analysis described in Chapter 2.1. have been applied in this chapter.

The Commission agreed with a comment to revise the descriptors for consequence (Table 2) and added trade and economic impacts at the aquaculture establishment level throughout the table.

#### **Article 4.X.8. Biosecurity plan development**

For point 1 regarding development of a biosecurity plan, the Commission agreed with a comment to add text in points (f) and (g) to include examples of emergency procedures in the event of a biosecurity failure and documentation of essential contact information.

For point 2 regarding key components of a biosecurity plan, the Commission did not agree to clarify the term 'escapee' as they considered this term to be widely understood.

The revised Chapter 4.X. Biosecurity for Aquaculture Establishments is presented as [Annex 3 A](#) (clean text) and [Annex 3 B](#) (track changes), respectively for Member comments and is intended to be proposed for adoption at the 88th General Session in May 2020.

### **EU comment**

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

#### **6.2. Revised Article 1.3.3. of Chapter 1.3. Diseases listed by the OIE**

##### **Assessment of infection with Decapod iridescent virus-1 (DIV1) (previously named infection with shrimp haemocyte iridescent virus)**

Comments were received from Australia, Canada, China (People's Rep. of), Chinese Taipei, New Zealand, Thailand and the USA.

The Commission, at its February 2019 meeting, assessed infection with shrimp haemocyte iridescent virus (SHIV) against the criteria for listing aquatic animal diseases in Article 1.2.2. and agreed that infection with SHIV meets the OIE criteria for listing and should be added to Article 1.3.3. diseases of crustaceans listed by the OIE.

The Commission reviewed Member comments received regarding the assessment.

The Commission agreed with comments that the name of the disease should be changed to 'Infection with Decapod iridescent virus-1 (DIV1)' in accordance with the classification of the pathogenic agent in the database of the International Committee of Taxonomy of Viruses (ICTV) (<https://talk.ictvonline.org/taxonomy/>).

Regarding Criterion 2: 'At least one country may demonstrate country or zone freedom from the disease in susceptible aquatic animals': The Commission did not agree with a comment that the conclusion that this criterion was met was based on a lack of surveillance evidence. The Commission noted that the disease has been listed by the Regional Advisory Group on Aquatic Animal Health in the 'Quarterly Aquatic Animal Disease report' (Asia and Pacific Region) (<https://enaca.org/?id=8>) since 2017. The Commission also noted



that if the disease had spread widely, it would be likely to have resulted in clinical disease that would lead to reporting. The disease has still only been reported in China (People's Rep. of).

The Commission maintained its conclusion from February 2019 that criterion 2 is met.

Regarding Criterion 4(b): ‘The disease has been shown to affect the health of cultured aquatic animals at the level of a country or a zone resulting in significant consequences, e.g. production losses, morbidity or mortality at a zone or country level’: the Commission did not agree with a comment that the disease does not meet this criterion as the disease has been reported to cause severe mortalities in shrimp farms in China (People’s Rep. of).

The Commission reiterated its proposal to list infection with DIV1 in Article 1.3.3. of Chapter 1.3. Diseases listed by the OIE.

The Commission encouraged Members to investigate significant mortality and morbidity events in crustaceans and should infection with DIV1 be detected, to report this as an emerging disease in accordance with Article 1.1.4. of the *Aquatic Code*.

Given the impact of this new emerging disease and the need to provide information about the diagnosis and control of this pathogenic agent, the Commission has developed a technical disease card for infection DIV1, which will be uploaded onto the OIE official website by the end of November 2019.

The revised assessment for infection with DIV1 is presented as [Annex 14](#) for Member information in support of the proposed listing.

The revised Article 1.3.3. is presented as [Annex 4](#) for Member comments and intended to be proposed for adoption at the 88th General Session in May 2020.

**EU comment :**

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

**6.3. Model Article 10.X.13. Importation of disinfected eggs for aquaculture from a country, zone or compartment not declared free from infection with pathogenic agent X**

Comments were received from Canada, the USA and the EU.

In response to a comment, the Commission amended point 1(b) by adding ‘including testing of’ before ‘ovarian fluid and milt’ to clarify that in this context material for diagnostic testing is not restricted to ovarian fluid and milt but may also include tissues from the broodstock.

The Commission agreed to amend text in point 2(a) to clarify that the Competent Authority of the importing country should only request risk mitigation measures which are outlined in Chapter 4.4. (Recommendations for surface disinfection of salmonid eggs).

The revised Model Article 10.X.13. and Article 10.4.17. for Chapter 10.4. Infection with infectious salmon anaemia virus are presented as [Annex 5](#) for Member comments and are intended to be proposed for adoption at the 88th General Session in May 2020.

**EU comment:**

**The EU thanks the OIE and supports the proposed changes to this model article.**

**6.4. Article 10.10.2. of Chapter 10.10. Infection with viral haemorrhagic septicaemia virus**

The Aquatic Animals Commission reviewed the report of the *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases which had applied the criteria for listing species as susceptible to infection with a specific pathogenic agent in accordance with Chapter 1.5. of the *Aquatic Code* for infection with viral haemorrhagic septicaemia virus (VHSV) (see also [Annex 15](#)).

The Commission reviewed the individual species assessments and considered whether Article 1.5.9. ‘Listing susceptible species at a taxonomic ranking of Genus or higher’ should be applied to infection with VHSV. It was noted that there are numerous susceptible species within many taxonomic groups, including some diverse taxonomic groups with few species found susceptible to VHSV. The Commission acknowledged that the geographic distribution of multiple VHSV genotypes combined with the diversity of susceptible host species

across disparate taxonomic groups (including representatives of several classes of fishes) contributed significant complexity. In consideration of these issues, the Commission agreed that it would not be appropriate to apply the criteria in Article 1.5.9. 'Listing susceptible species at a taxonomic ranking of Genus or higher' to infection with VHSV.

The Commission agreed to amend the list of susceptible species in Article 10.10.2. in line with recommendations made by the *ad hoc* Group. It noted that of the 12 species currently listed in Article 10.10.2. as susceptible to infection with VHSV, two species, haddock (*Gadus aeglefinus*) and rockling (*Onos mustelus*), did not meet the criteria for listing as a susceptible species and would be deleted from Article 10.10.2. In addition, 61 new susceptible species met the criteria for listing as a susceptible species and would be added to Article 10.10.2.

The Commission recognised that systematic analysis of the available scientific literature using the criteria in Chapter 1.5. for infection with VHSV has resulted in a significant expansion of the number of susceptible species from 12 to 71. The Commission considered this demonstrated the importance of applying these criteria to ensure an appropriate scope of application of sanitary standards in the *Aquatic Code*.

The Commission noted that the considerable inconsistency in the lists of susceptible species for infection with VHSV between the Chapter 10.10. of the *Aquatic Code* and Chapter 2.3.10. of the *Aquatic Manual* will be addressed by the application of the recommendations of the *ad hoc* Group to the relevant articles of these two chapters (see also Item 6.8.4.).

The report of the *ad hoc* Group on susceptibility of fish species to infection with OIE listed diseases (Infection with VHSV) is presented as [Annex 15](#) for Member information.

The revised Article 10.10.2. is presented as [Annex 6](#) for Member comments and is intended to be proposed for adoption at the 88th General Session in May 2020.

**EU comment:**

**The EU thanks the OIE and supports the proposed changes to this article.**

**6.5. Article 10.9.2. of Chapter 10.9. Infection with spring viraemia of carp virus**

The Commission reminded Members that the updated list of susceptible species in Article 10.9.2. Infection with spring viraemia of carp virus (SVCV) was adopted at the 87<sup>th</sup> General Session in May 2019.

Based on previous assessments undertaken by the *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases, zebrafish (*Danio rerio*) did not meet the criteria for listing as susceptible species to infection with SVCV. The rationale for this conclusion was that the conditions of experimental challenge did not meet the requirements of Article 1.5.7. concerning evidence of a route of transmission that is consistent with natural pathways for the infection. The relevant study (Sanders *et al.*, 2003) had used temperatures outside of the natural range of zebrafish and the authors had acknowledged that this had likely resulted in immunosuppression.

Since adoption of the revised Article 10.9.2, the Commission had become aware of other scientific publications which had reported on the susceptibility of zebrafish to infection with SVCV (Lopez-Munoz *et al.*, 2010; Martinez-Lopez *et al.*, 2014; Medina-Gali *et al.*, 2018a; Medina-Gali *et al.*, 2018b; Bello-Perez *et al.*, 2019). In light of this information, the Commission requested that the *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases review its previous assessments for zebrafish against the criteria in Chapter 1.5. taking into consideration these scientific studies.

The *ad hoc* Group reviewed its previous assessments taking into account the additional publications and agreed that zebrafish did meet the criteria for listing as a susceptible species and should be included in Article 10.9.2.

The Commission reviewed the *ad hoc* Group's revised assessments and agreed with the *ad hoc* Group's revised assessment that zebrafish be proposed for inclusion in Article 10.9.2. of the *Aquatic Code*.

The *ad hoc* Group's revised assessments of zebrafish against the criteria in Chapter 1.5. is presented as [Annex 16](#).

The revised Article 10.9.2. of Chapter 10.9. Infection with spring viraemia of carp virus is presented as [Annex 7](#) for Member comments and will be proposed for adoption at the 88th General Session in May 2020.

**EU comment:**

**The EU thanks the OIE and supports the proposed changes to this article.**

References:

BELLO-PEREZ, M., MEDINA-GALI, R., COLL, J. & PEREZ, L. (2019). Viral interference between infectious pancreatic necrosis virus and spring viremia of carp virus in zebrafish. *Aquaculture*, **500**, 370-377.

LÓPEZ-MUÑOZ, A., ROCA, F. J., SEPULCRE, M. P., MESEGUER, J. & MULERO, V. (2010). Zebrafish larvae are unable to mount a protective antiviral response against waterborne infection by spring viremia of carp virus. *Developmental and Comparative Immunology*, **34**, 546-552.

MARTINEZ-LOPEZ, A., GARCIA-VALTANEN, P., ORTEGA-VILLAIZAN, M., CHICO, V., GOMEZ-CASADO, E., COLL, J. M. & ESTEPA, A. (2014). VHSV G glycoprotein major determinants implicated in triggering the host type I IFN antiviral response as DNA vaccine molecular adjuvants. *Vaccine*, **32**, 6012-6019.

MEDINA-GALI, R. M., ORTEGA-VILLAIZAN, M., MERCADO, L., NOVOA, J. C. & PEREZ, L. (2018a). Beta-glucan enhances the response to SVCV infection in zebrafish. *Developmental and Comparative Immunology*, **84**, 307-314.

MEDINA-GALI, R. M., BELLÓ-PÉREZA, M., MARTÍNEZ-LÓPEZ, A., FALCÓ, A., ORTEGA-VILLAIZANA, M. M., ENCINARA, J. A., NOVOA, B., COLL, J. & PEREZ, L. (2018b). Chromatin immunoprecipitation and high throughput sequencing of SVCV infected zebrafish reveals novel epigenetic histone methylation patterns involved in antiviral immune response. *Fish and Shellfish Immunology*, **82**, 514-521.

SANDERS, G. E., BATTS, W. N. & WINTON, J. R. (2003). Susceptibility of Zebrafish (*Danio rerio*) to a Model Pathogen, Spring Viremia of Carp Virus. *Comparative Medicine*, **53**(5), 514-521.

**6.6. Discussion paper on approaches for determining periods required to demonstrate disease freedom**

A discussion paper on approaches for determining periods required to demonstrate disease freedom, developed by the Commission, had been circulated in the Commission's September 2018 report for Member comments.

The Commission, at its February 2019 meeting acknowledged the extensive number of comments submitted by Members and appreciated the quality of comments and the high level of engagement on this topic.

Since February 2019, the Commission worked on the development of a revised paper, taking into account Member comments. Version 2 of the discussion paper includes summaries of the responses provided by Member Countries on Version 1, and revised recommendations throughout the paper. These recommendations form the basis of the proposed approach by the Aquatic Animals Commission to the revision of Aquatic Code articles relevant to declaration of freedom.

Following this consultation with Members, the Aquatic Animals Commission proposes to retain the broad framework of requirements in the Aquatic Code for declaration of freedom for reasons of simplicity and practicality and to provide confidence among Members in the strength of self-declarations of freedom. However, the revised approaches recommended in the discussion paper will also provide additional flexibility (e.g. to use secondary sources of data) and improve rigour in determining the required surveillance periods specified in disease-specific chapters of the *Aquatic Code*. Chapter 1.4. Aquatic Animal Health Surveillance will be revised substantially to better support the approaches recommended in the discussion paper.

For the convenience of Members, the summary of the discussion paper highlights the key recommended approaches that are suggested throughout the paper.

The revised document 'Approaches for determining periods required to demonstrate disease freedom' is presented as [Annex 8](#) for Member comments. The Commission encouraged Members to review this document and provide comments, particularly on the recommended approaches that are provided in each section of the discussion paper.

**EU comment:**

**The EU thanks the OIE and supports the proposed changes by the discussion paper.**

#### **6.7. Revised Glossary definition for ‘Aquatic animal waste’**

In response to a comment and given that the term ‘aquatic animal waste’ is used extensively in the new draft chapter on Biosecurity for Aquaculture Establishments (Chapter 4.X.) as well as in Chapter 4.7. Handling, disposal and treatment of aquatic animal waste, the Commission agreed to include a new definition for ‘aquatic animal waste’ in the Glossary (see also Item 6.1.).

Because of the inclusion of a new definition for ‘aquatic animal waste’ in the Glossary, the Commission also agreed that once the new definition is adopted, the current definition for ‘aquatic animal waste’ in Article 4.7.3. will be deleted.

The revised Glossary definition for ‘aquatic animal waste’ is presented in [Annex 9](#) for Member comment.

**EU comment:**

**The EU thanks the OIE and supports the proposed changes to the glossary definition of aquatic animal waste.**

OIE MANUAL OF DIAGNOSTICS TESTS FOR AQUATIC ANIMALS

#### **6.8. Reformatting of *Aquatic Manual* chapters into the new template**

Comments were received from Canada, China (People’s Rep. of), Japan, New Zealand, Singapore, Thailand and the EU.

The Aquatic Animals Commission has commenced the process of progressively reformatting the disease-specific chapters of the *Aquatic Manual* into a new template that had been developed by the *ad hoc* Group on the *Aquatic Manual*. The template had been provided to Members previously in the February 2018 report of the Commission.

Reformatting of *Aquatic Manual* chapters into the new template – together with revisions to update the content – involves substantial changes to the currently adopted text. The Commission considered how best to indicate the changes proposed in the revised chapters compared with the currently adopted versions. The Commission agreed that revisions indicated in the usual style (i.e. strikethrough for deletions and double underline for additions) would be of limited use to Members due to the extensive nature of the changes. The Commission agreed that for these major revisions of *Aquatic Manual* chapters (when reformatted into the new template) only clean versions of the chapters would be provided in the report of the Commission. Subsequent changes made to these initial revisions following Member comments would be indicated in the usual style and highlighted in yellow. Each draft chapter will also include a note specifying that marked edits are indicated against the initial revision of the revised chapter rather than the currently adopted text.

A software generated document that compares the adopted version of a chapter and the proposed new text will be created. This comparison document will not be included in the Commission's report but will be available on request from the OIE Standards Dept ([standards.dept@oie.int](mailto:standards.dept@oie.int)). The first two chapters to which the new template has been applied include Infection with spring viraemia of carp virus (revised chapter) and Infection with *Batrachochytrium salamandrivorans* (new chapter). Both chapters were provided in the February 2019 report of the Commission for Member comments.

#### 6.8.1. Infection with spring viraemia of carp virus (Chapter 2.3.9.)

Two versions of the chapter were annexed to the February 2019 report: Annex 21A (a version showing changes against the currently adopted version) and Annex 21B (a clean version of the chapter). As described above, the Commission took the decision to only annex the “clean” version of the chapter, and to show amended text (highlighted in grey) from this “clean” version that was sent for comment in February 2019.

In response to a comment proposing some terms for inclusion in the glossary, the Commission noted that there is currently no glossary in the *Aquatic Manual*.

The Commission included zebrafish (*Danio rerio*) in Section 2.2.1. *Susceptible host species*, consistent with its decision to include this species in Article 10.9.2. of the *Aquatic Code* (see Item 2.7.). As a result, the species was deleted from Section 2.2.2. *Species with incomplete evidence for susceptibility*.

When applying the new template to this chapter, the Commission agreed to add a new Section 2.2.3. *Non-susceptible species* to the template as this was considered important information for Members. The Commission noted that the *ad hoc* Group had found largemouth bass (*Micropterus salmoides*), muskellunge (*Esox masquinongy*) and walleye (*Sander vitreus*) to be non-susceptible to infection with SVCV and decided include these species in the new Article 2.2.3.

The Commission did not agree with comments proposing the inclusion of further species in Section 2.2.1. *Susceptible host species* and Section 2.2.2. *Species with incomplete evidence for susceptibility* as these species had been found not to be susceptible to infection with SVCV by the *ad hoc* Group on susceptibility of fish species to infection with OIE listed diseases (<https://www.oie.int/en/standard-setting/specialists-commissions-working-ad-hoc-groups/ad-hoc-groups-reports/>).

In response to comments, the Commission removed text on vertical and horizontal transmission from Section 2.2.4 *Distribution of the pathogen in the host*, as the information is already included in Section 2.3.4. *Modes of transmission and life cycle*.

For clarity on the information being sought, the Commission agreed to amend the title of Section 2.2.5 from “Reservoirs of infection” to “Aquatic animal reservoirs of infection”. This change would be made to the chapter template.

In Section 2.3.1. *Mortality, morbidity and prevalence*, the sentence “Co-infections with koi herpesvirus or carp oedema virus can increase levels of mortality” was deleted as the Commission is not aware of any published information to substantiate the statement.

The Commission agreed to include in Section 2.3.5. *Environmental and management factors*, a sentence clarifying that a combination of the stressors of temperature and confinement, in conjunction with spawning, are key factors resulting in outbreaks of infection with SVCV.

In Section 2.4.1. *Vaccination*, the text on DNA vaccines was amended to better reflect the current situation.

In response to Member comments, the last paragraph in 3.1. Selection of populations and individual specimens was amended to clarify the use of the terms “group” and “lot”.

In Section 3.2. Selection of organs or tissues, the first sentence on which organs should be sampled from subclinically infected fish was reinstated, and the word “alevin” was replaced by the word “fry”. This latter amendment would be made as appropriate in other fish disease chapters as they are revised.

The Commission reworded the last paragraph in Section 3.3. Samples or tissues not suitable for pathogen detection for clarity. It also agreed to replace the text in Section 3.6. Pooling of samples with the standard text from the template as there is no evidence warrant any alternative recommendation.

Following review of comments and discussion with the Reference Laboratory experts, the Commission revised some of the scores given in Table 4.1 OIE recommended diagnostic methods and their level of validation for surveillance of healthy animals and investigation of clinically affected animals. The scores and text of Section 4 Diagnostic methods now corresponds with the recommendations given in Section 6 Corroborative diagnostic criteria. The changes to Table 4.1 are: cell or culture and conventional PCR<sup>1</sup> were given higher scores (“++”) in all three columns as they are suitable methods but may require further validation. *In-situ* hybridisation and LAMP<sup>2</sup> were deleted as they have not been well validated. IFAT<sup>3</sup> and immunohistochemistry were added.

The Commission agreed to split Section 4.6 *In-situ* hybridisation (and histoimmunochemistry) into two sections: Section 4.6. *In-situ* hybridisation and Section 4.7. Immunohistochemistry. This change would be made to the chapter template.

Edits presented in the document are those made against the revised “clean” version” provided for comment in February 2019 and are highlighted in grey. A software generated comparison document between the proposed new text and the adopted version of the chapter is available on request from the OIE Standards Dept ([standards.dept@oie.int](mailto:standards.dept@oie.int)).

The revised Chapter 2.3.9. Infection with spring viraemia of carp virus is presented as [Annex 10](#) for Member comments and is intended to be proposed for adoption at the 88th General Session in May 2020.

### **EU comment:**

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

#### **6.8.2. Infection with *Batrachochytrium salamandrivorans* (Chapter 2.1.3.)**

In the absence of an OIE Reference Laboratory for *Batrachochytrium salamandrivorans*, the chapter had been drafted by experts from a research institution. The Aquatic Animals Commission noted their appreciation for the contribution by the experts to draft the chapter. The Commission also reiterated that it would welcome applications from suitable laboratories to become a Reference Laboratory for *B. salamandrivorans* (Bsal).

In response to comments to alter the scope of the chapter, the Commission reiterated that the scope of the chapter follows exactly that of the scope of the chapter in the *Aquatic Code*. The comments were taken into account in Section 2.1.1. Aetiological agent.

A proposal to include a sentence that desiccation is fatal to all Bsal life stages in Section 2.1.3. *Survival and stability outside the host* was not accepted as the reference cited did not confirm such a statement.

Comments on Section 2.2.1. Susceptible host species will be addressed when the *ad hoc* Group on susceptibility of amphibian species to infection with OIE listed diseases is convened. Section 2.2.1. will remain under study until the assessments can be completed.

The Commission did not agree to add a sentence on an Asian species of wild frog to Section 2.2.6. *Vectors* because the wild frog species is likely to be susceptible species for infection with Bsal, albeit one that does not necessarily show clinical signs.

<sup>1</sup> PCR: polymerase chain reaction

<sup>2</sup> LAMP: loop-mediated isothermal amplification

<sup>3</sup> IFAT: indirect immunofluorescent antibody test

The Commission declined a request to add a specific reference to Section 2.3.1. Mortality, morbidity and prevalence as it would not add any new information. A proposal to mention heat treatment in the same section was also not accepted as this information is more appropriate in Section 2.4.7. *General husbandry*.

The Commission agreed to delete the sentence in Section 2.4.4. *Breeding resistant strains* and replace with a statement that there is no information available.

The Commission agreed to keep the text in Section 3.6. *Pooling of samples* noting that the information on pooling is confirmed by the cited reference.

In response to a comment and to be consistent with Section 6 *Corroborative diagnostic criteria*, the Commission considered that conventional PCR, amplicon sequencing, *in-situ* hybridisation, LAMP and immunohistochemistry should not be included in Table 4.1 *OIE recommended diagnostic methods and their level of validation for surveillance of healthy animals and investigation of clinically affected animals* due to insufficient information on their suitability. Recommendations for wet mounts, histopathology and lateral flow assay were removed from column C: *Confirmatory diagnosis of a suspect result from surveillance or presumptive diagnosis* as the text does not support their use for this purpose. Section 6 was amended accordingly.

The Commission did not agree with a request to add a sentence to the description of the real-time PCR stating that sensitivity of the test is lower for trade or field purposes, because the cited reference did not support this conclusion.

The Commission acknowledged a comment that the chapter includes a number of references to trademarked reagents and commercially available laboratory kits. The Commission will include in the instructions to experts a request to avoid registered or trademarked proprietary names.

Edits presented in [Annex 11](#) are those made against the draft version of this new chapter provided for comment in February 2019.

The revised new Chapter 2.1.3 Infection with *Batrachochytrium salamandrivorans* is presented as [Annex 11](#) for Member comments and is intended to be proposed for adoption at the 88<sup>th</sup> General Session in May 2020.

**EU comment:**

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

**6.8.3. Chapter 2.3.4. Infection with infectious haematopoietic necrosis virus**

The Commission reviewed Chapter 2.3.4. *Infection with infectious haematopoietic necrosis*, which had been updated by the OIE Reference Laboratory experts and reformatted using the new disease chapter template. The main amendments include updated lists of susceptible host species and species with incomplete evidence for susceptibility in accordance with the findings of the *ad hoc* Group on susceptibility of fish species to infection with OIE listed diseases (<https://www.oie.int/en/standard-setting/specialists-commissions-working-ad-hoc-groups/ad-hoc-groups-reports/>); updated protocols for the real-time and conventional PCR; and revised definitions of suspect and confirmed case in apparently healthy and clinically affected animals.

As described above at item 6.8, the Commission took the decision to only annex a clean version of the chapter. A software generated comparison document between the proposed new text and the adopted version of the chapter is available on request from the OIE Standards Dept ([standards.dept@oie.int](mailto:standards.dept@oie.int)).

The revised Chapter 2.3.4. Infection with infectious haematopoietic necrosis virus is presented as [Annex 12](#) for Member comments.

**EU comment:**

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



#### 6.8.4. Chapter 2.3.10. Infection with viral haemorrhagic septicaemia virus

The Commission reviewed a revised draft Chapter 2.3.10. *Infection with viral haemorrhagic septicaemia virus*, which had been updated by the OIE Reference Laboratory experts and reformatted using the new disease chapter template. The main amendments include an updated description of the genotypes; updated lists of susceptible host species and species with incomplete evidence for susceptibility in accordance with the findings of the *ad hoc* Group on susceptibility of fish species to infection with OIE listed diseases; revised description of cell culture for virus isolation, and the protocols for the real-time reverse-transcription and conventional PCR; and the definitions of suspect and confirmed case in apparently healthy and clinically affected animals.

As described above at item 6.8, the AAC took the decision to only annex a clean version of the chapter. A software generated comparison document between the proposed new text and the adopted version of the chapter is available on request from the OIE Standards Dept ([standards.dept@oie.int](mailto:standards.dept@oie.int)).

The revised Chapter 2.3.10. Infection with viral haemorrhagic septicaemia virus is presented as [Annex 13](#) for Member comment.

#### EU comment:

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

### 7. ITEMS FOR MEMBER INFORMATION

#### OIE AQUATIC ANIMAL HEALTH CODE

##### 7.1. Glossary definitions for ‘Competent Authority’, ‘Veterinary Authority’ and ‘Veterinary Services’. Information about work in the Terrestrial Code commission.

The OIE Secretariat provided the Commission with background information about the Terrestrial Animal Health Standards Commission’s decision to amend the definitions for Competent Authority, Veterinary Authority and Veterinary Services in the Glossary of the *Terrestrial Code*. The proposed amendments were circulated for Member comments in the Code Commission’s September 2018 report and comments received were considered by the *ad hoc* Group on Veterinary Services in July 2019.

The Aquatic Animals Commission was informed that the OIE Secretariat is seeking input from all Specialist Commissions on the proposed amended definitions in relation to the work of each Commission and any consequences of these amendments.

The Aquatic Animals Commission considered the proposed definitions and provided comments to OIE Headquarters.

##### 7.2. Acute hepatopancreatic necrosis disease (AHPND) (Chapter 9.1.)

The Aquatic Animals Commission considered published information of new non-*Vibrio parahaemolyticus* species that have been associated with acute hepatopancreatic necrosis disease (AHPND) to see determine if a revision of the scope of Article 9.1.1. was necessary. The Commission reviewed the available literature and agreed to seek further advice from the Reference Laboratory expert and to revisit the item at its next meeting in February 2020.

##### 7.3. Assessment for Infection with Decapod iridescent virus-1 (DIV1) against the criteria for listing an aquatic animal disease (Chapter 1.2.)

The Aquatic Animals Commission revised its assessment for infection with DIV1 from the February 2019 meeting.

The revised assessment for infection with DIV1 is presented as [Annex 14](#) for Member information in support of the proposed listing (see also Item 6.2.).

#### EU comment:

**The EU thanks the OIE and supports the revised assessment for infection with DIV1.**

## AD HOC GROUPS

### 7.4. Report of the *ad hoc* Group on the susceptibility of fish species to infection with OIE listed diseases

The Aquatic Animals Commission reviewed the report of the *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases which had applied the criteria for listing species as susceptible to infection with a specific pathogenic agent in accordance with Chapter 1.5. of the Aquatic Code for infection with viral haemorrhagic septicaemia virus (VHSV). The Commission acknowledged and thanked the *ad hoc* Group members for their extensive work to conduct these assessments.

The report of the OIE *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases is presented at [Annex 15](#) for Member information.

#### **EU comment:**

**The EU thanks the OIE and welcomes the report of the OIE *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases. We have included a clarification question.**

### 7.5. Revised assessments for susceptibility of zebrafish to infections with SVCV against the criteria in Chapter 1.5. Criteria for listing species as susceptible to infection with a specific pathogen

Since adoption of the revised Article 10.9.2, the Commission had become aware of other scientific publications which had reported on the susceptibility of zebrafish to infection with SVCV (Lopez-Munoz *et al.*, 2010; Martinez-Lopez *et al.*, 2014; Medina-Gali *et al.*, 2018a; Medina-Gali *et al.*, 2018b; Bello-Perez *et al.*, 2019). In light of this information, the Commission requested that the *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases review its previous assessments for zebrafish against the criteria in Chapter 1.5. taking into consideration these scientific studies.

The *ad hoc* Group reviewed its previous assessments taking into account the additional publications and agreed that zebrafish did meet the criteria for listing as a susceptible species and should be included in Article 10.9.2. (see also Item 6.5.).

The revised assessment of the OIE *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases is presented at [Annex 16](#) for Member information.

#### **EU comment:**

**The EU thanks the OIE and supports the revised annex 16.**

### 7.6. Electronic *ad hoc* Group on Tilapia lake virus

The Aquatic Animals Commission reviewed the progress report of the *ad hoc* Group on Tilapia lake virus (TiLV) which had met electronically since February 2019 to progress its work on the development of a robust and reliable diagnostic test for TiLV. The Commission expressed its gratitude for the extensive work carried out by this Group.

The *ad hoc* Group had reported on its successful propagation of TiLV and production of positive samples for proficiency testing, and the results of an inter-laboratory comparison to evaluate and compare the most promising of the TiLV molecular assays by using a panel of selected samples from infected and non-infected animals. Results indicated that the real-time qPCR was the most sensitive and reliable test, however, only two laboratories participated in the inter-laboratory comparison.

The Commission agreed with the *ad hoc* Group that given only two laboratories participated in the inter-laboratory comparison, the results could only be considered as preliminary. The Commission, therefore, requested that the *ad hoc* Group organise another round of inter-laboratory comparison to corroborate the results.

The Commission also reviewed the *ad hoc* Group's results against the criteria for listing aquatic animal diseases as described in Chapter 1.2. and agreed that the amount of data gathered did not provide enough

evidence for the Commission to recommend the listing of TiLV. The Commission acknowledged the issue of laboratory participation and noted that further inter-laboratory comparison work is required. The *ad hoc* Group was asked to continue its work and to report back to the next meeting of the Commission in February 2020.

The Commission noted that collaboration among Members is essential for effective responses to emerging diseases. The Commission encouraged the relevant laboratories to participate in the work of the *ad hoc* Group, in particular for further inter-laboratory comparisons.

#### **7.7. *Ad hoc* Group on susceptibility of mollusc species to infection with OIE listed diseases**

The Aquatic Animals Commission was informed that the *ad hoc* Group on Susceptibility of mollusc species to infection with OIE listed diseases had been established and will meet in December 2019 and January 2020 to start work on the application of Chapter 1.5. Criteria for listing species as susceptible to infection with a specific pathogen to OIE listed mollusc diseases. The Commission will consider the *ad hoc* Group's report at its February 2020 meeting.

### **OIE REFERENCE CENTRES**

#### **7.8. Evaluation of applications for OIE Reference Centres for Aquatic Animal Health issues or change of experts**

A nomination had been submitted to the OIE for a change of expert at an OIE Reference Laboratory by the Delegate of the Member concerned. The Commission recommended its acceptance:

*Infection with Koi herpesvirus disease*

Dr Takafumi Ito to replace Dr Kei Yuasa at the Diagnosis and Training Center for Fish Diseases, National Research Institute of Aquaculture, Japan Fisheries Research and Education Agency, JAPAN

### **OIE AQUATIC ANIMAL HEALTH STRATEGY**

#### **7.9. Development of an OIE Strategy for Aquatic Animal Health**

The OIE Director General had committed to developing an OIE Strategy for Aquatic Animal Health at the 4th OIE Global Conference on Aquatic Animal Health in Chile (2-4 April 2019). The Director General reiterated this commitment at the 2019 OIE General Session and requested that the Aquatic Animals Commission assist Headquarters to develop the strategy.

The Aquatic Animals Commission discussed progress toward development of an OIE Strategy for Aquatic Animal Health including the proposed outcomes, structure, milestones and consultation. The Commission also considered a number of proposed objectives and activities for the strategy, many of which are drawn from previous consideration by Members and experts; for example, the outcomes of the OIE Global Conference on Aquatic Animal Health in Chile. The Commission further developed and refined the objectives and activities, noting that it would be important that these be further prioritised to include only well-defined and achievable tasks.

The Commission noted that further consultation with Members and experts was necessary to ensure the plan would enable the OIE to address the highest priority needs of OIE Member countries. The Commission considered the consultation that had occurred at the Conference of the OIE Regional Commission for Asia, the Far East and Oceania in Sendai, Japan in September 2019. The Aquatic Animals Commission noted that participants at the Conference engaged in the development of the strategy in an interactive session; in particular, to consider prospective objectives and priority activities. The Commission noted that it would not be possible to conduct this level of consultation in all regions due the timing of regional commission conferences and the need to finalise a draft of the strategy by early 2019. However the Commission agreed to the following consultation activities: consultation at regional commission conferences where possible, consultation with delegates and focal points through the teleconferences within each region that Commission members will provide to communicate the major points of this report, and an online survey that will be provided to all Members in November 2019.

The Commission agreed on milestones for development of the strategy and to hold regular teleconferences to track progress and maintain momentum for development of the strategy. The next teleconference of the Commission would be in November 2019.

## **8. ACTIVITIES OF THE AQUATIC ANIMALS COMMISSION MEMBERS**

**9. NEXT MEETING**

The next meeting of the Aquatic Animals Commission is scheduled for 19 to 26 February 2020.

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.../Annexes

**MEETING OF THE OIE  
AQUATIC ANIMAL HEALTH STANDARDS COMMISSION  
Paris, 25 September–2 October 2019**

**List of participants**

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**MEETING OF THE OIE  
AQUATIC ANIMAL HEALTH STANDARDS COMMISSION  
Paris, 25 September–2 October 2019**

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**Adopted agenda**

- 1. WELCOME FROM THE DEPUTY DIRECTOR GENERAL**
- 2. MEETING WITH THE DIRECTOR GENERAL**
- 3. ADOPTION OF THE AGENDA**
- 4. PROJECT ON ANTIMICROBIAL RESISTANCE IN AQUACULTURE**
- 5. WORK PLAN OF THE AQUATIC ANIMAL HEALTH STANDARDS COMMISSION**
- 6. ITEMS FOR MEMBER COMMENTS**

*Aquatic Code*

- 6.1. New draft chapter on Biosecurity for Aquaculture Establishments (Chapter 4.X.)
- 6.2. Revised Article 1.3.3. of Chapter 1.3. Diseases listed by the OIE
- 6.3. Model Article 10.X.13.
- 6.4. Article 10.10.2. of Chapter 10.10. Infection with viral haemorrhagic septicaemia virus
- 6.5. Article 10.9.2. of Chapter 10.9. Infection with spring viraemia of carp virus
- 6.6. Discussion paper on approaches for determining periods required to demonstrate disease freedom
- 6.7. Revised Glossary definition for ‘Aquatic animal waste’

*Aquatic Manual*

- 6.8. Reformatting of *Aquatic Manual* chapters into the new template
  - 6.8.1 Chapter 2.3.9. Spring viraemia of carp
  - 6.8.2. New draft Chapter 2.1.X. Infection with *Batrachochytrium salamandrivorans*
  - 6.8.3. Chapter 2.3.4. Infectious haematopoietic necrosis
  - 6.8.4. Chapter 2.3.10. Viral haemorrhagic septicaemia
- 7. ITEMS FOR MEMBER INFORMATION**

*Aquatic Code*

- 7.1. Glossary definitions for ‘Competent Authority’, ‘Veterinary Authority’ and ‘Veterinary Services’. Information about work in the Terrestrial Code Commission.

Annex 2 (contd)

- 7.2. Acute hepatopancreatic necrosis disease (AHPND) (Chapter 9.1.)
- 7.3. Assessment for Infection with Decapod iridescent virus-1 (DIV1) against the criteria for listing an aquatic animal disease (Chapter 1.2.)

***Ad hoc* Groups**

- 7.4. Report of the *ad hoc* Group on susceptibility of fish species to infection with OIE listed diseases
- 7.5. Revised assessments for zebrafish against the criteria in Chapter 1.5. Criteria for listing species as susceptible to infection with a specific pathogen
- 7.6. Electronic *ad hoc* Group on Tilapia lake virus
- 7.7. *Ad hoc* Group on susceptibility of mollusc species to infection with OIE listed diseases

**OIE Reference Centres**

- 7.8. Evaluation of applications for OIE Reference Centres for Aquatic Animal Health issues or change of experts

**OIE Aquatic Animal Health Strategy**

- 7.9. Development of an OIE Strategy for Aquatic Animal Health

**8. ACTIVITIES OF THE AQUATIC ANIMALS COMMISSION MEMBERS**

**9. NEXT MEETING**

19–26 February 2020



## CHAPTER 4.X.

**BIOSECURITY  
FOR AQUACULTURE ESTABLISHMENTS**

**EU comment:**

**The EU thanks the OIE and supports the proposed changes to Chapters 4.X. (Annexes 3A and 3B).**

**Particular comments are inserted within the body of the text of these two annexes.**

Article 4.X.1.

**EU comment:**

**This article refers to ‘specific pathogenic agents’ whilst Article 4.X.3 refers to ‘pathogenic agents’. It would be beneficial to use the same approach throughout the Chapter.**

**Purpose**

To provide recommendations on the development and implementation of biosecurity measures primarily to mitigate the *risk* of the introduction of specific *pathogenic agents* into *aquaculture* establishments, and if *pathogenic agents* are introduced, to mitigate the *risk* of further spread within, or release from the *aquaculture establishment*.

Article 4.X.2.

**Scope**

Biosecurity principles are relevant to application of the standards in the Aquatic Code at the level of country, zone, compartment or aquaculture establishment as appropriate. This chapter describes recommendations on biosecurity to be applied to aquaculture establishments, including semi-open, semi-closed and closed systems. The chapter describes general principles of *biosecurity* planning, categories of *aquaculture* production systems, mitigation measures for transmission pathways, the application of *risk analysis* and approaches for *biosecurity plan* development.

For further guidance on disease prevention and control refer to Section 4 of the *Aquatic Code*.

Article 4.X.3.

**Introduction**

*Biosecurity* at the level of an *aquaculture establishment* is integral to effective *biosecurity* at the level of a country, zone or compartment and thus the optimal health status of *aquatic animal* populations. This chapter describes *biosecurity* principles designed to mitigate the *risks* associated with the introduction of *pathogenic agents* into, the spread within, or the release from *aquaculture establishments*.

Given the unique challenges posed by varied aquaculture production systems and the vast diversity of farmed aquatic animal species, the development of biosecurity plans for aquaculture establishments requires the assessment of disease risks posed by specific pathogenic agents and their potential transmission pathways. A biosecurity plan describes physical and management measures to mitigate the identified risks according to the circumstances of the aquaculture establishment. Aquaculture establishment staff, service providers and aquatic animal health professionals or veterinarians should be engaged in developing and implementing the biosecurity plan to ensure it is practical and effective.

The outcome achieved through the implementation of *biosecurity* at *aquaculture establishments* is improved health and welfare of *aquatic animals* throughout the production cycle. The benefits include improved market access and increased productivity, directly through improved survival, growth rates and *feed* conversion and indirectly through a reduction in the use of veterinary medicinal products (including *antimicrobial agents*) and associated production costs.

Article 4.X.4.

**General principles**

Biosecurity is a set of physical and management measures which, when used together, cumulatively reduce the risk of infection in aquatic animal populations within an aquaculture establishment. Implementation of biosecurity within an aquaculture establishment requires planning to identify risks and cost-effective measures to achieve the identified biosecurity objectives of the plan. The measures required will vary among aquaculture establishments, depending on factors such as likelihood of exposure to pathogenic agents, the species of aquatic animal, category of aquaculture production system, husbandry practices and geographical location. Although different approaches may be used to achieve an identified objective, the general principles for developing and implementing a *biosecurity plan* are described below:

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**EU comment:**

**We suggest removing text in first paragraph of General principles as follows:**

**Biosecurity is a set of physical and management measures which, when used together, cumulatively reduce the risk of infection in aquatic animal populations ~~within an aquaculture establishment.~~**

**Justification: We appreciate that this chapter is specifically aimed at biosecurity at the aquaculture establishments level. However, biosecurity when applied effectively, will in many cases also reduce the risk of infection in wild aquatic animal populations.**

- 1) Potential pathways for pathogenic agents to be transmitted into, spread within and released from the aquaculture establishment must be identified, as described in Articles 4.X.5. and 4.X.6., giving consideration to the category of aquaculture production system and design of the aquaculture establishment.
- 2) Risk analysis should be undertaken to evaluate biosecurity threats and ensure that the plan addresses risks appropriately and efficiently. The risk analysis may range from a simple to a complex analysis depending on the objectives of the biosecurity plan and the circumstances of the aquaculture establishment and disease risks, as described in Article 4.X.7.
- 3) Biosecurity measures to address identified disease risks should be evaluated on the basis of their potential effectiveness, initial and ongoing costs (e.g. building works, maintenance), and management requirements, as described in Article 4.X.7.
- 4) Management practices should be integrated into the aquaculture establishment's operating procedures and relevant training provided to personnel, as described in Articles 4.X.7. and 4.X.8.
- 5) Appropriate records and documentation are essential to demonstrate effective implementation of the *biosecurity plan*. Examples are provided in Article 4.X.8.
- 6) A schedule for routine reviews and audits of the biosecurity plan should be described. Triggers for ad hoc review must be determined (e.g. changes to infrastructure, production techniques, disease outbreaks, or risk profiles). Third party audits may be required where recognition of the biosecurity measures is required by customers, or regulators, or for market access, as provided in Article 4.X.8.

## Article 4.X.5.

**Categories of aquaculture production systems**

Four different categories of aquaculture production systems are defined based on the capacity to treat water entering and exiting the system, and the level of control of *aquatic animals* and *vectors*. These factors need to be considered in *biosecurity* planning.

Open systems

In an open *aquaculture* production system, it is not possible to have control of water, environmental conditions, animals and *vectors*. These production systems may include stock enhancement of wild populations with animals originating from *aquaculture establishments* or from the wild. As these systems cannot be considered 'establishments', they are not considered further in this chapter. However, movements of *aquatic animals* to open systems should still be subject to disease mitigation measures.

Semi-open

In a semi-open *aquaculture* production system, it is not possible to have control over the water entering or exiting the system, or the environmental conditions. Some *aquatic animals* and *vectors* may also enter and exit the system. Examples of semi-open *aquaculture* production systems are net pens for finfish and suspended baskets for molluscs in natural water bodies.

Semi-closed

In a semi-closed *aquaculture* production system, there is some control over the water entering and exiting the system and the environmental conditions. *Aquatic animals* and *vectors* may be prevented from entering and exiting the

system; however, there is limited control to prevent the entry or exit of *pathogenic agents*. Examples of semi-closed *aquaculture* production systems are ponds, raceways, enclosed floating pens, and flow-through tanks.

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Closed

**EU comment:**

**We would recommend that the first sentence of the paragraph below should be re-worded as follows:**

**‘In a closed aquaculture production system, the control of water entering and exiting the system ~~can~~ will exclude aquatic animals, vectors and pathogenic agents’.**

In a closed *aquaculture* production system, the control of water entering and exiting the system can exclude *aquatic animals*, *vectors* and *pathogenic agents*. Examples of closed *aquaculture* systems include recirculating *aquaculture* production systems, production systems with a safe water supply free from *pathogenic agents* or *aquatic animals* (e.g. ground water), or those with high levels of treatment (and redundancy) of water entering or exiting the system. Environmental conditions can also be controlled.

Article 4.X.5. bis

**Area Management**

It may not be possible to control the transmission of *pathogenic agents* among semi-open or semi-closed *aquaculture establishments* that are in close proximity within shared water bodies. In these circumstances, a consistent set of *biosecurity* measures should be applied by all of the *aquaculture establishments* considered to be epidemiologically linked. Area management agreements can formalise the common *biosecurity* measures among all of the epidemiologically linked *aquaculture establishments*.

Article 4.X.6.

**Transmission pathways and mitigation measures**

*Pathogenic agents* can move into, spread within, and be released from *aquaculture establishments* via various transmission pathways. The identification of all potential transmission pathways is essential for the development of an effective *biosecurity plan*. Mitigation of pathways that are likely to result in transmission of specific *pathogenic agents* should be prioritised.

The *risks* associated with introduction, spread, and release of pathogenic agents from the *aquaculture establishment* need to be considered for each of the following transmission pathways.

1. Aquatic animals

Movement of *aquatic animals* into, within and from *aquaculture establishments*, either intentionally or unintentionally, may pose a high likelihood of *pathogenic agent* transmission. This is particularly the case when clinically and sub-clinically infected *aquatic animals*, or *aquatic animals* with unknown health status are moved into a susceptible population.

*Aquatic animals* intentionally introduced into, or moved within, an *aquaculture establishment* may include broodstock, juvenile stock for on-growing, and genetic material such as eggs. Both horizontal and vertical transmission mechanisms should be considered for *aquatic animals*. The *risk* of transmitting *pathogenic agents* via *aquatic animals* should be managed, giving consideration to the following mitigation measures:

- a) Only introducing into the *aquaculture establishment* *aquatic animals* with known health status, which is of equal or higher status than the existing animals in the establishment.

**EU Comment:**

We suggest including the following either at the end of 1a) or as an additional point to ensure consideration is given to Chapter 5.1 of the code:

**For imported animals, the Competent Authority in the exporting country should assure that animals dispatched to another country comply with OIE standards in line with the articles laid out in Chapter 5.1 of the Aquatic Code.**

**Justification:** this point makes reference to the fact that animals imported into an aquaculture establishment should be certified as disease free or at least meet the animal health requirements of the Competent Authority of that country.

b) Placing introduced *aquatic animals* of unknown *disease status* into *quarantine*.

**EU Comment:**

We suggest expanding point b) as follows:

**Placing introduced *aquatic animals* of unknown *disease status* into *quarantine* where the conditions in which the aquatic animals are kept are conducive to clinical expression of disease and appropriate testing of animals is carried out to determine their health status.**

**Justification:** placing animals into quarantine on its own is not sufficient to ensure that they are not infected with a pathogen of interest. The conditions within the quarantine should be maintained to be conducive to the disease in question. For example, for KHVD, temperatures must be maintained at temperatures permissive to KHVD expression. Furthermore, testing of animals in line with OIE standards should be carried out to ensure animals are free from disease.

On this point, we would like to ask the OIE AAC to consider reviewing the glossary definition of quarantine to reflect the points above. We would suggest a rewording of the definition of quarantine as follows:

**‘Quarantine means maintaining a group of *aquatic animals* in isolation with no direct or indirect contact with other *aquatic animals*, in order to undergo observation for a specified length of time. The animals should be kept in conditions conducive to clinical expression of disease, with appropriate testing and treatment, including proper treatment of the effluent waters.’**

c) Where appropriate, treating *quarantined aquatic animals* to mitigate *disease risks* (for example, treatment for external parasites).

d) Ensuring biosecure transport of *aquatic animals* that avoids exposure to *pathogenic agents*.

e) Only moving *aquatic animals* between different populations within the establishment following consideration of the *disease risks* and with a view to maintaining the high health status of the *aquatic animal* population.

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- f) Isolating *aquatic animal* populations that display clinical signs of *disease* from other populations until the cause is known and the situation is resolved.
- g) Removing sick or dead *aquatic animals* from production units as soon as possible and disposing of them in a biosecure manner in accordance with Chapter 4.7.
- h) Reporting of unexplained or unusual mortalities, or suspicion of a notifiable *disease* in *aquatic animals* to the *Competent Authority* in accordance with local requirements. Investigation and *diagnosis* of the cause of mortality should be undertaken.

**EU Comment:**

**An addition to point h) is suggested, to clarify who should be responsible for investigating mortality.**

**Investigation and diagnosis of the cause of mortality should be undertaken by the Aquatic Animal Health Services under the direction of the Competent Authority.**

**Justification: point h currently leaves the responsibility of who should conduct a disease investigation open to interpretation. This should be clarified with additional text. Under the OIEs definition of an early detection system the Aquatic Animal Health Services should have the ability to undertake a rapid and effective disease investigation based on a national chain of command.**

- i) If possible, totally depopulating the *aquaculture establishment* at intervals, for instance between *aquatic animal* generations or production cycles, followed by cleaning and *disinfection* of production installations. Sites should be fallowed for a period sufficient to interrupt *infection* cycles and reduce or eliminate pathogen challenge to restocked *aquatic animals*. *Fallowing* should be coordinated for *aquaculture establishments* that are epidemiologically linked through shared water bodies.
- j) Considering physical measures to minimise the likelihood of escape of farmed *aquatic animals* or the entry of wild *aquatic animals* into the *aquaculture establishment*. The likelihood of entry or escape of *aquatic animals* will be higher for semi-open than for closed or semi-closed systems.

**EU Comment:**

**We suggest adding an additional point to cover animal movements records:**

**k) Aquaculture establishments should maintain accurate records of movements of live and dead aquatic animals on, off and within the site**

**Justification: while point 5 of 4. X. 4 mentions appropriate documentation and record keeping, this suggested point k) adds greater clarity and detail on the keeping of animal movement records which are essential to ensure that an effective and rapid disease investigation is conducted if required.**



## 2. Aquatic animal products and aquatic animal waste

*Aquatic animal products* may also be brought into, moved within or moved out of *aquaculture establishments*; for example, *aquatic animal products* derived from *aquatic animals* harvested at other sites. *Aquatic animal waste* may be generated when *aquatic animals* have died or been killed for *disease control* purposes or through killing and processing well as slaughtered of *aquatic animals* for human consumption or other purposes.

### EU comment:

**The proposed wording of the second sentence does not read correctly.**

**We propose ‘Aquatic animal waste may be generated when aquatic animals have died or been killed for disease control purposes, or through killing and processing, as well as ~~slaughtered of aquatic animals~~ slaughtering for human consumption or other purposes’.**

Movement of *aquatic animal products* and *aquatic animal waste* into, within and out of *aquaculture establishments* may pose a *risk of pathogenic agent* transmission. This is particularly the case when a susceptible population is exposed to *aquatic animal products* and *aquatic animal waste* derived from clinically or sub-clinically infected *aquatic animals*. High *risk waste* includes *aquatic animal waste* that constitutes, or is suspected of constituting, a significant health *risk* to *aquatic animals*. Movement of *aquatic animal waste* into *aquaculture establishments* should be avoided where possible. Waste should be stored, transported, disposed of and treated following the guidance in Chapter 4.7. Handling, disposal and treatment of *aquatic animal waste*.

For intentional movements of *aquatic animal products* and *aquatic animal waste*, the likelihood of presence of *pathogenic agents* in the *aquatic animals* from which products and waste are derived should be evaluated giving consideration to the species, source, and health status.

The *risk* of transmitting *pathogenic agents* via *aquatic animal products* and *aquatic animal waste* should be managed giving consideration to the following mitigation measures:

- a) determining the potential *disease risk* of *aquatic animal products* and *aquatic animal waste* to the establishment and the environment;
- b) managing *aquatic animal products* and *aquatic animal waste* in areas within the *aquaculture establishment* that are isolated from *aquatic animal* populations to minimise identified *disease transmission risks*;
- c) ensuring systems are implemented for appropriate collection, treatment (inactivating *pathogenic agents*), transport, storage or disposal of *aquatic animal products* and *aquatic animal waste* to minimise the *risks* of transmitting *pathogenic agents*.

3. Water

Water is an important asset that supports productivity and *aquatic animal* health but may present a *risk* of introduction of *pathogenic agents* into, spread within, and release from *aquaculture establishments*. The source of the water, and how it provides an epidemiological link between the *aquaculture establishment* and other farmed or wild populations or processing plants, should be identified and considered. Exposure to transport water and ballast water should be considered.

The *risk* of the *aquaculture establishment* being exposed to water containing *pathogenic agents* may be influenced by the category of *aquaculture* production system, the likelihood being higher for semi-open than for closed systems. Any water that is flowing from *aquatic animals* with lower or unknown health status presents a potential *risk* of transmitting *pathogenic agents* to *aquatic animals* of a higher health status.

The *risk* of transmitting *pathogenic agents* via water should be managed giving consideration to the following mitigation measures:

- a) Where possible, choosing water sources that are entirely free of susceptible *aquatic animal* populations and *pathogenic agents* of concern. Such water sources may include saline or fresh groundwater, dechlorinated municipal water, and artificial seawater. These water sources may be particularly suitable for high health status *aquatic animals* such as broodstock.
- b) Providing an appropriate level of screening, filtration or disinfection (in accordance with Chapter 4.3.) of water from sources that are likely to contain *susceptible species* and may present a *risk* of *pathogenic agent* transmission (e.g. oceans, streams or lakes). The level of treatment required will depend on the identified *risks*.
- c) Provide an appropriate level of filtration, disinfection or holding (in accordance with Chapter 4.3.) of effluent water from *aquaculture establishments* (or associated slaughterhouses or processing facilities) where it may present a *risk* of *pathogenic agent* transmission to wild *aquatic animals* or other *aquaculture establishments* with *susceptible species*. The level of treatment required will depend on the identified *risks*.

**EU Comment:**

**It is unclear what ‘holding’ refers to in point (c) above.**

**It may mean holding the effluent water for a period until it can be subsequently disinfected. If this is the case, it would be better to delete it altogether because disinfection is already referred to in the first sentence.**

**If the action of ‘holding’ is for any other purpose, it may be beneficial to specify for which purpose, or provide an example.**

- d) Ensuring the position of water intakes and outlets for semi-closed and closed *aquaculture establishments*, and the location of semi-open *aquaculture establishments*, minimises contamination from other farmed or wild populations or processing plants, taking into account factors such as distance and water currents.
- e) The likelihood of ingress of contaminated water either through flooding from external sources or from defective infrastructure (e.g. leaking pipes, blocked drains, bund wall failure) should be assessed and appropriate management or infrastructure measures applied.

4. Feed

*Feed* can be an important pathway for transmission of *pathogenic agents* to *aquatic animals*. *Feed* may be initially infected with *pathogenic agents* or contaminated during harvest, transport, storage and processing of commodities used as feed ingredients. Poor hygiene may contribute to contamination during manufacture, transport, storage and use of *feed*.

In closed or semi-closed production systems there can be a high level of control on *aquatic animal feed*. However, in semi-open production systems, *aquatic animals* may obtain food from their environment (e.g. filter-feeding molluscs or wild fish which may be preyed on in net pens).

The *risk* of transmitting *pathogenic agents* via *aquatic animal feed* should be managed by mitigation measures as provided in Chapter 4.8., for example using *feed* and *feed* ingredients that:

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- a) have undergone sufficient processing to inactivate *pathogenic agents* of concern;
- b) are from sources that are declared free from the *pathogenic agents* of concern or have been confirmed (e.g. by testing) that *pathogenic agents* are not present in the *feed or feed ingredients* ;
- c) have been processed, manufactured, stored, transported and delivered during feeding to *aquatic animals* in a manner to prevent contamination by *pathogenic agents*.

**EU Comment:**

**In 2018, we suggested the addition of a bullet point (d) above to stress the importance of appropriate storage and management of feed on site, and practices to mitigate the risk of contamination of feed.**

**Our comment has been partially taken on board with the addition of the last sentence in the first paragraph related to poor hygiene. However, we still believe that it is important to be more specific and include detailed activities such as the storage in secure, dry locations and in containers with lids kept on and protected from the risk of splashing / contamination of water; and during feeding making sure that feed is not delivered in a way that risks contamination or subsequent spread across the site.**

**Moreover, it is also important that the risk of contamination from other feedstuffs which may be medicated, must be avoided to avoid the possibility of under-dose medication being inadvertently provided to fish.**

**These measures are important for ensuring effective management of risk associated with feed prior to being used on site, or during feeding practice and we would request that they should be considered for inclusion.**

5. Fomites

Equipment, *vehicles*, packaging material, clothing, footwear, sediments, infrastructure and other fomites can mechanically transfer *pathogenic agents* into, within and from an *aquaculture establishment*.

The likelihood of transferring *pathogenic agents* will depend on the stability of the pathogenic agent in the environment, the presence and nature of organic matter on the fomite surface, as well as the type of surface and its capacity to hold water. The likelihood of transferring *pathogenic agents* may be higher for fomites which are difficult to clean and disinfect. Sharing equipment between *aquaculture establishments*, between *aquaculture establishments* and processing facilities, or between different production units with unequal health status within an *aquaculture establishment* may result in the spread of *pathogenic agents*. The likelihood of transmitting *pathogenic agents* via fomites should be managed—giving consideration to the following mitigation measures:

- a) Assessing the *disease risk* associated with any fomites brought into the *aquaculture establishment*
- b) Ensuring procedures and infrastructure are in place to clean and disinfect fomites, including at designated delivery and loading areas, prior to entry into the *aquaculture establishment*. Recommendations for the cleaning and *disinfection* of fomites are described in Chapter 4.3.
- c) Wherever possible, dedicating items that are difficult to disinfect ~~items~~, or those with a high likelihood of contamination, to a specific *aquaculture establishment* rather than moving them between *aquaculture establishments* after *disinfection*.
- d) Applying the mitigation measures described at points a) to c) above to the movement of *fomites* between production units within an *aquaculture establishment* with the measures determined based on an evaluation of the *disease risks*.

6. Vectors

*Vectors* can transport *pathogenic agents* to susceptible *aquatic animals* in *aquaculture establishments*. They include wild *aquatic animals* entering via the water supply, predators, wild birds, scavengers, pest animals such as rodents and people. *Vectors* can transfer *pathogenic agents* into, within and from an *aquaculture*

*establishment*, either by mechanical transfer or as a developmental stage of the *pathogenic agent* within the *vector*.

**EU comment:**

**Because of some organisms like viruses there will not be a developmental stage, we would recommend amending the sentence above to read:**

**'...either by mechanical transfer or as a developmental stage of the *pathogenic agent* within the *vector*.**

The *risk* of transferring *pathogenic agents* via *vectors* varies with the type of *vector*, the nature of the *pathogenic agent*, the category of *aquaculture* production system, and the level of *biosecurity*.

**EU comment:**

**We would recommend that the sentence below be re-worded as follows:**

**The risk of transmitting *pathogenic agents* via *vectors* should be managed depending on the identified risks and giving consideration to the following mitigation measures.**

The *risk* of transmitting *pathogenic agents* via *vectors* should be managed giving consideration to the following mitigation measures:

- a) Physical mitigation measures should be used to prevent the access of *vectors* to *aquaculture establishments* including:

**EU comment:**

**The wording in 6(a) above does not seem to fit with the preceding sentence. The following is suggested instead 'Physical mitigation measures ~~should be used to prevent the access of vectors to aquaculture establishments including~~ may include:'**

b)

- i) filtering or screening of water entering semi-closed and closed *aquaculture* production systems to prevent entry of wild *aquatic animals*;
- ii) surrounding land-based *aquaculture* production systems by a fence or a wall to prevent entry of animals and people, with a gate for controlled access;
- iii) surrounding floating *aquaculture* production systems by barriers on the establishment perimeter to prevent contact with or entry of wild *aquatic animals* and other animals;
- iv) covering outdoor *aquaculture* production systems with nets to prevent access by birds

- b) Access of personnel to *aquaculture establishments* should be controlled by creating a defined border between the outer risk area and the inner biosecure area comprising facilities for:
- i) changing of clothes and shoes, or use of disposal coverings (hoods, coats, shoe coverings);
  - ii) cleaning and *disinfection* of hands, and the use of foot baths;
- c) Pest control.

**EU Comment:**

**The expansion of physical mitigation measures to cover a new point on signage as a means to limit transfer of vector borne pathogens is recommended:**

**d) The use of signage as means to raise awareness of biosecure or disease control areas on a farm**

**Justification: signage can be an effective tool in raising awareness to members of the public and farm personnel that farms or certain units within the farm are operating to biosecure conditions or they are subject to disease control measures.**

Article 4.X.7.

**Risk analysis**

*Risk analysis* is an accepted approach for evaluating *biosecurity* threats and is used to support the development of mitigation measures. A formal *risk analysis* has four components: *hazard* identification, *risk assessment*, *risk management* and *risk communication*. This article elaborates the principles in Chapter 2.1. and applies them for the development of *biosecurity plans* for *aquaculture establishments*.

A *biosecurity plan* may not necessarily require a comprehensive *risk analysis* to evaluate *disease risks* linked to transmission pathways. The chosen approach may depend on the objectives of the *biosecurity plan*, the level of *biosecurity* that is appropriate for the specific production requirements of the *aquaculture establishment*, the complexity of the threats to be addressed, and the availability of information and resources. Depending on these circumstances, a partial analysis may be appropriate, and can build on previous experiences to identify the *hazards* associated with relevant transmission pathways.

The three formal steps of the *risk analysis* process to underpin a *biosecurity plan* are:

**Step 1 – Hazard identification**

*Hazard* identification determines which *pathogenic agents* should be the subject of the *risk assessment*. A *hazard* may include a specific *pathogenic agent* or be defined in more general terms as a group of *pathogenic agents*. This step includes identifying and collecting relevant information on the *pathogenic agents* that have potential to cause *diseases* in *aquatic animal* populations within an *aquaculture establishment*. This process must consider the *aquatic animal health status* of the establishment and, for semi-open and semi-closed *aquaculture* production systems, the *aquatic animal health status* of the epidemiologically linked environments. The following step is to identify both known and *emerging diseases*, not present in the *aquaculture establishment*, which may negatively impact the farmed population.

To complete the next steps of the *risk assessment*, information on the identified *hazards* is required and includes: i) the frequency of occurrence, ii) the biophysical characteristics, iii) the likelihood of detection if present and iv) the possible transmission pathways (described in Article 4.X.6.). Many of the *hazards* will share the same pathways.

**Step 2 – Risk assessment**

A *risk assessment* can be initiated once it has been identified that a *hazard* exists and the required information listed under step 1 has been gathered. The aim of the *risk assessment* is to establish a *risk* estimate, which is the product of the likelihood and consequences of entry of a *pathogenic agent* into, spread within or release from the *aquaculture establishment*.

A *risk assessment* can be quantitative or qualitative. Both methods require the same conceptual pathway which identifies the necessary steps for *hazard* introduction, establishment and spread to be constructed. In a qualitative assessment, introduction and establishment are estimated using descriptors of likelihood. A quantitative assessment

requires data on which to estimate likelihood. In most circumstances, transmission pathways will be assessed qualitatively but within a formal *risk assessment* framework. Examples of descriptors for qualitative estimates of likelihood and consequence are given in Tables 1 and 2. Table 3 illustrates how estimates of likelihood and consequence can be combined in a matrix to give an estimate of *risk*.

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**Table 1. Qualitative descriptors of likelihood**

Estimate	Descriptor
Remote	Never heard of, but not impossible.
Unlikely	May occur here, but only in rare circumstances.
Possible	Clear evidence to suggest this is possible in this situation.
Likely	It is likely, but not certain, to occur here.
Certain	It is certain to occur.

**Table 2. Qualitative descriptors of consequences**

Estimate	Descriptor of consequences at level of the aquaculture establishment
Insignificant	Impact not detectable or minimal. No trade impact.
Minor	Limited decreased production affecting only a small number of units or short-term, and/or very limited and transitory disruption to trade.
Moderate	Decreased production (e.g. sustained increased mortality or decreased growth rate) and/or some short-term to medium-term disruption to trade, resulting in financial loss.
Major	Considerable, decreased production, and/or some medium-term to long-term disruption to trade, resulting in significant financial loss.
Catastrophic	Complete production loss, possibly barriers to resumption of production, and/or complete loss of trade, resulting in extreme financial loss.

**Table 3. Matrix for assessing risk**

	Consequence rating					
		insignificant	minor	moderate	major	catastrophic
Likelihood estimate	remote	negligible	low	low	low	medium
	unlikely	low	low	medium	medium	high
	possible	low	medium	medium	high	high
	likely	low	medium	high	high	extreme
	certain	medium	high	high	extreme	extreme

*Risk assessments* inform which biological *hazards* need to be addressed, which critical control points on the transmission pathway should be targeted, and the measures that are most likely to be effective in reducing *risk*.

**Table 4. Interpretation of risk estimates**

Risk level*	Explanation and management response
Negligible	Acceptable level of <i>risk</i> . No action required.
Low	Acceptable level of <i>risk</i> . On-going monitoring may be required.
Medium	Unacceptable level of <i>risk</i> . Review and strengthen the risk mitigation measures.
High	Unacceptable level of <i>risk</i> . Identify and implement additional mitigation measures.
Extreme	Unacceptable level of <i>risk</i> . Take immediate action to mitigate the risk.

\*The *risk* level is determined from a combination of the likelihood and consequence scores obtained using the *risk* matrix (Table 3).

### Step 3 – Risk Management

*Risk management* is used to determine the appropriate management response for the assessed level of *risk* as described in Table 4. The *risk assessment* process identifies the steps within transmission pathways necessary for a *risk* to be realised and thus allows the most effective mitigation measures to be determined. Many of the *hazards* will share the same pathways and therefore mitigation measures may be effective against more than one *hazard*. Information on hazards and their pathways of introduction (step 1) should be combined with the assessment of the pathways (step 2) to identify the most appropriate and cost-effective risk mitigation measures.

Article X.X.6. describes some possible mitigation measures relevant to different transmission pathways. The most appropriate mitigation measures for a specific *aquaculture establishment* will depend on the *hazards* identified, the effectiveness and reliability of the mitigation measure, the category of *aquaculture* production system and cost.

After the implementation of the *biosecurity plan*, *hazards* should be regularly reassessed, and measures adjusted according to any changed *risk* estimates.

Article 4.X.8.

#### Biosecurity plan development

The purpose of a *biosecurity plan* is primarily to reduce the *risk* of introducing *pathogenic agents* into an *aquaculture* establishment, and if *pathogenic agents* are introduced, to reduce the *risk* of further spread within or release from the *aquaculture establishment*. The plan will document identified transmission pathways and the outputs of any *risk analysis* performed (*hazards*, *risk* estimate and mitigation measures), and information relevant to ongoing implementation, monitoring and review of the plan.

#### 1. Development of a biosecurity plan

The process of developing a *biosecurity plan* will vary depending on its objectives, the level of *biosecurity* appropriate to the specific production system requirements, the complexity of the *disease risks* to be addressed, and availability of information and resources. Consideration and documentation of the following issues are recommended:

- a) objectives and regulatory requirements for the *biosecurity plan*;
- b) information about the *aquaculture establishment* including an up-to-date plan of the layout of buildings and production units (including *epidemiological units*, if any, and structures and processes to maintain separation), loading/ unloading, unpacking, processing, feed storage, waste storage, reception areas, and maps showing major movements of *aquatic animals*, *aquatic animal products* and *aquatic animal waste*, water, *feed* and fomites;
- c) the potential pathways for entry of *pathogenic agents* into, spread within or release from the *aquaculture establishment* (refer to Article X.X.6. above);
- d) a *risk analysis*, including identification of the major *disease hazards* to the *aquaculture establishment* (refer to Article X.X.7. above);
- e) the mitigation measures that have been determined to address identified *risks*;
- f) emergency procedures in the event of a *biosecurity* failure. These may include reporting requirements, emergency measures to eradicate *pathogenic agents* such as *aquatic animal* depopulation and disposal, and site *disinfection*, in accordance with Chapters 4.3. and 7.4.;



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- g) internal and external communication procedures, roles and responsibilities of *aquaculture establishment* staff and essential contact information, e.g. for staff, farm *veterinarian* and the *Competent Authority*;
- h) monitoring and audit schedule;
- ij) performance evaluation;
- j) standard operating procedures required to support implementation of the mitigation measures, emergency procedures and the training requirements of personnel.

2. Key components of a biosecurity plan

a) Standard operating procedures (SOPs)

SOPs describe routine management processes that must be performed to support the effectiveness of the *biosecurity plan*. Each SOP should clearly describe its objectives, staff responsibilities, the procedure (including record keeping), precautions and a review date.

Staff should be trained in the application of the SOPs including completion of forms, checklists and other records associated with each procedure, as well as routine communication requirements.

b) Documentation and record keeping

The *biosecurity plan* describes the documentation necessary to provide evidence of compliance with the mitigation measures. The level of detail required in the documentation depends on the outcomes of the transmission pathway assessment.

Examples of documentation required include: *aquaculture establishment* layout, movements of *aquatic animals*, origin and destination and health status of the *aquatic animals* introduced to the *aquaculture establishment*, records of visitors to the establishment, escapees, stocking densities, feeding and growth rates, records of staff training, treatments/vaccination, water quality, cleaning and *disinfection* events, morbidity and mortality (including removal and disposal of mortalities), *surveillance* and laboratory records.

c) Emergency procedures

Procedures should be developed and, when necessary, implemented to minimise the impact of emergencies, *disease* events, or unexplained mortality in *aquatic animals*. These procedures should include clearly defined thresholds that help to identify an emergency incident and activate response protocols, including reporting requirements.

d) Health monitoring

Health monitoring as part of the *biosecurity plan* involves monitoring of the health status of *aquatic animals* in *aquaculture establishments*. Activities may include *disease surveillance*, routine monitoring of stock for important health and production parameters (e.g. by staff, an *aquatic animal health professional* or a *veterinarian*), recording of clinical signs of *disease*, morbidity and mortality, and analysis of these data (e.g. calculation of rates of morbidity and mortality).

e) Routine review and auditing

The *biosecurity plan* should describe a systematic auditing schedule to verify implementation and compliance with the requirements of the *biosecurity plan*. Routine revision of the *biosecurity plan* is necessary to ensure that it continues to effectively address *biosecurity risks*.

The *biosecurity plan* should also be reviewed at least annually or in response to changes to the *aquaculture establishment* operations, changes in facility design, changes in husbandry approaches, identification of a new *disease risk*, or the occurrence of a *biosecurity* incident. *Biosecurity* incidents, and actions taken to remedy them, should be documented to enable re-assessments of SOPs.

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UNOFFICIAL VERSION

## CHAPTER 4.X.

## BIOSECURITY FOR AQUACULTURE ESTABLISHMENTS

## Article 4.X.1.

**Purpose**

To provide recommendations on the development and implementation of biosecurity measures primarily to mitigate the *risk* of the introduction of specific *pathogenic agents* into *aquaculture* establishments, and if *pathogenic agents* are introduced, to mitigate the *risk* of further spread within, or release from, the *aquaculture establishment*.

## Article 4.X.2.

**Scope**

Biosecurity principles are relevant to application of the standards in the Aquatic Code at the level of country, zone, compartment or aquaculture establishment as appropriate. This chapter describes recommendations on biosecurity to be applied to aquaculture establishments, including semi-open, semi-closed and closed systems. The chapter describes general principles of *biosecurity* planning, categories of *aquaculture* production systems, major transmission pathways, mitigation measures for transmission pathways, the use of the application of risk analysis and approaches for biosecurity plan development, to develop a biosecurity plan, and the key components of a plan.

For further guidance on disease prevention and control refer to Section 4 of the Aquatic Code.

## Article 4.X.3.

**Introduction**

~~The fundamental measures that underpin aquatic animal disease prevention at the level of country, zone or compartment is the application of biosecurity. Biosecurity at the level of an aquaculture establishment is integral to effective biosecurity at the level of a country, zone or compartment and thus the optimal health status of aquatic animal populations. This chapter describes biosecurity principles designed to mitigate the risks associated with the introduction of pathogenic agents into, the spread within, or the release from aquaculture establishments. The application of biosecurity at the level of an aquaculture establishment may be integral to effective biosecurity at the level of a country, zone or compartment to maintain the optimal health status of aquatic animal populations.~~

Given the unique challenges posed by varied aquaculture production systems and the vast diversity of farmed aquatic animal species, the development of biosecurity plans for aquaculture establishments requires the assessment of disease risks posed by specific pathogenic agents and their potential transmission pathways. A biosecurity plan describes physical and management measures to mitigate the identified risks according to the circumstances of the aquaculture establishment. Aquaculture establishment staff, and service providers and aquatic animal health professionals or veterinarians should be engaged in developing and implementing the biosecurity plan to ensure it is practical and effective.

The outcome achieved through the implementation of *biosecurity* at *aquaculture establishments* is improved health and welfare status of *aquatic animals* throughout the production cycle. The benefits include improved market access and increased productivity, directly through improved survival, growth rates and *feed* conversion and indirectly through the a reduction in the use in treatments of veterinary medicinal products (including antimicrobial agents) and associated production costs.

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## Article 4.X.4.

**General principles**

Biosecurity is a set of physical and management measures which, when used together, cumulatively reduce the risk of infection in aquatic animal populations at within an aquaculture establishment. Implementation of biosecurity within an aquaculture establishment requires planning to identify risks and ~~consider~~ cost effective measures to achieve the identified biosecurity objectives of the plan. The measures required will vary among between aquaculture establishments, depending on factors such as risk likelihood of exposure to pathogenic agents, the species of aquatic animal species, category of aquaculture production system, husbandry practices and geographical location. Although different approaches may be used to achieve an identified objective, the general principles for developing and implementing a *biosecurity plan* are described as below:

- 1) Planning is necessary to document the objectives of the biosecurity plan, the identified risks to be managed, the measures that will be put in place to manage the disease risks, required operating procedures and monitoring, as described in Articles 4.X.6. and 4.X.7.
- 21) Potential pathways for pathogenic agents to be transmitted into, spread within and released from the aquaculture establishment must be identified, as described in Articles 4.X.5. and 4.X.6., and giving consideration to the category of aquaculture production system and design of the aquaculture establishment.
- 32) Risk analysis should be undertaken to evaluate biosecurity threats and ensure that the plan addresses risks appropriately and efficiently. The risk analysis may range from a simple to a complex analysis depending on the objectives of the biosecurity plan and the circumstances of the aquaculture establishment and disease risks, as described in Article 4.X.7.
- 43) Biosecurity measures to address identified disease risks should be evaluated based on the basis of their potential effectiveness, initial and ongoing costs (e.g. building works, maintenance), and management requirements, as described in Article 4.X.7.
- 45) Management practices should be integrated into the aquaculture establishment's operating procedures and associated relevant training ~~are~~ is provided to personnel, as described in Articles 4.X.7. and 4.X.8.
- 5) Appropriate records and documentation are essential to demonstrate effective implementation of the biosecurity plan. Examples are provided in Article 4.X.8.
- 6) A routine review schedule for routine reviews and audits of the biosecurity plan should be described, and identified triggers for ad hoc review must be determined (e.g. changes to infrastructure, production techniques, disease outbreaks, or risk profiles). Third party audits may be required where recognition of the biosecurity measures is required by customers, or regulators, or for market access, as described provided in Article 4.X.8.

## Article 4.X.5.

**Categories of aquaculture production systems**

~~Aquatic animals can be produced in~~ Four different categories of aquaculture production systems, ~~which~~ are defined based on the capacity to treat water entering and exiting the system, and the level of control of *aquatic animals* and *vectors*. These measures factors need to be considered in *biosecurity* planning.

Open systems

In an open Open aquaculture production systems, it is not possible to have no control of water, environmental conditions, and animals and *vectors*. These production systems may include stock enhancement of wild populations with animals originating from aquaculture establishments or from the wild. As these systems cannot be considered 'establishments', they are not considered further in this chapter. However, movements of aquatic animals to open systems should still be subject to disease mitigation measures.

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### Semi-open

In a semi-open *aquaculture* production system, it is not possible to have control over the water entering or exiting the system, or ~~of~~ the environmental conditions. Some *aquatic animals* and *vectors* may also enter and exit the system. Examples of semi-open *aquaculture* production systems are net pens for finfish and suspended baskets for molluscs aquaculture in natural water bodies and ~~mollusc aquaculture, either suspended in the water column or on the ocean floor.~~

### Semi-closed

In a semi-closed *aquaculture* production system, there is some control ~~of~~ over the water entering and exiting the system and ~~of the~~ environmental conditions. *Aquatic animals* and *vectors* may be prevented from entering and exiting the system; however, there is limited control to prevent the entry or exit of *pathogenic agents*. Examples of semi-closed *aquaculture* production systems are ponds, raceways, enclosed floating pens, and flow-through tanks.

### Closed

In a closed *aquaculture* production system, the control of water entering and exiting the system can exclude *aquatic animals*, *vectors* and *pathogenic agents*. Examples of closed *aquaculture* systems include recirculating *aquaculture* production systems, production systems with a safe water supply free from *pathogenic agents* or *aquatic animals* (e.g. ground water), or those with high levels of treatment (and redundancy) of water entering or exiting the system. Environmental conditions can also be controlled.

### Article 4.X.5. bis

### Area Management

It may not be possible to control the transmission of *pathogenic agents* transmission among semi-open or semi-closed *aquaculture establishments* that are in close proximity within shared water bodies. In these circumstances, a consistent set of *biosecurity* measures should be applied by all of the *aquaculture establishments* considered to be epidemiologically linked. Area management agreements can formalise the common *biosecurity* measures among all of the epidemiologically linked *aquaculture establishments*.

### Article 4.X.6.

### Transmission pathways, and associated risks and mitigation measures

*Pathogenic agents* can move into, spread within, and be released from *aquaculture establishments* via various transmission pathways. The identification of all potential transmission pathways is essential for the development of an effective *biosecurity plan*. Mitigation of pathways that are likely to result in transmission of specific ~~may expose~~ susceptible *aquatic animals* to high loads of *pathogenic agents* should be prioritised.

The *risks* associated with introduction, spread, and release of *pathogenic agents* from the *aquaculture establishment* need to be considered for each of the following transmission pathways.

#### 1. Aquatic animals

Movement of *aquatic animals* into, within and from *aquaculture establishments*, either intentionally or unintentionally, may usually may pose ~~pose~~ has a high likelihood *risk* of *pathogenic agent* transmission. This is particularly the case when clinically and sub-clinically infected *aquatic animals*, or *aquatic animals* with unknown health status are moved into a susceptible population.

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*Aquatic animals* intentionally brought introduced into, or moved within, an *aquaculture establishment*, or moved within it, may include broodstock, juvenile stock for on-growing, and genetic material such as eggs. Both horizontal and vertical transmission mechanisms should be considered for *aquatic animals*. The risk of transmitting *pathogenic agents* via *aquatic animals* should be managed; possible mitigation measures include the giving consideration to the following mitigation measures can be managed by:

- a) Only introducing into the aquaculture establishment *aquatic animals* with known health status into the aquaculture establishment with known health status, which is of equal or higher status than the existing animals in the establishment.
- b) Quarantining Placing introduced *aquatic animals* of unknown disease status into quarantine from other farm populations in separate production units or dedicated *quarantine* facilities.
- c) Where appropriate, treating treatment of *quarantined aquatic animals* to mitigate disease risks (for example, treatment for external parasites).
- d) Ensuring biosecure transport of *aquatic animals* that avoids exposure to *pathogenic agents*.
- e) Only moving *aquatic animals* between different populations within the establishment following consideration of the *disease risks* and with a view to maintaining the high health status of the *aquatic animal* population.
- f) Isolating *aquatic animal* populations that display clinical signs of *disease* from other populations until the cause is known and the situation is resolved.
- g) Removing sick or dead *aquatic animals* from production units as soon as possible and disposing of them in a biosecure manner in accordance with Chapter 4.7.
- h) Reporting of unexplained or unusual mortalities, or suspicion of a notifiable disease in aquatic animals to the Competent Authority in accordance with local requirements. Investigation and diagnosis of the cause of mortality should be undertaken.
- i) If possible, totally depopulating the aquaculture establishment at intervals, for instance between aquatic animal generations or production cycles, followed by cleaning and disinfection of production installations. Sites should be fallowed for a period sufficient to interrupt infection cycles and reduce or eliminate pathogen challenge to restocked aquatic animals. Fallowing should be coordinated for aquaculture establishments that are epidemiologically linked through shared water bodies.
- j) Where possible, preventing unintended movement of aquatic animals into, within or from the establishment. Considering physical measures to minimise the likelihood of escape of farmed aquatic animals or the entry of wild aquatic animals into the aquaculture establishment. The likelihood of entry or escape of aquatic animals will be higher for semi-open than for closed or semi-closed systems.

The risk of unintentional movements of aquatic animals will be influenced by the category of *aquaculture* production system, with the likelihood being higher for semi-open than closed systems. If risks are found to be high, physical mitigation measures may be necessary.

## 2. Aquatic animal products and aquatic animal waste

*Aquatic animal products* may also be brought into, moved within and or moved out of an *aquaculture establishments* or moved within it; for example, *aquatic animal products* derived from *aquatic animals* harvested at other sites. *Aquatic animal waste* waste may include the be generated entire body or parts of when *aquatic animals* that have died or been killed for *disease* control purposes, as or through killing and processing well as slaughtered of *aquatic animals*, and their parts, that are not intended for human consumption or other purposes.

Movement of *aquatic animal products* and *aquatic animal waste* into, within and out of *aquaculture establishments* may pose a *risk* of *pathogenic agent* transmission. This is particularly the case when a susceptible population is exposed to *aquatic animal products* and *aquatic animal waste* waste derived from clinically or sub-clinically infected *aquatic animals*. High *risk* waste includes aquatic animal waste waste that constitutes, or is suspected of constituting, a high significant health *risk* to *aquatic animals*. Movement of aquatic animal waste into aquaculture establishments should be avoided where possible. Waste should be stored, transported, disposed of and treated following the guidance in Chapter 4.7. Handling, disposal and treatment of aquatic animal waste.

For intentional movements of *aquatic animal products* and *aquatic animal waste*, the likelihood of presence of *pathogenic agents* in the *aquatic animals* from which products and waste are derived should be evaluated giving consideration to the species, source, and health status.

The *risk* of transmitting *pathogenic agents* via *aquatic animal products* and *aquatic animal waste* waste should be managed; possible mitigation measures include the giving consideration to the following mitigation measures can be managed by:

- a) determining the potential *disease risk* of *aquatic animal products* and aquatic animal waste to the establishment and the environment;
- b) mManaging aquatic animal products and aquatic animal waste in areas within the aquaculture establishment that are isolated isolating areas within the aquaculture establishment where aquatic animal products and aquatic animal waste waste are managed from *aquatic animal* populations to minimise identified *disease transmission risks*;
- c) ensuring systems are implemented for appropriate collection, treatment (inactivating *pathogenic agents*), transport, storage or disposal of *aquatic animal products* and aquatic animal waste waste to minimise the *risks* of transmitting *pathogenic agents*.

### 3. Water

Water is an important asset that supports productivity and *aquatic animal* health but may present a *risk* of introduction of *pathogenic agents* into, spread within, and release from *aquaculture establishments*. The source of the water, and how it provides an epidemiological link between the *aquaculture establishment* and other farmed or wild populations or processing plants, should be identified and considered. Exposure to transport water and ballast water should be considered.

The *risk* of the *aquaculture establishment* being exposed to water containing *pathogenic agents* may be influenced by the category of *aquaculture* production system, the likelihood being higher for semi-open than for closed systems. Any water that is flowing from *aquatic animals* with lower or unknown health status presents a potential *risk* of transmitting *pathogenic agents* to *aquatic animals* of a higher health status.

The *risk* of transmitting *pathogenic agents* via water should be managed; possible mitigation measures include the giving consideration to the following mitigation measures can be managed by:

- a) Where possible, choosing water sources that are entirely free of susceptible *aquatic animal* populations and *pathogenic agents* of concern. Such water sources may include saline or fresh groundwater, de-chlorinated municipal water, and artificial seawater. These water sources may be particularly suitable for high health status *aquatic animals* such as broodstock.
- b) Providing an appropriate level of screening, filtration or disinfection (in accordance with Chapter 4.3.) of water from sources that are likely to contain *susceptible species* and may present a *risk* of *pathogenic agent* transmission (e.g. oceans, streams or lakes). The level of treatment required will depend on the identified *risks*.



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- c) Provide an appropriate level of filtration, disinfection or holding (in accordance with Chapter 4.3.) of effluent water from aquaculture establishments (or associated slaughterhouses or processing facilities) where it may present a risk of pathogenic agent transmission to wild aquatic animals or other aquaculture establishments with susceptible species. The level of treatment required will depend on the identified risks.
- ed) Ensuring the position of water intakes and outlets for semi-closed and closed aquaculture establishments, and the location of semi-open aquaculture establishments, minimises contamination from other farmed or wild populations or processing plants, taking into account factors such as distance and water currents.
- e) The likelihood of ingress of contaminated water either through flooding from external sources or from defective infrastructure (e.g. leaking pipes, blocked drains, bund wall failure) should be assessed and appropriate management or infrastructure measures applied.

## 4. Feed

Feed can be an important pathway for transmission of pathogenic agents to aquatic animals. Feed may be initially infected with pathogenic agents or contaminated during harvest, transport, storage and processing of commodities used as feed ingredients. Poor hygiene may contribute to contamination during manufacture, transport, storage and use of feed.

In closed or semi-closed production systems there can be a high level of control on aquatic animal feeds. However, in semi-open production systems, aquatic animals may obtain food from their environment (e.g. filter-feeding molluscs or wild fish which may be preyed on/predated in net pens).

The risk of transmitting pathogenic agents via aquatic animal feed can should be managed by mitigation measures as described/provided in Chapter 4.8., for example using feed and feed ingredients that:

- a) have undergone sufficient processing to inactivate pathogenic agents of concern;
- b) are from sources that are declared free from the pathogenic agents of concern or have been confirmed (e.g. by testing) that pathogenic agents are not present in the feed or feed ingredients commodity;
- c) have been processed, manufactured, stored, and transported and delivered during feeding to aquatic animals in a manner to prevent contamination by pathogenic agents.

## 5. Fomites

Equipment, vehicles, packaging material, clothing, footwear, sediments, infrastructure and other fomites can mechanically transfer pathogenic agents into, within and from an aquaculture establishment.

The level of risk/likelihood of transferring pathogenic agents will depend on the stability of the pathogenic agent in the environment, the presence and nature of organic matter on the fomite surface, as well as the type of surface and its ability/capacity to hold water. The risk/likelihood of transferring pathogenic agents may be higher for fomites which are difficult to clean and disinfect. Sharing e/Equipment that is shared between aquaculture establishments, between aquaculture establishments and processing facilities, or between different production units with unequal health status within an aquaculture establishment with unequal health status, may result in the spread of pathogenic agents present a higher risk than compared to new or dedicated equipment. The risk/likelihood of transmitting pathogenic agents via fomites should be managed; possible mitigation measures include the giving consideration to the following mitigation measures can be managed by:

- a) Assessing the disease risk associated with any fomites brought into the aquaculture establishment for their disease risk.



- b) Ensuring procedures and infrastructure are in place to clean and disinfect fomites, including at designated delivery and loading areas, prior to entry into the aquaculture establishment. Recommendations for the cleaning and ~~disinfection~~ disinfection of fomites are described in Chapter 4.3.
- ~~e) Assigning dedicated equipment for use in production units of different health status. Where equipment must be used in multiple production units it should be cleaned and disinfected prior to movement between units.~~
- c) Wherever possible, dedicating items that are difficult to disinfect items, or those with a high likelihood of contamination, to a specific aquaculture establishment rather than moving them between aquaculture establishments after disinfection.
- d) Applying the mitigation measures described at points a) to c) above to the movement of fomites between production units within an aquaculture establishment with the measures determined based on an evaluation of the disease risks.

## 6. Vectors

Vectors can transport *pathogenic agents* to susceptible *aquatic animals* in *aquaculture establishments*. These They include wild *aquatic animals* entering via the water supply, predators, wild birds, and scavengers, pest animals such as rodents, and people. Vectors can transfer *pathogenic agents* into, within and from an *aquaculture establishment*, either by mechanical transfer or as a developmental stage of the *pathogenic agent* within the vector. The risk of unintentional exposure to vectors will be influenced by the category of aquaculture production system.

The *risk* of transferring *pathogenic agents* via *vectors* varies with the type of vector species, the nature of the *pathogenic agent*, the category of *aquaculture* production system, and the level of *biosecurity*. Measures identified to mitigate risks associated with aquatic animals, as described in point 1, can also be applied to mitigate risks associated with vectors. Mitigation measures for other vectors include:

The risk of transmitting pathogenic agents via vectors should be managed giving consideration to the following mitigation measures:

- a) netting (to prevent access by birds): Physical mitigation measures should be used to prevent the access of vectors to aquaculture establishments including:
  - i) filtering or screening of water entering semi-closed and closed aquaculture production systems to prevent entry of wild aquatic animals;
  - ii) surrounding land-based aquaculture production systems by a fence or a wall to prevent entry of animals and people, with a gate for controlled access;
  - iii) surrounding floating aquaculture production systems by barriers on the establishment perimeter to prevent contact with or entry of wild aquatic animals and other animals;
  - iv) covering outdoor aquaculture production systems with nets to prevent access by birds;
- b) barriers on the establishment perimeter to prevent entry by of other animals (e.g. electric fencing);

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- b) Controlling Access of personnel to aquaculture establishments should be controlled by creating a defined border between the outer risk area and the inner biosecure area comprising facilities for:
- i) changing of clothes and shoes, or use of disposal coverings (hoods, coats, shoe coverings);
  - ii) cleaning and disinfection of hands, and the use of foot baths for shoe disinfection;
- c) Pest control and secure storage of feed and mortalities.

Article 4.X.7.

**Risk analysis**

*Risk analysis* is an accepted approach for evaluating *biosecurity* threats and is used to support the development of mitigation measures. A formal *risk analysis* has four components: *hazard* identification, *risk assessment*, *risk management* and *risk communication* (see Chapter 2.1.). This article elaborates the principles in Chapter 2.1. and applies them for the development of *biosecurity plans* for *aquaculture establishments*.

A *biosecurity plan* may not necessarily require a comprehensive *risk analysis* to evaluate *disease risks* linked to transmission pathways. The chosen approach may depend on the objectives of the *biosecurity plan*, the level of *biosecurity* that is appropriate for the specific production requirements of the *aquaculture establishment*, the complexity of the threats to be addressed, and the availability of information and resources. Depending on these circumstances, a partial analysis may be appropriate, and can build on previous experiences to identify the *hazards* associated with relevant transmission pathways.

The three formal steps of the *risk analysis* process to underpin a the *biosecurity plan* are:

**Step 1 – Hazard Identification**

*Hazard* identification determines which *pathogenic agents* should be the subject of the *risk assessment*. A hazard may include a specific *pathogenic agent* or be defined in more general terms as a group of *pathogenic agents*. This step includes identifying and collecting relevant information on the *pathogenic agents* that have a potential to cause *diseases* in *aquatic animal* populations within an *aquaculture establishment*. This process must consider the *aquatic animal health status* of the establishment and, for semi-open and semi-closed *aquaculture* production systems, the *aquatic animal health status* of the epidemiologically linked environments. The following step is to identify both known and *emerging diseases*, not present in the *aquaculture establishment*, which may negatively impact the farmed population.

To complete the next steps of the *risk assessment*, required information on the identified *hazards* is required needed and includes: i) the frequency of occurrence, ii) the biophysical characteristics, iii) the likelihood of detection if present and iv) the possible transmission pathways (described in Article 4.X.6.). Many of the *hazards* will share the same pathways. ~~A hazard may include a specific *pathogenic agent* or be defined in more general terms as a group of *pathogenic agents*.~~

**Step 2 – Risk Assessment**

A *risk assessment* can be initiated once it has been identified that a biological *hazard* exists and the required information listed under step 1 has been gathered. The aim of the *risk assessment* is to establish a *risk estimate*, which is the product of the likelihood and consequences of entry of a *pathogenic agent* entry into, spread within or release from the *aquaculture establishment*.

A *risk assessment* can be quantitative or qualitative. Both methods require the same conceptual pathway which identifies the necessary steps for *hazard* introduction, establishment and spread to be constructed. In a qualitative assessment, introduction and establishment are estimated using descriptors of likelihood. A quantitative assessment requires data on which to estimate likelihood. In most circumstances, transmission pathways will be assessed qualitatively but within a formal *risk assessment* framework. Examples of descriptors for **qualitative** estimates of likelihood and consequence are given in Tables 1 and 2. Table 3 illustrates how estimates of likelihood and consequence can be combined in a matrix to give an estimate of *risk*.

**Table 1. Qualitative descriptors of likelihood**

Estimate	Descriptor
Remote	Never heard of, but not impossible.
Unlikely	May occur here, but only in rare circumstances.
Possible	Clear evidence to suggest this is possible in this situation.
Likely	It is likely, but not certain, to occur here.
Certain	It is certain to occur.

**Table 2. Qualitative descriptors of consequences**

Estimate	Descriptor <b>of consequences at level of the aquaculture establishment</b>
Insignificant	Impact not detectable or minimal. <b>No trade impact.</b>
Minor	<b>Impact Limited decreased production on aquaculture establishment productivity limited to some affecting only a small number of production-units or short-term, and/or very limited and transitory disruption to trade, only.</b>
Moderate	<b>Widespread impact on aquaculture establishment productivity due to increased mortality or decreased performance. Decreased production (e.g. sustained increased mortality or decreased growth rate) and/or some short-term to medium-term disruption to trade, resulting in financial loss.</b>
Major	<b>Considerable, decreased impact on aquaculture establishment production, and/or some medium-term to long-term disruption to trade, resulting in significant financial loss, resulting in serious supply constraints and financial impact.</b>
Catastrophic	<b>Complete depopulation, production loss, in of the aquaculture establishment and possibly barriers to resumption of production, and/or complete loss of trade, resulting in extreme financial loss.</b>

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**Table 3. Matrix for assessing risk**

	Consequence rating					
Likelihood estimate		insignificant	minor	moderate	major	catastrophic
	remote	negligible	low	low	low	medium
	unlikely	low	low	medium	medium	high
	possible	low	medium	medium	high	high
	likely	low	medium	high	high	extreme
	certain	medium	high	high	extreme	extreme

Results of *rRisk* assessments informs which biological hazards need to be addressed, which critical control points on the transmission pathway should be targeted, and the measures which that are most likely to be effective in reducing risk.

**Table 4. Interpretation of risk estimates**

Risk level*	Explanation and management response
Negligible	Acceptable level of risk. No action required.
Low	Acceptable level of risk. On-going monitoring may be required.
Medium	Unacceptable level of risk. <u>Active management Review and strengthen the risk mitigation measures is required to reduce the level of risk.</u>
High	Unacceptable level of risk. <u>Intervention Identify and implement additional mitigation measures is required to mitigate the risk.</u>
Extreme	Unacceptable level of risk. <u>Take immediate action to mitigate the risk. Urgent intervention is required to mitigate the level of risk.</u>

\*The *Risk* level is determined by from a combination of the likelihood and consequence scores obtained using the risk matrix (Table 3).

**Step 3 – Risk Management**

*Risk management* is used to determine the appropriate management response for the assessed level of risk as described in Table 4. The *risk assessment* process identifies the steps within transmission pathways necessary for a risk to be realised and thus allows the most effective mitigation measures to be determined. Many of the hazards will share the same pathways and thus therefore mitigation measures may be effective against more than one hazard. Information on hazards and their pathways of introduction (step 1) should be combined with the assessment of the pathways (step 2) to identify the most appropriate and cost-effective risk mitigation measures.

Article X.X.6. describes some possible mitigation measures relevant for to different transmission pathways. The most appropriate mitigation measures for a specific *aquaculture establishment* will depend on the risks hazards identified, the effectiveness and reliability of the mitigation measure, the category of *aquaculture* production system and cost.

After the implementation of the *biosecurity plan*, *hazards* should be regularly reassessed, and measures adjusted according to any changed *risk* estimates.

#### Article 4.X.8.

##### Biosecurity plan development

The purpose of a *biosecurity plan* is primarily to reduce the *risk* of introducing *pathogenic agents* into an *aquaculture establishment*, and if *pathogenic agents* are introduced, to reduce the *risk* of further spread within or release from the *aquaculture establishment*. The plan will document identified transmission pathways and the outputs of any *risk analysis* performed (*hazards*, *risk estimate* and mitigation measures), and information relevant to ongoing implementation, monitoring and review of the plan.

##### 1. Development of a biosecurity plan

The process to of developing a *biosecurity plan* will vary depending on its objectives of the biosecurity plan, the level of *biosecurity* appropriate to the specific production system requirements, the complexity of the *disease risks* to be addressed, and availability of information and resources. Consideration and documentation of the following issues are recommended:

- a) objectives and regulatory requirements for the *biosecurity plan*;
- b) information about the *aquaculture establishment* including an up-to-date plan of the layout of buildings and production units (including epidemiological units, if any, and structures and the processes to maintain separation methods), loading/ unloading, unpacking, processing, feed storage, waste storage, reception areas, and maps showing major movements of *aquatic animals*, *aquatic animal products* and *aquatic animal waste waste*, water, feed and fomites (including staff, equipment and vehicles);
- c) the potential pathways for entry of *pathogenic agents* into, spread within or release from the *aquaculture establishment* (refer to Article X.X.6. above);
- d) a *risk analysis*, including identification of the major *disease hazards* to the *aquaculture establishment* (refer to Article X.X.7. above);
- e) the mitigation measures that have been determined to address identified *risks*;
- f) emergency procedures in the event of a *biosecurity* failure. These They may include reporting requirements, emergency measures to eradicate pathogenic agents such as aquatic animal depopulation and disposal, and site disinfection, in accordance with Chapters 4.3. and 7.4.;
- g) standard operating procedures required to support implementation of the mitigation measures, emergency procedures and the training requirements of personnel;
- g#) internal and external communication procedures, and roles and responsibilities of personnel aquaculture establishment staff and essential contact information, e.g. for personnel, staff, farm veterinarian and the Competent Authority;

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- h) monitoring and audit schedule;
- ij) performance evaluation;
- i) standard operating procedures required to support **all** implementation of the mitigation measures, emergency procedures and the training requirements of personnel.

2. Key components of a biosecurity plan

## a) Standard operating procedures (SOPs)

SOPs describe routine management processes that must be performed to support the effectiveness of the *biosecurity plan*. Each SOP should clearly describe its objectives, staff responsibilities, the procedure (including record keeping), precautions and a review date.

Staff should be trained in the application of the SOPs including completion of forms, checklists and other records associated with each procedure, as well as routine communication requirements.

## b) Documentation and record keeping

The *biosecurity plan* describes **the** documentation necessary to provide evidence of compliance with the mitigation measures. The level of detail required in the documentation depends on the outcomes of the transmission pathway assessment.

Examples of documentation required **may** include: *aquaculture establishment* layout, movements of *aquatic animals*, ~~escapees~~, origin and destination and health status of the *aquatic animals* introduced to the *aquaculture establishment*, records of visitors to the establishment, ~~escapees~~, stocking densities, feeding and growth rates, records of staff training, treatments/vaccination, water quality, cleaning and disinfection events, morbidity and mortality (including removal and disposal of mortalities), *surveillance* and laboratory records.

## c) Emergency procedures

Procedures should be developed and, when necessary, implemented to minimise the impact of emergencies, *disease events*, or unexplained mortality in *aquatic animals*. These procedures should include clearly defined thresholds that help to identify an emergency incident and activate response protocols, including reporting requirements.

## d) Health monitoring

Health monitoring as part of the *biosecurity plan* involves monitoring of the health status of *aquatic animals* in *aquaculture establishments*. Activities may include *disease surveillance*, routine monitoring of stock for important health and production parameters (e.g. by staff, an *aquatic animal health professional* or a *veterinarian*), recording of clinical signs of *disease*, morbidity and mortality, and analysis of these data (e.g. calculation of rates of morbidity and mortality and diseases).

## e) Routine review and auditing

The *biosecurity plan* should describe a systematic auditing schedule to verify implementation and compliance with the requirements of the *biosecurity plan*. Routine revision of the *biosecurity plan* is necessary to ensure that it continues to effectively address *biosecurity risks*.

The *biosecurity plan* should also be reviewed at least annually or in response to changes to the *aquaculture establishment* operations, changes in facility design, changes to in husbandry approaches, identification of a new *disease risk*, or the occurrence of a *biosecurity* incident. *Biosecurity* incidents, and actions taken to remedy them, should be documented to enable SOP re-assessments of SOPs.





## CHAPTER 1.3.

## DISEASES LISTED BY THE OIE

**EU Comment:**

**The EU thanks the OIE for listing ‘Infection with decapod iridescent virus 1’ and supports the proposal for its inclusion in the list.**

[...]

## Article 1.3.3.

The following *diseases* of crustaceans are listed by the OIE:

- Acute hepatopancreatic necrosis disease
- Infection with *Aphanomyces astaci* (crayfish plague)
- Infection with *Hepatobacter penaei* (necrotising hepatopancreatitis)
- Infection with infectious hypodermal and haematopoietic necrosis virus
- Infection with infectious myonecrosis virus
- Infection with *Macrobrachium rosenbergii* nodavirus (white tail disease)
- Infection with shrimp haemocyte iridescent virus Infection with decapod iridescent virus 1
- Infection with Taura syndrome virus
- Infection with white spot syndrome virus
- Infection with yellow head virus genotype 1.

[...]



**Model Article 10.X.13. for the fish disease-specific  
Chapters 10.5., 10.6. and 10.10. (and ~~or~~ Article  
10.4.17. for  
Chapter 10.4. Infection with infectious  
salmon anaemia virus)**

**EU Comment:**

**The EU thanks the OIE and supports the proposed changes to Model Article 10.X.13.**

**Particular comments are inserted within the body of the text.**

[...]

Article 10.X.13.

Importation of disinfected eggs for aquaculture from a country, zone or compartment not declared free from infection with [pathogenic agent X]

- 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 [pathogenic agent X], the Competent Authority of the importing country should assess ~~the risk associated with~~ at least the following:

**EU comment:**

**It isn't possible for the importing country to assess the points raised in 1) without gaining assurances from the exporting country. We therefore suggest a rewording of the statement as follows:**

- 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 [pathogenic agent X], the Competent Authority of the importing country should assess and gain assurances from the Competent Authority of the exporting country that the risk associated with at least the following has been considered:

- a) ~~the infection with pathogenic agent X likelihood that status of the water to be used during the disinfection of the eggs is contaminated with~~ [pathogenic agent X];

**EU Comment:**

**It is suggested that point a) should make reference to chapter 4.4 – Recommendation for**

surface disinfection of salmonid eggs

~~a )the infection with pathogenic agent X~~ likelihood that status of the water to be used during the *disinfection* of the eggs is contaminated with [pathogenic agent X]; by gaining assurance that the protocol in Article 4.4.2. of Chapter 4.4 has been applied by the exporting country

**Justification:** the importing country should seek assurances that the protocol in 4.4 is followed, which specifically states that pathogen free water should be used. This would add clarity to this specific point.

- b) the prevalence of infection with [pathogenic agent X] in broodstock (including by testing of ovarian fluid and milt); and
- c) the temperature and pH of the water to be used for *disinfection*.

**EU Comment:**

We would suggest further clarification on point c). Reference should be made clearly to Chapter 4.4 as this gives more detail on pH requirements. We would also note that there is no mention of the temperature of the disinfection water anywhere in the chapter and that this should be clarified.

A style comment: strictly speaking water is not used as a disinfectant, it is used for the dilution of the disinfectant. For this reason we have added some text to the phrase to improve clarity.

c) the temperature and pH of the water to be used to dilute the disinfectant for disinfection as described in Article 4.4.1 of Chapter 4.4.

- 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, in accordance with recommendations in Chapter 4.4. of 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.

The *Competent Authority* may wish to consider internal measures, such as additional ~~renewed~~ *disinfection* of the eggs upon arrival in the *importing country*.

**EU Comment:**

We propose changing the sentence above to the following:

The *Competent Authority* ~~may wish to~~ must consider internal measures, such as

additional ~~renewed~~ *disinfection* of the eggs upon arrival in the *importing country*.

**Justification:** Given the points outlined in Section 1 and the last sentence of 2 it is clear that the trade in disinfected eggs from countries, zones or compartments not declared disease free presents a significant risk and should be treated with caution . Furthermore, evidence suggests that viral titres for IHN, can be so high in ovarian fluid that disinfection protocols can be insufficient (Roberts, 1993).

**Reference:** Roberts SD. IHN at Lyons Ferry Hatchery: a case study of vertical transmission. *Fish Health Sect Am Fish Soc Newslett.* 1993;21:13–14.

- 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 pathogenic agent X, the *Competent Authority* of the *importing country* should require that the consignment be accompanied by an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* certifying that the procedures described in point 2 of this article have been fulfilled.

[...]

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## CHAPTER 10.10

INFECTION WITH VIRAL HAEMORRHAGIC  
SEPTICAEMIA VIRUS**EU Comment:**

The EU thanks the OIE ad hoc group for the work which has been completed in relation to the susceptibility of fish species to infection with VHS and welcomes the outcome from their assessments.

The EU supports the amendment to the list of susceptible species in Article 10.10.2 and also requests that *Salvelinus fontinalis* and *Lota lota* should be considered for listing as species which are susceptible to VHS. Mortalities in *S. fontinalis* have been reported under natural conditions in a hatchery within the EU and the cause of infection was confirmed to be VHS genotype Ia. This virus was subsequently used to infect *Lota lota* using experimental bath challenge and the virus was subsequently isolated from all dead or euthanised fish.

For *Danio rerio*, Zebra fish, we believe that natural infection has not been proven, only experimental infection by immersion as well as injection.

[...]

Article 10.10.2.

**Scope**

The recommendations in this chapter apply to the following species that meet the criteria for listing as susceptible in accordance with Chapter 1.5: rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), grayling (*Thymallus thymallus*), white fish (*Coregonus* spp.), pike (*Esox lucius*), turbot (*Scophthalmus maximus*), herring and sprat (*Clupea* spp.), Pacific salmon (*Oncorhynchus* spp.), Atlantic cod (*Gadus morhua*), Pacific cod (*Gadus macrocephalus*), haddock (*Gadus aeglefinus*) and rockling (*Onos mustelus*). These recommendations also apply to any other susceptible species referred to in the Aquatic Manual when traded internationally.

<u>Family</u>	<u>Scientific name</u>	<u>Common name</u>	<u>Genotype</u>
<u>Ammodytidae</u>	<u><i>Ammodytes hexapterus</i></u>	<u>Pacific sand lance</u>	<u>IVa</u>
<u>Aralichthyidae</u>	<u><i>Paralichthys olivaceus</i></u>	<u>Bastard halibut</u>	<u>IVa</u>
<u>Carangidae</u>	<u><i>Trachurus mediterraneus</i></u>	<u>Mediterranean horse mackerel</u>	<u>Ie</u>
<u>Centrarchidae</u>	<u><i>Ambloplites rupestris</i></u>	<u>Rock bass</u>	<u>IVb</u>
	<u><i>Lepomis gibbosus</i></u>	<u>Pumpkinseed</u>	<u>IVb</u>
	<u><i>Lepomis macrochirus</i></u>	<u>Bluegill</u>	<u>IV, IVb</u>
	<u><i>Micropterus dolomieu</i></u>	<u>Smallmouth bass</u>	<u>IVb</u>
	<u><i>Micropterus salmoides</i></u>	<u>Largemouth bass</u>	<u>IVb</u>

<b><u>Family</u></b>	<b><u>Scientific name</u></b>	<b><u>Common name</u></b>	<b><u>Genotype</u></b>
	<u><i>Pomoxis nigromaculatus</i></u>	<u>Black crappie</u>	<u>IVb</u>
<b><u>Clupeidae</u></b>	<u><i>Alosa immaculata</i></u>	<u>Pontic shad</u>	<u>Ie</u>
	<u><i>Sardina pilchardus</i></u>	<u>Pilchard</u>	
	<u><i>Clupea harengus</i></u>	<u>Atlantic herring</u>	<u>Ib, III</u>
	<u><i>Clupea pallasii pallasii</i></u>	<u>Pacific herring</u>	<u>IVa</u>
	<u><i>Dorosoma cepedianum</i></u>	<u>American gizzard shad</u>	<u>IVb</u>
	<u><i>Sardinops sagax</i></u>	<u>South American pilchard</u>	<u>IVa</u>
	<u><i>Sprattus sprattus</i></u>	<u>European sprat</u>	<u>Ib</u>
<b><u>Cyclopteridae</u></b>	<u><i>Cyclopterus lumpus</i></u>	<u>Lumpfish</u>	<u>IVd</u>
<b><u>Cyprinidae</u></b>	<u><i>Danio rerio</i></u>	<u>Zebra fish</u>	<u>IVa</u>
	<u><i>Notropis hudsonius</i></u>	<u>Spottail shiner</u>	<u>IVb</u>
	<u><i>Notropis atherinoides</i></u>	<u>Emerald shiner</u>	<u>IVb</u>
	<u><i>Pimephales notatus</i></u>	<u>Bluntnose minnow</u>	<u>IVb</u>
	<u><i>Pimephales promelas</i></u>	<u>Fathead Minnow</u>	<u>IVb</u>



## Annex 6 (contd)

<u>Family</u>	<u>Scientific name</u>	<u>Common name</u>	<u>Genotype</u>
<u>Embiotocidae</u>	<u><i>Cymatogaster aggregata</i></u>	<u>Shiner perch</u>	<u>IVa</u>
<u>Engraulidae</u>	<u><i>Engraulis encrasicolus</i></u>	<u>European anchovy</u>	<u>Ie</u>
<u>Esocidae</u>	<u><i>Esox lucius</i></u>	<u>Northern pike</u>	<u>IVb</u>
	<u><i>Esox masquinongy</i></u>	<u>Muskellunge</u>	<u>IVb</u>
<u>Fundulidae</u>	<u><i>Fundulus heteroclitus</i></u>	<u>Mummichog</u>	<u>IVc</u>
<u>Gadidae</u>	<u><i>Gadus macrocephalus</i></u>	<u>Pacific cod</u>	<u>IVa</u>
	<u><i>Gadus morhua</i></u>	<u>Atlantic cod</u>	<u>Ib, III</u>
	<u><i>Merlangius merlangus</i></u>	<u>Whiting</u>	<u>Ie</u>
	<u><i>Micromesistius poutassou</i></u>	<u>Blue Whiting</u>	<u>Ib, III</u>
	<u><i>Trisopterus esmarkii</i></u>	<u>Norway pout</u>	<u>Ib, III</u>
<u>Gasterosteidae</u>	<u><i>Gasterosteus aculeatus</i></u>	<u>Three-spine stickleback</u>	<u>IVc</u>
<u>Gobiidae</u>	<u><i>Neogobius melanostomus</i></u>	<u>Round goby</u>	<u>IVb</u>
	<u><i>Pomatoschistus minutus</i></u>	<u>Sand goby</u>	<u>Ib</u>
<u>Ictaluridae</u>	<u><i>Ictalurus nebulosus</i></u>	<u>Brown bullhead</u>	<u>IVb</u>
<u>Labridae</u>	<u><i>Centrolabrus exoletus</i></u>	<u>Rock cook wrasse</u>	<u>III</u>
	<u><i>Ctenolabrus rupestris</i></u>	<u>Goldsinny wrasse</u>	<u>III</u>
	<u><i>Labrus bergylta</i></u>	<u>Ballan wrasse</u>	<u>III</u>
	<u><i>Labrus mixtus</i></u>	<u>Cuckoo wrasse</u>	<u>III</u>
	<u><i>Symphodus melops</i></u>	<u>Corkwing wrasse</u>	<u>III</u>
<u>Lotidae</u>	<u><i>Gaidropsarus vulgaris</i></u>	<u>Three-bearded rockling</u>	<u>Ie</u>
<u>Moronidae</u>	<u><i>Morone americana</i></u>	<u>White Perch</u>	<u>IVb</u>
	<u><i>Morone chrysops</i></u>	<u>White Bass</u>	<u>IVb</u>
	<u><i>Morone saxatilis</i></u>	<u>Striped bass</u>	<u>IVb, IVc</u>
<u>Mullidae</u>	<u><i>Mullus barbatus</i></u>	<u>Red mullet</u>	<u>Ie</u>
<u>Osmeridae</u>	<u><i>Thaleichthys pacificus</i></u>	<u>Eulachon</u>	<u>IVa</u>
<u>Percidae</u>	<u><i>Sander vitreus</i></u>	<u>Walleye</u>	<u>IVb</u>
	<u><i>Perca flavescens</i></u>	<u>Yellow perch</u>	<u>IVb</u>
<u>Petromyzontidae</u>	<u><i>Lampetra fluviatilis</i></u>	<u>River lamprey</u>	<u>II</u>
<u>Pleuronectidae</u>	<u><i>Limanda limanda</i></u>	<u>Common dab</u>	<u>Ib</u>
	<u><i>Platichthys flesus</i></u>	<u>European flounder</u>	<u>Ib</u>
	<u><i>Pleuronectes platessus</i></u>	<u>European plaice</u>	<u>III</u>
<u>Rajidae</u>	<u><i>Raja clavata</i></u>	<u>Thornback ray</u>	<u>Ie</u>

<u>Family</u>	<u>Scientific name</u>	<u>Common name</u>	<u>Genotype</u>
<u>Salmonidae</u>	<u><i>Coregonus artedii</i></u>	<u>Lake cisco</u>	<u>IVb</u>
	<u><i>Coregonus clupeaformis</i></u>	<u>Lake whitefish</u>	<u>IVb</u>
	<u><i>Coregonus lavaretus</i></u>	<u>Common whitefish</u>	<u>Ia</u>
	<u><i>Oncorhynchus kisutch</i></u>	<u>Coho salmon</u>	<u>Iva</u>
	<u><i>Oncorhynchus mykiss</i></u>	<u>Rainbow trout</u>	<u>Ia-e, III, IVb</u>
	<u><i>Oncorhynchus mykiss X Oncorhynchus kisutch hybrids</i></u>	<u>Rainbow trout X coho salmon hybrids</u>	<u>Ia</u>
	<u><i>Oncorhynchus tshawytscha</i></u>	<u>Chinook salmon</u>	<u>IVa, IVb</u>
	<u><i>Salmo marmoratus</i></u>	<u>Marble trout</u>	<u>Ia</u>
	<u><i>Salmo salar</i></u>	<u>Atlantic salmon</u>	<u>Ia, Ib, II, III, IVa</u>
	<u><i>Salmo trutta</i></u>	<u>Brown trout</u>	<u>Ia, Ib</u>
	<u><i>Salvelinus namaycush</i></u>	<u>Lake trout</u>	<u>Ia, IVa, IVb</u>
	<u><i>Thymallus thymallus</i></u>	<u>Grayling</u>	<u>I</u>
	<u>Scophthalmidae</u>	<u><i>Scophthalmus maximus</i></u>	<u>Turbot</u>
<u>Sciaenidae</u>	<u><i>Aplodinotus grunniens</i></u>	<u>Freshwater drum</u>	<u>IVb</u>
<u>Scombridae</u>	<u><i>Scomber japonicus</i></u>	<u>Pacific Chub mackerel</u>	<u>IVa</u>
<u>Soleidae</u>	<u><i>Solea senegalensis</i></u>	<u>Senegalese sole</u>	<u>III</u>
<u>Uranoscopidae</u>	<u><i>Uranoscopus scaber</i></u>	<u>Atlantic stargazer</u>	<u>Ie</u>

[...]

## CHAPTER 10.9.

INFECTION WITH SPRING VIRAEMIA OF  
CARP VIRUS**EU Comment:**

**The EU thanks the OIE and supports the proposed changes to Chapter 10.9**

[...]

Article 10.9.2.

**Scope**

The recommendations in this chapter apply to the following species that meet the criteria for listing as susceptible in accordance with Chapter 1.5.:

all varieties and subspecies of common carp (*Cyprinus carpio*), bighead carp (*Aristichthys nobilis*), bream (*Abramis brama*), Caspian white fish (*Rutilus kutum*), fathead minnow (*Pimephales promelas*), golden shiner (*Notemigonus crysoleucas*), goldfish (*Carassius auratus*), grass carp (*Ctenopharyngodon idella*), roach (*Rutilus rutilus*) and sheatfish (also known as European or wels catfish) (*Silurus glanis*).

<u>Family</u>	<u>Scientific name</u>	<u>Common name</u>
Cyprinidae	<i>Abramis brama</i>	Bream
	<i>Aristichthys nobilis</i>	Bighead carp
	<i>Carassius auratus</i>	Goldfish
	<i>Ctenopharyngodon idella</i>	Grass carp
	<i>Cyprinus carpio</i>	Common carp (all varieties and subspecies)
	<u><i>Danio rerio</i></u>	<u>Zebrafish</u>
	<i>Notemigonus crysoleucas</i>	Golden shiner
	<i>Pimephales promelas</i>	Fathead minnow
	<i>Rutilus kutum</i>	Caspian white fish
	<i>Rutilus rutilus</i>	Roach
Siluridae	<i>Silurus glanis</i>	Sheatfish (also known as European or wels catfish)

[...]

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UNOFFICIAL VERSION

## PATHWAYS FOR CLAIMING FREEDOM FROM DISEASE IN THE OIE *AQUATIC ANIMAL HEALTH CODE*

A discussion paper developed by the OIE Aquatic Animal Health Standards Commission  
for Member Countries' comment.

Version 2: October 2019

### EU Comment:

**The EU thanks the OIE and supports the proposed changes.**

**We also appreciate the work the OIE have put into this paper, however we are disappointed to note the apparent change in purpose of it as stated in the report - that it 'will provide additional flexibility (e.g. to use secondary sources of data) and improve rigour in determining the required surveillance periods specified in the disease-specific chapters of the Aquatic Code.' This appears to be a significant change to its original intended purpose (to be used to revise the Aquatic Code articles relevant to declaration of freedom). It is important that the intended purpose of the discussion paper is clarified to ensure the strength of the current standards relating to declarations of disease freedom, is not weakened.**

**Other comments are inserted in the text below.**

### SUMMARY

This paper aims to explore improvements to the standards of the *Aquatic Code*<sup>4</sup> for demonstration of freedom from OIE *listed diseases*. These standards are provided through several inter-related parts of the *Aquatic Code*, for example: articles X.X.4. (*free country*), X.X.5. (*free zone or free compartment*) and X.X.6. (maintenance of freedom) of each disease-specific chapter (except Infection with ISAV, for which numbering differs); Chapter 1.4. *Aquatic animal health surveillance*; and relevant definitions in the glossary (e.g. *basic biosecurity conditions* and *early detection system*).

Version 1 of this discussion paper was circulated to Member Countries in the September 2018 meeting report of the Aquatic Animal Health Standards Commission (hereafter called Aquatic Animals Commission). Member Countries were invited to provide responses to 15 questions regarding different provisions of the *Aquatic Code* relevant to declaration of freedom (refer to Appendix 1). Responses were received from the African Union – Interafrican Bureau for Animal Resources, Australia, Canada, China (The Peoples Rep. of), Chinese Taipei, the European Union, Japan, Malaysia, Mexico, New Caledonia, New Zealand, Norway, Thailand, and Vietnam, representing 94 member states of the OIE.

This Version 2 of the discussion paper includes summaries of the responses provided by Member Countries on Version 1 and revised recommendations throughout the paper based on those responses. These recommendations form the basis of the proposed approach by the Aquatic Animals Commission to the revision of *Aquatic Code* articles relevant to declaration of freedom.

Following this consultation with Member Countries, the Aquatic Animals Commission proposes to retain the broad framework of requirements in the *Aquatic Code* for declaration of freedom for reasons of simplicity and practicality and to provide confidence among *Member Countries* in the strength of self-declarations of freedom (principles A to D of section 2 below). However, the revised approaches recommended in this discussion paper will also provide additional flexibility (e.g. to use secondary sources of data) and improve rigour in determining the required surveillance periods specified in disease-specific chapters of the *Aquatic Code* (principles A, D, E of section 2 below). Some of the key approaches recommended include:

1. Pathway 1 – absence of *susceptible species* (refer to section 3.1.)

<sup>4</sup> For the purposes of this paper, some terms are italicised to indicate that they have a defined meaning in the *Aquatic Code*

- This pathway will be retained in the *Aquatic Code* and further guidance on its application, including appropriate circumstances of use and required standards of evidence, will be incorporated into Chapter 1.4.
- This pathway will not be available for pathogens that are considered to have a broad and uncertain range of *susceptible species*.

**EU Comment:**

**The EU would like to suggest further reflection on the above position where it says that this pathway is not available for species with a broad host range (e.g. VHS).**

**We would like the OIE Aquatic Commission to consider defining the key information that should be included in a self-declaration of freedom (as referred to in Part 6 of the document) and allow countries to put forward relevant evidence and data sources as part of that process, given that there may at least be zones or compartments in their territory where the relevant species do not exist.**

2. Pathway 2 – historical freedom (refer to section 3.2.)

- Requirements for this pathway will aim to achieve 95% confidence of disease freedom for countries and zones—primarily through passive surveillance data. The pathway will not be available for *compartments*.

**EU Comment:**

**We believe that the option to obtain disease-freedom on historical grounds should also be available for compartments.**

**A practical example of how this might be useful is for example, a compartment in which *O.mykiss* are grown which has been free of VHS for more than 10 years and where historical freedom from IHN would now be possible, given that basic biosecurity conditions have been in place during the period and increased mortality or the suspicion of the presence of disease has been investigated.**

**We would also like to suggest that if it is to be used properly, guidance will be required in relation to Table 1 included later at the end of section 3.2 . in relation to the likelihood of pathogenic agents being detected by passive surveillance based on an annual likelihood of detection and duration of surveillance. In addition, we believe that further guidance is required in relation to the process whereby the annual likelihood of pathogen detection is assessed.**

- Requirements for evaluating the performance of *early detection systems* (which provide passive surveillance data) will be elaborated in Chapter 1.4.
- It will be possible to use secondary sources of evidence for this pathway (i.e. *targeted surveillance* data).
- The pathway will not be available for pathogenic agents under circumstances where infection would not manifest clinically and subsequently be observed, reported and investigated as part of the country's *early detection system* (e.g. ISAV HPR0).

Annex 8 (contd)

3. Pathway 3 – unknown disease status (refer to section 3.3.)

- Prior to commencement of *targeted surveillance*, *basic biosecurity conditions* will need to be in place for a period of time to prevent introduction and establishment of the disease. Criteria for defining this period for each disease-specific chapter of the *Aquatic Code* will be included in Chapter 1.4 but the default minimum will be one year.
- *Targeted surveillance* for countries and zones will be required to occur over a minimum period of time. Criteria for defining this period for each disease-specific chapter of the *Aquatic Code* will be included in Chapter 1.4. but the default minimum will be two years.
- *Targeted surveillance* for compartments will be required to occur over a minimum period of time. Criteria for defining this period for each disease-specific chapter of the *Aquatic Code* will be included in Chapter 1.4. but the default minimum will be one year.

**EU Comment:**

**We do not support the shortening of the testing period for compartments with an unknown disease status, to obtain disease-freedom.**

**We acknowledge that the text provides a caveat that the default period should only be shortened to one year ‘if warranted by the epidemiology of the disease’ but we would advise a testing period the same as the one that applies in countries and zones, for compartments.**

- It will be possible to use passive surveillance data as additional evidence of freedom where it can be demonstrated to be an appropriately sensitive method for detection of the disease in populations of *susceptible species*.

4. Pathway 4 – Return to freedom (refer to section 3.4.)

**EU Comment:**

**This section deals with a country, zone or compartment which has previously made a self-declaration of freedom but which has subsequently had a disease outbreak. The scenario which appears to be missing from the document is freedom in a country, zone or compartment which was previously infected and which has now eradicated the disease, but which is making its first declaration of disease-freedom.**

**We believe that these two scenarios could be combined in this pathway, which could be re-titled ‘Freedom following infection’**

- For compartments, a mechanism to regain freedom following an outbreak will be included in each of the disease-specific chapters of the *Aquatic Code*. Surveillance will be required within the *compartment* following depopulation and decontamination to demonstrate that eradication has been successful and to test the reviewed biosecurity conditions.
- For countries and zones, surveillance to regain freedom will be required over a period of time that is determined by the epidemiology of the specific disease and will be included in each of the disease-specific chapters of the *Aquatic Code*. The default minimum period of surveillance will be consistent with requirements for the original declaration of freedom. However, a general provision in Chapter 1.4. of the *Aquatic Code* will allow a self-declaration of freedom to be made sooner if Member Countries can demonstrate that the approach would provide an appropriate standard of evidence for the circumstances of the outbreak and the disease.

**EU Comment:**

**Pathway 4 puts a strong emphasis on determining if countries and zones should be allowed to return to freedom more quickly following an eradication programme, than in the initial programme to obtain freedom.**

**We welcome the fact that the default minimum period of surveillance for countries and zones will however, remain consistent with the surveillance period required in relation to the original declaration of freedom.**

- Clearer guidance will be provided on establishing *infected* and *protection zones* and sampling within them (for farmed and wild animals) in Chapter 1.4. of the *Aquatic Code*.

**EU Comment:**

**We would like to suggest an addition to the text (or perhaps the provision of guidance) which would be very useful: a description of the steps which should be taken when ‘re-defining’ a country into a zone , or a zone into a smaller one, following a disease outbreak, or a number of outbreaks in a country or zone which has been declared disease-free.**

## 5. Maintaining freedom (refer to section 4)

- For pathways 2, 3 and 4, maintenance of free status will require evidence that *basic biosecurity conditions* have been continuously met.
- If *targeted surveillance* that was required for initial demonstration of freedom is to be discontinued for any identified population, evidence must be provided that passive surveillance, as provided by the country’s *early detection system*, would detect the disease in those populations should it occur.
- Any ongoing *targeted surveillance* to maintain freedom should be undertaken at a level necessary to maintain confidence of freedom and should take into account the likelihood of infection.
- Additional guidance on what constitutes ‘conditions conducive to clinical expression of infection’ will be included in Chapter 1.4 of the *Aquatic Code*, as will guidance on how to evaluate or test an *early detection system*.

## 6. Chapter 1.4. Aquatic Animal Surveillance (refer to section 5)

This chapter will be substantially revised to provide improved guidance and support the recommendations described above, specifically to:

- Include the criteria recommended in this paper for determining the relevant time periods (for *basic biosecurity conditions* and periods of surveillance) in each of the disease-specific chapters of the *Aquatic Code*.
- Provide flexibility to use secondary forms of surveillance data for relevant pathways.

Annex 8 (contd)

- Include guidance on regaining freedom following eradication of a disease at the level of a country, zone or *compartment* (not currently included), and maintaining freedom; including guidance on a risk-based approach to survey design.



- Include additional guidance on what constitutes an *early detection system*, including how it may be evaluated.
- Include guidance on what constitutes ‘conditions conducive to clinical expression of infection’.
- Include guidance on establishing *infected* and *protection zones* and sampling within them for farmed and wild animals.

Member Countries are invited to comment on this Version 2 of the discussion paper, in particular on the recommended approaches included throughout. Member Countries need not reiterate comments made previously as these have been, and will continue to be considered by the Aquatic Animals Commission. However, new perspectives or information to inform aspects of this work are welcomed.

## 1. BACKGROUND

Chapter 1.4. of the *OIE Aquatic Animal Health Code (Aquatic Code)* describes four pathways through which Member Countries can make self-declarations of freedom from a disease. These four pathways are also reflected in each of the disease-specific chapters of the *Aquatic Code* in Article X.X.4. (Country free from disease X), and Article X.X.5. (*Zone* or *compartment* free from disease X). Additionally, Article X.X.6. describes the requirements for maintenance of free status. An example of these articles is provided at [Appendix 2](#).

Within Articles X.X.4. and X.X.5., several periods of time are specified for which a country must have *basic biosecurity conditions*<sup>5</sup> in place or for which surveillance must be conducted. These periods are applied differently across the four pathways for claiming freedom at the country, *zone* or *compartment* level and among various *listed diseases*. [Appendix 3](#) provides a summary for all *listed diseases* of the relevant periods for declaration of country freedom that are included in the *Aquatic Code*. There has not previously been any documented rationale on the considerations or criteria for determining these periods.

Member Countries have previously requested that the Aquatic Animals Commission explain how these periods are determined. In 2016, the Commission requested that an OIE *ad hoc* Group be established to consider this issue and provide advice on appropriate criteria or guidelines for determining the periods in Articles X.X.4. and X.X.5. The *ad hoc* Group on Demonstration of disease freedom met twice in 2017. It found that the requested task could not be separated from a broader review of the structure of Articles X.X.4. and X.X.5. in each disease-specific chapter of the *Aquatic Code*. Although it progressed consideration of these issues, the *ad hoc* Group was not able to develop recommendations that were sufficiently advanced to provide to Member Countries for their comments.

At its February 2018 meeting, the Aquatic Animals Commission considered progress that had been made and agreed that the next step would be for the Commission to prepare a discussion paper to explore the rationale for determining the time periods included in Articles X.X.4. and X.X.5. of each disease-specific chapter of the *Aquatic Code*. The present document serves this purpose, and has been developed in consideration of the *ad hoc* Group’s discussions and the guidance documents that had been provided to it by the Commission.

While the primary task of this paper is to consider the time periods in Articles X.X.4. and X.X.5., there are other related issues that are explored. For example, the Aquatic Animals Commission has also recognised that Articles X.X.4. and X.X.5. are somewhat inflexible. For instance, certain types of surveillance data are specified which may not be appropriate or practical in all circumstances, e.g. for *compartments*. Revision of *Aquatic Code* provisions for declaration of freedom may provide an opportunity to reflect more flexible, outcome-based surveillance methods.

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<sup>5</sup> Basic Biosecurity Conditions is a defined term in the OIE *Aquatic Code* which means:  
 “a set of conditions applying to a particular disease, and a particular zone or country, required to ensure adequate disease security, such as:

- the disease, including suspicion of the disease, is compulsorily notifiable to the Competent Authority; and
- an early detection system is in place within the zone or country; and
- import requirements to prevent the introduction of disease into the country or zone, as outlined in the *Aquatic Code*, are in place.”

Annex 8 (contd)**2. OBJECTIVES OF THIS PAPER**

The primary objectives of this paper are to:

1. Define criteria and recommend guidelines that can be applied for determining the time periods included in Articles X.X.4. and X.X.5. of the disease-specific chapters of the *Aquatic Code*;
2. Explore possible improvements to Articles X.X.4., X.X.5. and X.X.6.

Secondary objectives of the paper include:

3. Identifying the nature of revisions that may be required to Chapter 1.4. (consistent with objectives 1 and 2).
4. Determine whether guidance is required within the *Aquatic Code* for Member Countries on the approach to designing surveillance for and making a self-declaration of freedom.

In addressing the objectives described above, several principles are proposed to achieve these objectives. Any changes to the *Aquatic Code* guidance on self-declaration of freedom at the country, zone or *compartment* level should:

- A. provide confidence among Member Countries in the strength of self-declarations of freedom that are made in accordance with any proposed approaches in the *Aquatic Code*;
- B. be fit for the intended purpose at the level of either country, zone or *compartment*;
- C. be as uncomplicated as possible and readily understandable by Member Countries;
- D. be practical and developed considering the resource constraints of Member Countries;
- E. be sufficiently flexible to allow efficient approaches that meet principle A.

**3. ANALYSIS OF EXISTING PATHWAYS FOR CLAIMING FREEDOM**

The pathways within the disease-specific chapters of the *Aquatic Code* that Member Countries can use to make a self-declaration of freedom are described for countries, zones or *compartments* which have:

1. Absence of *susceptible species*
2. Had no occurrence for at least the last ten years (historical freedom)
3. Unknown disease status
4. Previously made a self-declaration of freedom, but lost their free status due to a detection.

In all cases, *basic biosecurity conditions* (compulsory notification of the disease or suspicion of the disease to the Competent Authority, an *early detection system*<sup>6</sup> and measures to prevent disease introduction) need to be in place to claim freedom and, for Pathways 3 and 4, *targeted surveillance* is also required.

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<sup>6</sup> *Early detection system* is a defined term in the OIE *Aquatic Code* which means: an efficient system for ensuring the rapid recognition of signs that are suspicious of a listed disease, or an emerging disease situation, or unexplained mortality, in aquatic animals in an aquaculture establishment or in the wild, and the rapid communication of the event to the Competent Authority, with the aim of activating diagnostic investigation by the Aquatic Animal Health Services with minimal delay. Such a system will include the following characteristics:

- broad awareness, e.g. among the personnel employed at aquaculture establishments or involved in processing, of the characteristic signs of the listed diseases and emerging diseases;
- veterinarians or aquatic animal health professionals trained in recognising and reporting suspicions of disease occurrence;
- ability of the Aquatic Animal Health Services to undertake rapid and effective disease investigation based on a national chain of command;
- access by the Aquatic Animal Health Services to laboratories with the facilities for diagnosing and differentiating listed diseases and emerging diseases;

Sections 3.1. to 3.4. below analyse the four existing pathways. Each section includes a description of the current approach in the *Aquatic Code*, an evaluation of that approach, a summary of Member Country responses on Version 1 of this paper, and recommendations for improvement.

### 3.1. PATHWAY 1 - ABSENCE OF SUSCEPTIBLE SPECIES

#### *Current situation in the Aquatic Code*

Unless otherwise specified in the relevant disease chapter, a country, zone or *compartment* may be recognised as being free from disease without applying *targeted surveillance* if there are no *susceptible species*.

#### **EU Comment:**

**We believe the sentence below should read as follows:**

**‘This pathway is not currently available for some species pathogens that have a broad host range (e.g. infection with viral haemorrhagic septicaemia virus, infection with *Aphanomyces invadans*; refer to Appendix 2)**

This pathway is not currently available for some species that have a broad host range (e.g. infection with viral haemorrhagic septicaemia virus, infection with *Aphanomyces invadans*; refer to Appendix 2).

#### *Evaluation*

Although this pathway is epidemiologically sound, it would appear to be of practical application in few circumstances. For example, if a country has no species susceptible to a specific disease (as defined in Article X.X.2. of each disease-specific chapter of the *Aquatic Code*), it would have no reason to claim freedom because it would have no domestically produced aquatic animals or products to trade that fall within the scope of the *Aquatic Code*'s sanitary standards for that disease.

However, there may be some circumstances where this pathway may be of practical application, for example:

- a) where a country wishes to self-declare freedom prior to introducing a new species (susceptible to the disease in question) for aquaculture purposes in accordance with Articles X.X.7 or X.X.8. of each disease-specific chapter.
- b) where aquatic animals or products either transit through a country or are imported into the country for processing and reexport.

For this pathway, *basic biosecurity conditions* would need to be in place for a period of time prior to the introduction of the *susceptible species*, sufficient to ensure that i) no pathogenic agent introduced via aquatic animal commodities remained present in the environment, ii) the pathogenic agent *early detection system* was properly established.

This pathway relies on confidence that *susceptible species* are in fact absent from a country, *zone* or *compartment*. To be confident that *susceptible species* are absent there must be a) sound knowledge of the range of *susceptible species* of a pathogenic agent and b) sufficient knowledge of the local aquatic animal fauna to be confident that *susceptible species* are absent.

#### *Member Country comments - February 2019*

A small majority of Member Country responses were in favour of retaining the pathway and some countries indicated that they were likely to use it.

- 
- the legal obligation of private veterinarians or aquatic animal health professionals to report suspicions of disease occurrence to the Competent Authority.

Member countries made the following suggestions regarding the appropriate standard of evidence that *susceptible species* are absent from a country:

- a) There has never been any report of the existence of the species in the country, *zone* or *compartment*.
- b) Documentation from the relevant authorities showing that those species have not been moved into the country or *zone* or *compartment*
- c) Provision of documentation which sets out scientific evidence indicating that the likelihood of the presence of animals of *susceptible species* in the country, *zone* or *compartment* is negligible.

#### Annex 8 (contd)

Member countries also provided suggestions on the data sources that could be utilised to demonstrate that a *susceptible species* is absent, including:

- Historical fisheries data on known species distribution
- Data on physiological requirements
- Global Biodiversity database
- FAO databases on natural range of habitat
- Review reports in scientific literature, including fisheries and aquatic fauna surveys
- Trade data (historical records of transboundary movements of animals).

#### *Recommended approach*

The Aquatic Animals Commission recommends that this pathway be retained in the *Aquatic Code* as many countries have indicated that they are likely to use it to claim freedom. Additionally, the Commission recommends that:

- Member Countries would be expected to provide a reason for wanting to establish disease freedom in the absence of *susceptible species*.
- The pathway would not be available for some diseases where there may be uncertainty regarding the range of *susceptible species*. (This is currently the case in the *Aquatic Code* for Infection with *Aphanomyces invadans*.)
- Guidance be provided in Chapter 1.4 regarding the standard of evidence and data sources that could be utilised to demonstrate that a *susceptible species* is absent.
- The required period for *basic biosecurity conditions* for a country, *zone* or *compartment* prior to making a declaration of freedom using this pathway should be determined for each pathogen based on its epidemiology.

### **3.2. PATHWAY 2 - HISTORICAL FREEDOM**

#### *Current situation in the Aquatic Code*

This pathway for demonstrating freedom can be used for a country, *zone* or *compartment* provided certain conditions are met, such as:

- there has not been an observed occurrence of the disease for at least the last ten years (the period may be longer for some diseases; refer to Appendix 2); and
- *basic biosecurity conditions* have been in place for a specified period.

#### *Evaluation*

This pathway should only be available for diseases where sufficient confidence can be demonstrated that passive

surveillance, as a part of a country's *early detection system*, would detect the disease if it were to occur. Importantly, the disease would need to manifest clinically, be observed, reported and investigated as part of the country's *early detection system*. If diseases are not expected to manifest clinically (e.g. ISAV HPR0), this pathway would not be appropriate.

This pathway is appropriate for self-declaration of freedom for countries and zones but may have limited application for *compartments*. Areas outside of a *free compartment* would usually not be declared free (otherwise there may be no reason to have a *free compartment*); therefore, the requirement for the disease to 'have never been reported' could not be met. For self-declaration of freedom of *compartments*, *targeted surveillance* is the most appropriate approach to providing evidence to substantiate a self-declaration of freedom.

**EU Comment:**

**In relation to the statement above – ‘Areas outside of a free compartment would usually not be declared free (otherwise there may be no reason to have a free compartment); The EU would like to add that some businesses seek free compartment status despite being in a disease-free zone, as this can guarantee continuation of business without restriction if the zone loses its status.**

Passive surveillance is generally not effective in wild populations because they are either not observed, or the level of observation may be limited compared to farmed animals. This could mean that, as currently described, this pathway is not available even if passive surveillance provided robust evidence of freedom for most of the populations of *susceptible species* in a country or zone. This issue could be addressed if Member Countries could: a) supplement evidence from passive surveillance with *targeted surveillance* data for populations not adequately covered by passive surveillance; or b) demonstrate that wild populations are epidemiologically linked to farmed populations such that disease would be observed in farmed populations should it occur in linked wild populations. This issue is discussed further below under recommended approach.

For this pathway, the period required for *basic biosecurity conditions* to be in place varies among diseases. This period should be set appropriately such that passive surveillance (provided through a country’s *early detection system*) will establish sufficient evidence of freedom from the disease, and that import requirements are sufficient to prevent introduction of the disease during the period that evidence of freedom is being obtained. Many factors need to be considered to determine the sensitivity of a passive surveillance system (and thus the period that *basic biosecurity conditions* must be in place before freedom based on historic grounds can be demonstrated), such as the epidemiology of the disease (notably its clinical expression), host and environmental factors.

#### *Member Country comments February 2019*

Most Member Countries wished to retain the pathway for claiming historical freedom from a disease. A minority considered that the disease should never have been detected to be able to use this pathway but most believed that a minimum time period (e.g. 10 years) where *basic biosecurity conditions* are in place would be sufficient for this pathway.

Most Member Countries agreed that the factors for determining the required period of *basic biosecurity conditions* for *listed diseases* were appropriate. Additional factors were highlighted by one Member Country including the existence of latent spores or other life stages outside of the host. One Member Country noted that factors related to production systems and management practices are likely to change over time.

It was noted that quantifying the sensitivity of an *early detection system* could be technically difficult. It was acknowledged that epidemiological modelling is appropriate and defensible for determining overall sensitivity of surveillance and confidence in establishing freedom; however, this may exclude countries that lack sufficient epidemiology expertise from effectively applying these measures. The use of a default period would make this pathway for claiming freedom more accessible to Member Countries.

Some Member Countries indicated concern with allowing a period of passive surveillance less than 10 years due to the technical difficulties associated with accurately determining the sensitivity of passive surveillance.

A small number of Member Countries recommended that there be no default period at all and Member Countries should determine the required periods of passive surveillance to achieve 95% confidence of freedom. These views were contrary to many others (refer to paragraphs above) that requested that a default period be recommended for each disease.

Some Member Countries also requested additional clarity on key concepts relevant to passive surveillance; for example, defining observers and ‘conditions conducive to clinical expression of infection’. Member countries also requested that guidance be provided so that the relevant factors that contribute to determining the values in Table 1 (below) could be clearly described.

Member countries highlighted the lack of sensitivity of passive surveillance for wild aquatic animal populations; however, many also noted that it is applicable in some circumstances.

### *Recommended approach*

#### *Requirements for passive surveillance*

It is proposed that, similar to current provisions of the relevant disease-specific chapter of the *Aquatic Code*, a country or zone (but not a *compartment*) could be declared free from a disease on the basis of historical freedom. The evidence for historical freedom is passive surveillance data generated by a country's *early detection system* that should meet the following conditions.

#### Annex 8 (contd)

- the disease has not been reported in the country, *zone* or *compartment* (including in wild aquatic animal populations) for a default minimum period that would be determined based on factors specific to that disease (see section on requirements for *basic biosecurity conditions* below);
- the country has *basic biosecurity conditions* in place including an *early detection system* that is sufficiently sensitive to detect the disease should it occur and the following conditions (additional to the requirements of an *early detection system*) are met:
  - conditions within the country (biotic and abiotic) are conducive to clinical expression of the infection such that if the pathogenic agent were present, it would produce clinical signs of the disease in populations of susceptible animals;
  - for populations of susceptible farmed aquatic animals they must be under sufficient observation such that, if clinical signs of the disease were to occur, they would be observed;
  - there must be sufficient awareness by potential observers that observation of clinical signs of the disease would lead to reporting;
  - for populations of susceptible wild aquatic animals, they must:
    - be under sufficient observation such that if clinical signs of the disease were to occur they would be observed and reported, or
    - be epidemiologically linked to farmed populations such that the disease would occur and be observed and reported in farmed populations if it were to occur in wild aquatic animal populations;
  - there must be access to sufficient diagnostic capability to confirm or exclude cases of the disease.

#### *Need for targeted surveillance*

If passive surveillance for some susceptible aquatic animal populations would not meet the requirements for passive surveillance specified above (e.g. for wild populations), it is proposed that *targeted surveillance* could be used to provide additional evidence of freedom for those identified populations. However, for this pathway to be used it must be based primarily on historical freedom (i.e. passive surveillance data); alternatively, pathway 3 (section 3.3. below) should be used.

#### *Requirements for basic biosecurity conditions*

Prior to a self-declaration of freedom being made, *basic biosecurity conditions* must be in place for a period that is sufficient that, should the disease be present, it would manifest clinically and be detected by the country's *early detection system*. Additionally, during that period there must be effective controls to prevent the introduction and establishment of the disease. Each disease-specific chapter of the *Aquatic Code* should include a minimum period that *basic biosecurity conditions* must be in place prior to a self-declaration of freedom being made in accordance with this pathway.

It is proposed that the period for *basic biosecurity conditions* for a self-declaration of freedom on historical grounds, should be determined considering factors that would affect the sensitivity of passive surveillance, including:

- the maximum duration of the production cycle for the *susceptible species*;
- the life stages at which animals are susceptible;



- the expected severity and duration of clinical signs in the *susceptible species* (and therefore the likelihood of detection);
- environmental conditions that influence levels of infection and clinical expression, including seasonality of the disease (period of the year when clinical disease occurs, e.g. when water temperatures are permissive);
- factors specific to the pathogenic agent (e.g. production of spores)
- production systems and management practices that would affect observation of clinical signs if they were to occur;
- any other relevant factors that may influence presentation of clinical signs and observation of the disease should it be present.

#### Annex 8 (contd)

It is proposed that the level of confidence of the evidence provided for historical freedom (on the basis of passive surveillance) should be equivalent to that of other pathways for which the evidence is provided by *targeted surveillance*. The level of confidence of freedom should be set at 95%, consistent with the current requirements of the *Aquatic Code*. If a combination of surveillance data sources is to be used (e.g. passive and *targeted surveillance*) the level of confidence should also be set at 95%.

The default period of passive surveillance required to make a self-declaration of freedom for all diseases in the *Aquatic Code* will be ten years. This period is the minimum required to achieve 95% likelihood of detection if the annual likelihood of detection is 30%. If, following consideration of the factors affecting the sensitivity of passive surveillance (provided above), the annual likelihood of detection is considered likely to be less than 30%, the minimum period required for *basic biosecurity conditions* (including passive surveillance) defined in the relevant disease-specific chapter of the *Aquatic Code* will be set to a period greater than ten years, as appropriate.

A country making a self-declaration of freedom on the basis of historical freedom will need to provide an explanation of how the criteria (i.e. for *basic biosecurity conditions*) presented for this pathway have been met. Further, if the annual likelihood of detection is considered to be lower than 30% (where the required period is 10 years) due to a country's circumstances (e.g. nature of the *early detection system*, environmental conditions, nature of the aquaculture industry), this pathway will not be valid. Instead, an alternative pathway that utilises *targeted surveillance* data will be required.

Epidemiological methods, such as scenario tree modelling, are available to determine the sensitivity of a surveillance system and thus the likelihood that a pathogenic agent, if present, is detected (Martin, Cameron & Greiner, 2007).

**Table 1.** Likelihood that pathogenic agent is detected by passive surveillance based on annual likelihood of detection and duration of surveillance.

Annual likelihood of detection (pD)	Years (n)									
	10	9	8	7	6	5	4	3	2	1
0.5	1.00	1.00	1.00	0.99	0.98	0.97	0.94	0.88	0.75	0.50
0.4	0.99	0.99	0.98	0.97	0.95	0.92	0.87	0.78	0.64	0.40
0.3	0.97	0.96	0.94	0.92	0.88	0.83	0.76	0.66	0.51	0.30
0.25	0.94	0.92	0.90	0.87	0.82	0.76	0.68	0.58	0.44	0.25
0.2	0.89	0.87	0.83	0.79	0.74	0.67	0.59	0.49	0.36	0.20
0.1	0.65	0.61	0.57	0.52	0.47	0.41	0.34	0.27	0.19	0.10

### 3.3. PATHWAY 3 – UNKNOWN DISEASE STATUS

#### *Current situation in the Aquatic Code*

This pathway for demonstrating freedom can be used for a country, *zone* or *compartment*. The requirements of this pathway include that:

- *basic biosecurity conditions* have been continuously met for a certain period; and
- *targeted surveillance*, as described in Chapter 1.4., has been in place for a certain period without detection of *infection* with the relevant *pathogenic agent*.

Additionally, Chapter 1.4. of the *Aquatic Code* (refer to Article 1.4.6., point 3) requires that:

- basic biosecurity conditions* are in place and effectively enforced;
- no vaccination against the disease has been carried out unless otherwise provided in the *Aquatic Code*;



- c) the disease is not known to be established in wild aquatic animals within the country or zone intended to be declared free. (A country or zone cannot apply for freedom if there is any evidence of disease in wild aquatic animals. *Targeted surveillance* in wild aquatic animals of *susceptible species* is necessary to confirm *absence*.)

UNOFFICIAL VERSION

Annex 8 (contd)*Evaluation*

This pathway is appropriate for self-declaration of freedom for countries, *zones* and *compartments* and may be applied when less resource intensive pathways cannot be applied (e.g. historical freedom based on passive surveillance cannot be claimed due to previous occurrence of clinical disease).

This pathway has previously emphasised *targeted surveillance* as the sole form of evidence to support a self-declaration of freedom. However, through the application of *basic biosecurity conditions*, passive surveillance evidence will also be generated. The relative weight of evidence from passive and *targeted surveillance* would depend on a range of factors as described above for the historical freedom (refer to Section 3.2. above).

*Basic biosecurity conditions* must be in place so that there are effective controls to prevent disease introduction from the time surveillance commences. However, *basic biosecurity conditions* may be necessary for a period prior to the commencement of surveillance that is sufficient to ensure that the disease would be detected (i.e. has reached the design prevalence) if it had been introduced immediately prior to the implementation of measures to prevent introduction.

*Summary of Member Country comments February 2019*

In general, Member Countries supported the criteria for determining periods for *basic biosecurity conditions* for this pathway with some Member Countries reiterating comments provided for pathway 2.

The following suggestions were made to improve guidance:

- The terms ‘*targeted surveillance*’ and ‘active surveillance’ are clearly defined and distinguished.
- The design of a surveillance programme needs to:
  - consider the number of species in the country/zone that are susceptible to a given disease
  - consider the epidemiological links of the species (geographically/indirectly through water/anthropogenic movements etc.)
  - consider strain differentiation, ability to detect pathogen presence and different disease presentations/conditions conducive to clinical expression of infection
  - ensure that all or a representative sample of non-epidemiologically linked populations are sampled to determine their health status.

Member Country views varied on the appropriate minimum period for *basic biosecurity conditions* to be in place prior to the commencement of *targeted surveillance* for declaring freedom for countries or zones. However, it appears (based on the rationale provided) that some Member Countries did not recognise that the purpose of implementing *basic biosecurity conditions* prior to the commencement of *targeted surveillance* is not to commence obtaining surveillance information, rather to ensure that import requirements are in place to prevent the introduction of disease.

Although views varied, most Member Countries supported the proposal of a 1-year minimum period for *basic biosecurity conditions* before surveillance. Other views are for *basic biosecurity conditions* to be implemented only at the point at which surveillance begins to a period of 3 years before surveillance begins.

One Member Country was against fixing a period, preferring that the OIE list criteria that Member Countries need to consider in determining the time required for *basic biosecurity conditions* prior to commencement of surveillance. Another Member Country suggested that: for a country or zone, the period should be set at least 2 years or 2 production cycles whichever is longer; and for *compartments* the period should be set at 1 year or 1 production cycle, whichever is longer.

There was general support from Member Countries that there should be a default frequency of *targeted surveillance* as a general requirement and the majority of Member Countries indicated a preference for two surveys per year for two years. However, most Member Countries also noted that a risk-based approach to maximise the likelihood of detection may provide sufficient evidence at a lower frequency of surveys and that sufficient flexibility should be available for this approach.

### **Recommended approach**

#### *Requirements for basic biosecurity conditions*

Prior to a self-declaration of freedom being made through this pathway, *basic biosecurity conditions* must be in place to ensure there are effective controls to prevent the introduction and establishment of the disease. The period of *basic biosecurity conditions* must be sufficient that, should the disease have been previously introduced, there would be sufficient time for it to reach design prevalence (based on assumptions of the survey design) by the time *targeted surveillance* has commenced. Each disease-specific chapter of the *Aquatic Code* should include a minimum period that *basic biosecurity conditions* must be in place prior to the commencement of *targeted surveillance*.

It is proposed that the minimum period that *basic biosecurity conditions* should be in place prior to commencement of *targeted surveillance* will generally be one year. It is expected that this period will be sufficient under most circumstances for a disease to reach a prevalence sufficiently high to be detected by a well-designed survey. However, different recommendations may be provided in the *Aquatic Code* for some diseases where it is considered that the epidemiology of a disease and nature of production systems would affect the expected rate of increase in prevalence and intensity of infection in the *susceptible species* following introduction of the disease. In setting an alternative period, the following criteria should be considered:

- the maximum duration of the production cycle for the *susceptible species*;
- the life stages at which animals are susceptible;
- seasonality of the disease (periods of the year when prevalence and intensity of infection is highest and most conducive to detection);
- production systems and management practices that would affect occurrence of infection;
- any other relevant factors that may influence the expected rate of increase in prevalence and intensity of infection in *susceptible species* following introduction of the disease.

#### *Requirements for targeted surveillance*

*Targeted surveillance* surveys should commence after a period of time following the implementation of *basic biosecurity conditions* (see section above).

The requirements for *targeted surveillance* will depend on epidemiology of the disease, the biology of *susceptible species*, the populations of *susceptible species* in the country, *zone* or *compartment* the epidemiological links of the populations and the nature of production practices and systems. In general, the same criteria proposed above for *basic biosecurity conditions* should also be considered for setting the period required for *targeted surveillance*.

For many diseases, there will be significant temporal variability in the prevalence and intensity of infection (and therefore likelihood of detection by *targeted surveillance*). For example, the likelihood of detection may be greatest for a particular life stage or during periods of the year when pathogenic agent replication and transmission are higher. Environmental variability from one year to another may also result in differences in prevalence and intensity between years that could affect likelihood of detection. Surveys must therefore be designed to account for such variability and sample populations in a manner to maximise the likelihood of detecting a disease should it occur. This may require targeting temporal windows such that sampling can only take place during limited periods within a single year.

## Annex 8 (contd)

For these reasons, it is proposed that *targeted surveillance* must occur over a period of at least two years for countries or zones. Each survey should occur under optimum conditions for detection of the pathogenic agent (e.g. seasons, temperatures, and life stages). The second survey should not commence within three months of completion of the first survey and, if there are breaks in production, the surveys should also ideally span two production cycles.

For compartments, it is proposed that *targeted surveillance* should generally occur for at least one year prior to a declaration of freedom. This shorter period for a *compartment* reflects the more clearly defined populations, the biosecurity controls on those populations and a likely narrower variation in environmental variables. However, a different period (more or less than one year) may be appropriate if warranted by the epidemiology of the disease and the criteria proposed above for *basic biosecurity conditions*. For example, different requirements may be appropriate for a *susceptible species* that has a three-year production cycle versus one that has a six-month production cycle; particularly if the disease is likely to occur at a very low prevalence until the third year of the production cycle.

**EU Comment:**

**We do not agree with the paragraph above – specifically on the period of targeted surveillance of one year for compartments. We do not feel that this time frame is adequate. The factors that make disease difficult to detect as described above for zones are potentially more problematic, not less, within a compartment. If environmental and husbandry conditions are optimal for the animals within a compartment, then infection rates and intensity will be very low, which will hinder disease detection. We therefore suggest that the time frame for targeted surveillance in a compartment should be at least the same as it is for a zone or a country.**

To maximise the likelihood of pathogen detection, surveys should target species and life stages most likely to be infected and take place at times of the year when temperature and season offer the best opportunity for detection. At least two surveys per year (for at least two consecutive years) need to be conducted three or more months apart to declare freedom, unless disease specific evidence supports an alternative strategy. The number of farms and animals sampled should be sufficient to generate an overall 95% confidence or greater. Design prevalence at the animal and higher levels of aggregation (i.e. pond, farm, village, etc.) should be 2% or lower (a higher design prevalence can be used if justified by epidemiological evidence). The design prevalence may be different for different diseases and guidance will be provided in the relevant disease-specific chapter in the *Aquatic Manual*). Guidance on survey design will be provided in Chapter 1.4.

The *early detection system* (implemented as part of the *basic biosecurity conditions*) contributes additional evidence of freedom from disease before and during the period of *targeted surveillance*. Member Countries could adopt a scenario tree modelling approach to combine evidence from targeted and passive surveillance and justify reducing the level of *targeted surveillance*. This may be particularly appropriate where passive surveillance can be demonstrated to be a sensitive method for detection of the disease in certain populations of *susceptible species* (in accordance with the criteria proposed above in Section 3.2. historical freedom).

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

**3.4. PATHWAY 4 – RETURNING TO FREEDOM***Current situation in the Aquatic Code*

If a previous *self-declaration of freedom* had been made but was subsequently lost due to the detection of infection, the following conditions need to be met to return to freedom:

- 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 (as described in Chapter 4.3.) have been completed; and
- c) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place since eradication of the *disease*; and
- d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of the relevant *pathogenic agent*.

Note that Chapter 1.4. of the *Aquatic Code* on *Aquatic Animal Health Surveillance*, includes no specific guidance for this pathway to return to freedom following eradication of a disease. A new *Aquatic Code* chapter on emergency disease response has been proposed for Section 4 of the *Aquatic Code* to guide Member Countries' emergency responses; however, it remains to be developed.

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### *Evaluation*

This pathway applies only to countries or zones for which a *self-declaration of freedom* had been made but free status was subsequently lost due to the detection of infection. The requirements of this pathway apply to circumstances where a disease will be contained and eradicated, and the risk of subsequent introduction addressed, with the view of re-establishing freedom from the disease.

Note that this pathway does not currently apply to *compartments* which have lost their free status following detection of the disease.

In a country or zone, the criteria applied to regaining freedom after a disease outbreak need to provide assurance (at an equivalent level to an initial *self-declaration of freedom*) that the eradication programme has been successful. Under circumstances of an eradication programme, affected populations will normally be well defined—affected farms would be depopulated and animals disposed of in an appropriate manner in accordance with Chapter 4.7 to prevent further spread of the disease; any in-contact farmed or wild populations would also require investigation to determine their disease status.

It may be possible to return to freedom more quickly under an eradication programme than in an initial *self-declaration of freedom* for a country or zone because study populations may be more narrowly defined. However, consideration must be given to the likely pathways of introduction and a review of *basic biosecurity conditions* to ensure that import requirements to prevent the re-introduction of disease are effective. The circumstances of the disease outbreak (e.g. affecting a small versus large geographic area), the type of production systems affected (e.g. open versus closed) and the epidemiology of the disease would also impact the surveillance period required for demonstration of freedom.

Chapter 1.4. does not provide specific guidance on surveillance required to regain freedom and no reference is made to *infected* or *protection* zones. The glossary of the *Aquatic Code* defines '*infected zone*' and '*protection zone*' as provided below.

- *INFECTED ZONE* means a zone in which a disease has been diagnosed.
- *PROTECTION ZONE* means a zone established to protect the health status of aquatic animals in a free country or free zone, from those in a country or zone of a different aquatic animal health status, using measures based on the epidemiology of the disease under consideration to prevent spread of the pathogenic agent into a free country or free zone. These measures may include, but are not limited to, vaccination, movement control and an intensified degree of surveillance.

Further guidance may be necessary to define how the zones should be established and the requirements for surveillance within them.

#### **EU Comment:**

**We agree that further guidance is required here to define how zones should be established and the surveillance within them. Furthermore, consideration should be given to the different categories of aquaculture production system e.g. open systems / closed systems and how this may impact upon the definition of these zones.**

#### *Member Country comments February 2018*

A majority of Member Countries considered that countries and zones should (under appropriate circumstances) be able to return to freedom more quickly following an eradication programme than in an initial self-declaration of freedom for a country or zone. The rationale provided included that the affected populations may be more narrowly defined than for the original declaration and that the country will have a good knowledge of the disease and study populations in connection with the original declaration. However, Member Countries noted that this will depend on the circumstances of the outbreak and the reason freedom was lost.

It was commented that there should not be a necessity to test wild stock when there are enough *aquaculture establishments* in the infected area that will result in the production of sound epidemiological data which accurately reflects the disease status of the system.

#### **EU Comment:**

**We believe that further clarification should be given on the point that 'there should not be a necessity to test wild stock when there are enough aquaculture establishments in the infected area.' Consideration should be given to wild and farmed stock population dynamics and the pathogen in question in terms of likely / expected**

**infection levels in different life stages of a host / susceptible species.**

Member Country views on whether *compartments* should be able to regain freedom immediately after destocking and successful decontamination were divided. Most indicated a preference for additional assurances that eradication had been successful (e.g. surveillance within the first production cycle under conditions favourable for the detection of the pathogen). Member Countries noted that it was important that *basic biosecurity conditions* be reviewed to ensure epidemiological isolation of the *compartment*.

Annex 8 (contd)

Member Countries universally supported provision of clearer guidance on establishing *infected* and *protection zones* and sampling within them (for farmed and wild animals). Views varied on where that guidance should be located – chapters on surveillance, zoning and compartmentalisation or emergency response. In addition, it was suggested that consideration could also be given to the concept of containment zones (i.e. zoning during a disease outbreak) (as provided for in the *Terrestrial Code*).

**Recommended approach***Compartments*

A pathway for *compartments* to regain freedom will be included in the disease specific chapters of the *Aquatic Code*. *Compartments* will be able to return to freedom relatively rapidly; however, a minimum period of time will be required (dependent on the nature of the specific disease) to test the reviewed biosecurity conditions and to undertake at least one round of testing to demonstrate that eradication has been successful.

*Countries and zones*

The default minimum period of surveillance for countries and zones to regain freedom will be consistent with requirements for the original declaration of freedom. However, a general provision in Chapter 1.4. of the *Aquatic Code* will allow a self-declaration of freedom to be made sooner if Member Countries can demonstrate that the approach would provide an appropriate standard of evidence for the circumstances of the outbreak and the disease.

*Infected and protection zones*

Clearer guidance on establishing *infected* and *protection zones* and sampling within them (for farmed and wild animals) will be developed. Aspects of this guidance will be included, as appropriate, in Chapter 1.4. Surveillance and the new chapter on emergency response in Section 4 of the *Aquatic Code*.

*Requirements for targeted surveillance*

Once all infected populations have been depopulated and disinfected (see Chapter 4.3.) and synchronously fallowed (see Chapter 4.6.) for a period determined by the biophysical properties (i.e. pathogenic agent survival in the environment), a surveillance programme within the *protection* and *infected zones* should commence. The programme should include both farmed and wild populations of *susceptible species* in the *protection* and *infection zones*. It is recommended that a risk-based approach to the design of the survey is adopted. The criteria used in Section 3.3. above would be used to determine the frequency and duration of surveillance. The following sites should be targeted for sampling:

- Farms which had been infected.
- Farms and wild populations at greatest risk of exposure to infection during the outbreak, i.e. in close proximity, with other epidemiological contacts such as equipment or aquatic animals.
- Wild populations of *susceptible species* downstream or in the immediate vicinity of previously infected farms must be included.

It is recommended that at least two negative surveys are conducted prior to reclaiming freedom. The second survey should start at least three months after completion of the first survey, and take place during optimum seasons, temperatures, and life stages to optimise pathogenic agent detection. If there are breaks in production, the surveys should also ideally span two production cycles. In each survey, the number of sites and the samples taken per site in a survey should be sufficient to demonstrate with 95% confidence that the pathogenic agent is not present above a prevalence of 2% (a higher design prevalence can be used if justified by epidemiological evidence).

**4. MAINTAINING FREEDOM**

For freedom to be maintained, *basic biosecurity conditions* need to remain in place; however, the possibility of introduction of the pathogenic agent may remain, albeit at a very low level. It is therefore important that the *early detection system* has sufficient sensitivity (i.e. capable of detecting pathogenic agent incursion) to ensure that a 95% confidence in *disease freedom* is maintained.



UNOFFICIAL VERSION

### *Current situation in the Aquatic Code*

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

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

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

### *Evaluation*

For declarations of freedom in accordance with Pathway 2 (Historical freedom: see Section 3.2. above), the critical aspects to maintaining disease freedom are that *basic biosecurity conditions* are continuously maintained. For *basic biosecurity conditions* to be maintained and remain effective: i) conditions must remain conducive to clinical expression of the disease, ii) measures to prevent disease introduction must be sustained and, iii) the *early detection system* must operate at such a level that introduction of the pathogenic agent would be detected rapidly if it were to be introduced.

For declarations of freedom in accordance with Pathway 3 (Unknown disease status: see Section 3.3. above), the critical aspects for maintaining freedom are that conditions remain conducive to clinical expression of the disease and that *basic biosecurity conditions* are continuously maintained. If these requirements are met (i.e. passive surveillance would be an effective means of detection should the disease occur), *targeted surveillance* may be discontinued. However, the *Aquatic Code* currently provides no guidance on an efficient means to maintain freedom should passive surveillance not be sufficiently sensitive to maintain freedom for some populations (e.g. populations of wild *susceptible species*).

For declarations of freedom in accordance with Pathway 4 (Returning to freedom: see Section 3.4. above), the *Aquatic Code* currently provides no guidance on the requirements for maintaining freedom.

### *Member country comments February 2019*

Most member countries believed that additional guidance on what constitutes ‘conditions conducive to clinical expression of infection’ would be useful. However, some countries believed that this was not necessary and that the definition of ‘*early detection system*’ and relevant information available in sections of the disease specific chapters of the *Aquatic Manual* were sufficient.

Most Member Countries agreed that additional guidance would be appropriate on how to evaluate or test their ‘*early detection system*’. It was suggested that this guidance could be provided in Chapter 1.4 and would assist with consistency and transparency.

### *Recommended approach*

For maintenance of free status following declarations of freedom in accordance with Pathways 2, 3 and 4, Member Countries must provide evidence that *basic biosecurity conditions* have been continuously maintained.

## Annex 8 (contd)

If *targeted surveillance* that was required for initial demonstration of freedom is to be discontinued for any identified population, evidence must be provided to demonstrate that conditions remain conducive to clinical expression of disease and that passive surveillance, as provided by the countries *early detection system*, would rapidly detect the disease in those populations should it occur.

Any ongoing *targeted surveillance* to maintain freedom should be undertaken at a level necessary to maintain confidence of freedom and should take into account the likelihood of infection.

Additional guidance on what constitutes 'conditions conducive to clinical expression of infection' will be included in Chapter 1.4 of the *Aquatic Code*, as will guidance on how to evaluate or test an *early detection system*.

### 5. REVISIONS REQUIRED TO CHAPTER 1.4.

Revisions will be required to Chapter 1.4. Surveillance of the *Aquatic Code* to bring effect to the recommendations proposed in this discussion paper. To avoid any conflicting guidance, the Aquatic Animals Commission considers that a revised Chapter 1.4 should be adopted in the *Aquatic Code* prior to revisions to the relevant articles on declaring and maintaining freedom in each disease specific chapters of the *Aquatic Code*. (i.e. X.X.4, X.X.5 and X.X.6).

Some required revisions to Chapter 1.4. include:

- Incorporate the proposed criteria (included in this document) relevant to setting periods in the disease-specific chapters required for *basic biosecurity conditions* and for periods of surveillance.
- Revise the proposed pathways for claiming or reclaiming freedom
- Include provision for flexibility to use different forms of surveillance data under each pathway rather than having a rigid requirement for one data type (e.g. targeted or passive surveillance).
- Provide guidance on acceptable mechanisms for maintaining disease freedom.
- Include guidance on regaining freedom following eradication of a disease at the level of country, zone or *compartment* (not currently included); including guidance on a risk-based approach to survey design.
- Provide guidance on defining the boundaries of *infection* and *protection zones*.
- Include guidance on what constitutes an *early detection system* and how it can be evaluated.
- Include guidance on what constitutes 'conditions conducive to clinical expression of infection'.
- Improved guidance on study design to demonstrate freedom.

### 6. REQUIREMENTS FOR MAKING A SELF-DECLARATION OF FREEDOM

#### *Current situation in the Aquatic Code*

Self-declaration of freedom is the only mechanism through which countries can establish freedom from a disease in accordance with the standards of the *Aquatic Code* (as there is no official disease recognition process for aquatic animal diseases).

There is currently no guidance within the *Aquatic Code* on the structure and contents of a self-declaration of freedom; however, the OIE has developed a procedure for the publication by the OIE of a self-declaration of freedom<sup>7</sup>. This document also includes information on the evidence that should be included in a self-declaration of freedom.

<sup>7</sup> [http://www.oie.int/fileadmin/Home/eng/Animal\\_Health\\_in\\_the\\_World/docs/pdf/Self-declarations/EN\\_Procedure\\_self\\_declaration.pdf](http://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/Self-declarations/EN_Procedure_self_declaration.pdf)

### Evaluation

Some Member Countries have requested improved guidance on the structure and content of *self-declarations of freedom*. The *Aquatic Code* provisions in Chapter 1.4. and in relevant disease-specific chapters, together with the relevant disease-specific chapters of the *Aquatic Manual*, define the key requirements that must be considered in a competent authority's *self-declaration of freedom*.

Guidance could be improved to ensure that sufficient and consistent standards of evidence are provided in *self-declarations of freedom*.

### Member country comments February 2019

Most Member Countries considered that the OIE procedure for the publication of a self-declaration of freedom provides sufficient guidance. However, others requested additional guidance in the form of a new chapter or other means.

### Recommended approach

Chapter 1.4. of the *Aquatic Code* will define the key information requirements that should be included in a self-declaration of freedom. More extensive guidance documents on making a self-declaration of freedom, possibly with examples, will be considered for development following the revision of Chapter 1.4.

## 7. DISCUSSION

The four existing pathways of the OIE *Aquatic Code* to demonstrate disease freedom are summarised in Table 2 below. One key change proposed in this paper is to allow Member Countries to combine evidence from targeted and passive surveillance in making the case for disease freedom. For Pathway 2 (historical freedom), this change would allow countries with aquatic animal populations that could not have their disease status established by passive surveillance to make a case for freedom through *targeted surveillance* in those defined populations. Additionally, for Pathway 3 (unknown disease status) and Pathway 4 (returning to freedom), passive surveillance data will be available through a country's *early detection system* and may provide additional evidence of freedom to that generated by *targeted surveillance* data.

**Table 2.** Summary of pathways to disease freedom and proposed forms of primary and secondary evidence.

Initial situation	Primary surveillance evidence to claim disease freedom	Proposed secondary evidence to claim freedom (if required)
1. Absence of <i>susceptible species</i>	Absence of <i>susceptible species</i>	Nil
2. Historical freedom	Passive surveillance	<i>Targeted surveillance</i> (in populations where passive surveillance is not appropriate)
3. Unknown disease status	<i>Targeted surveillance</i>	Passive surveillance (in appropriate populations)
4. Post eradication (returning to freedom)	<i>Targeted surveillance</i>	Passive surveillance (in appropriate populations)

Annex 8 (contd)

The factors which need to be considered when determining the time periods for *basic biosecurity conditions* and surveillance to demonstrate freedom have been discussed in this paper. It is proposed that these factors be used to set the default requirements specified in each disease-specific chapter of the *Aquatic Code*. Member Countries would also need to consider these factors when planning surveillance and developing self-declarations of freedom, including justifications for any deviation from the proposed requirements within the *Aquatic Code*.

Statistical methods for assessing the evidence needed to demonstrate freedom, based on the sensitivity of the surveillance system, are well established. This framework has been used to set the minimum period for passive surveillance (ten years) required to build sufficient evidence for a case for freedom using the historical freedom pathway. It is recognised that Member Countries are unlikely to have quantitative data needed to justify a period of surveillance using this model. However, the case for freedom should consider qualitative factors which influence the sensitivity of passive surveillance (and thus the duration of surveillance required), which are discussed in this paper. These factors will be reflected in a revised Chapter 1.4.

For all pathways, to maintain freedom, *basic biosecurity conditions* must be maintained. The quality of the *early detection system* and measures to prevent introduction of the pathogenic agent are crucial for both making a robust case for disease freedom and convincing trade partners that disease free status is being maintained. To this end, Member Countries declaring disease freedom need to provide evidence that the *early detection system* would identify any disease incursions, and that measures to prevent introduction are being rigorously applied.

## APPENDIX 1.

**DISCUSSION POINTS CONSIDERED BY MEMBER COUNTRIES  
ON VERSION 1 OF THIS DOCUMENT**

**Section 3.1. Pathway 1. Absence of *susceptible species***

1. Is Pathway 1 likely to be used by Member Countries?

2. What is an appropriate standard of evidence that *susceptible species* are absent from a country?

**Section 3.2. Pathway 2. Historical freedom**

3. Are the proposed requirements for passive surveillance in farmed and wild aquatic animals appropriate?

4. Should historic freedom require that the disease has never been detected (as proposed) or is a period of freedom (e.g. ten years) sufficient?

5. Are the factors for determining the required period of basic biosecurity conditions for *listed diseases* appropriate?

**Section 3.3. Pathway 3. Unknown disease status**

6. Are the proposed criteria for determining the periods for basic biosecurity conditions for this pathway appropriate?

7. Is one year an appropriate minimum period for *basic biosecurity conditions* to be in place prior to the commencement of active surveillance for declaring freedom for countries or zones?

8. Is one survey per year (at least three months apart) for two years an appropriate default requirement?

**Section 3.4. Pathway 4. Returning to freedom**

9. Should countries and zones be able to return to freedom more quickly following an eradication programme than in an initial *self-declaration of freedom* for a country or zone (if appropriate criteria are met)?

10. Should compartments be able to regain freedom immediately after destocking and successful decontamination (i.e. with surveillance at the level required to maintain freedom) if *basic biosecurity conditions* have been reviewed and modified and restocking is with disease free animals (e.g. from a free country, *zone* or *compartment*)?

11. When should the starting time point be for surveillance – e.g. commencement of sampling or at the conclusion of sampling for the first survey with negative results?

12. Should Chapter 1.4. provide clearer guidance on establishing *infected* and *protection zones* (perhaps in the proposed new chapter on emergency response) and sampling within them (for farmed and wild animals)?

**Section 4. Maintaining freedom**

13. Do Member Countries require additional guidance on what constitute ‘conditions conducive to clinical expression’?

14. Do Member Countries require additional guidance on how to evaluate or test their ‘early detection system’?

**Section 6. Requirements for making a self-declaration of freedom**

15. Is the OIE procedure for the publication of a self-declaration of freedom sufficient guidance for Member Countries for making self-declarations of freedom? If not, should a separate chapter be provided within the *Aquatic Code*?

## APPENDIX 2.

**EXAMPLE OF ARTICLES FOR CLAIMING FREEDOM  
(CRAYFISH PLAGUE, EXTRACTED FROM 2017 AQUATIC CODE)**

Article 9.1.4.

**Country free from crayfish plague**

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from crayfish plague if all the areas covered by the shared water bodies are declared countries or *zones* free from crayfish plague (see Article 9.1.5.).

As described in Article 1.4.6., a country may make a *self-declaration of freedom* from crayfish plague if:

- 1) none of the *susceptible species* referred to in Article 9.1.2. are present and *basic biosecurity conditions* have been continuously met for at least the last **two years**;

OR

- 2) any of the *susceptible species* referred to in Article 9.1.2. are present and the following conditions have been met:
  - a) there has been no observed occurrence of the *disease* for at least the last **25 years** despite conditions that are conducive to its clinical expression (as described in the corresponding chapter of the *Aquatic Manual*); and
  - b) *basic biosecurity conditions* have been continuously met for at least the last **10 years**;

OR

- 3) the disease status prior to *targeted surveillance* is unknown but the following conditions have been met:
  - a) *basic biosecurity conditions* have been continuously met for at least the last **five years**; and
  - b) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last **five years** without detection of crayfish plague;

OR

- 4) it previously made a *self-declaration of freedom* from crayfish plague and subsequently lost its *disease free* status due to the detection of crayfish plague but 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 (as described in Chapter 4.3.) have been completed; and
  - c) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place since eradication of the *disease*; and
  - d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last **five years** without detection of crayfish plague.

In the meantime, part or all of the non-affected area may be declared a *free zone* provided that such a part meets the conditions in point 3 of Article 9.1.5.

Article 9.1.5.

**Zone or compartment free from crayfish plague**

If a *zone* or *compartment* extends over more than one country, it can only be declared a *zone* or *compartment* free from crayfish plague if all the relevant *Competent Authorities* confirm that all relevant conditions have been met.

As described in Article 1.4.6., a *zone* or *compartment* within the *territory* of one or more countries not declared free from crayfish plague may be declared free by the *Competent Authority(ies)* of the country(ies) concerned if:

Annex 8 (contd)**APPENDIX 2.**

- 1) none of the *susceptible species* referred to in Article 9.1.2. are present in the *zone* or *compartment* and *basic biosecurity conditions* have been continuously met for at least the last **two years**;

OR

- 2) any of the *susceptible species* referred to in Article 9.1.2. are present in the *zone* or *compartment* and the following conditions have been met:
- a) there has not been any observed occurrence of the *disease* for at least the last **25 years** despite conditions that are conducive to its clinical expression (as described in the corresponding chapter of the *Aquatic Manual*); and
  - b) *basic biosecurity conditions* have been continuously met for at least the last **10 years**;

OR

- 3) the disease status prior to *targeted surveillance* is unknown but the following conditions have been met:
- a) *basic biosecurity conditions* have been continuously met for at least the last five years; and
  - b) *targeted surveillance*, as described in Chapter 1.4., has been in place, in the *zone* or *compartment*, for at least the last **five years** without detection of crayfish plague;

OR

- 4) it previously made a *self-declaration of freedom* for a *zone* from crayfish plague and subsequently lost its *disease free status* due to the detection of crayfish plague in the *zone* but 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 (as described in Chapter 4.3.) have been completed; and
  - c) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place since eradication of the *disease*; and
  - d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last **five years** without detection of crayfish plague.

## Article 9.2.6.

**Maintenance of free status**

A country, *zone* or *compartment* that is declared free from *infection* with *A. astaci* following the provisions of points 1 or 2 of Articles 9.2.4. or 9.2.5. (as relevant) may maintain its status as free from infection with *A. astaci* provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from infection with *A. astaci* following the provisions of point 3 of Articles 9.2.4. or 9.2.5. (as relevant) may discontinue *targeted surveillance* and maintain its free status provided that conditions that are conducive to clinical expression of infection with *A. astaci*, as described in the corresponding chapter of the *Aquatic Manual*, 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 infection with *A. astaci*, *targeted surveillance* should be continued at a level determined by the *Aquatic Animal Health Service* on the basis of the likelihood of infection.



APPENDIX 3. Summary of the periods required for *basic biosecurity conditions* and *targeted surveillance* in Article X.X.4. in each disease-specific chapter of the *Aquatic Code*.

**EU Comment:**

We assume the numbers in the table below relate to ‘years’ but it would be useful if this could be stated.

	Epizootic haematopoietic necrosis disease	Epizootic ulcerative syndrome	Infection with <i>Gyrodactylus salaris</i>	ISA virus HPR0 and HPR deleted	ISA virus HPR deleted	Infection with salmonid alphavirus	Infectious haematopoietic necrosis	Koi herpesvirus disease	Red sea bream iridoviral disease	Spring viraemia of carp	Viral haemorrhagic septicaemia	Infection with abalone herpesvirus	Infection with <i>Bonamia ostreae</i>	Infection with <i>Bonamia exitiosa</i>	Infection with <i>Martellia refringens</i>	Infection with <i>Perkinsus marinus</i>	Infection with <i>Perkinsus olseni</i>	Infection with <i>Xenobolus californiensis</i>	Acute hepatopancreatic necrosis disease	Crayfish plague ( <i>Aphanomyces astaci</i> )	Infection with yellow head virus	Infectious hypodermal and haematopoietic necrosis	Infectious myonecrosis	Necrotising hepatopancreatitis	Taura syndrome	White spot disease	White tail disease	Infection with <i>B. dendrobatidis</i>	Infection with <i>B. salamandrinus</i>	Infection with ranavirus	
<b>1. Absence of susceptible species</b>	2	NA	2	2	NA	2	2	2	2	2	NA	2	2	2	3	3	NA	3	2	2	2	2	2	2	2	2	2	2	2	2	2
<b>2. Historical freedom</b>																															
- Not observed	10	10	10	NA	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	25	10	10	10	10	10	10	10	10	10	10	10
- Basic biosecurity conditions	10	10	10	NA	10	10	10	10	10	10	10	2	2	2	3	3	3	3	2	10	2	2	2	2	2	2	2	2	10	10	10
<b>3. Targeted surveillance</b>																															
- Basic biosecurity conditions	2	2	5	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3	2	5	2	2	2	2	2	2	2	2	2	2	2

- Targeted surveillance	2	2	5	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3	2	2	5	2	2	2	2	2	2	2	2	2	2	2	2	2
<b>4. Return to freedom</b>	2	2	5	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3	2	2	5	2	2	2	2	2	2	2	2	2	2	2	2	2

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## G L O S S A R Y

**EU Comment**

**The EU thanks the OIE and supports the proposed change to the Glossary. A minor re-word suggestion and a comment are included below.**

For the purpose of the *Aquatic Code*:

**AQUATIC ANIMAL WASTE**

means the entire carcass of aquatic animals, their parts, or associated liquids which are intended for disposal or other purposes than for human consumption.

**We would like to suggest a slight re-wording as follows: ‘AQUATIC ANIMAL WASTE means the entire carcasse of aquatic animals, their parts, or associated liquids which are intended for disposal, or for ~~other~~ purposes other than ~~for~~ human consumption’.**

**We would however, also like consideration to be given to including pond sludge/ mud or other waste collected in ponds or in filtration units in aquaculture establishments.**



**Note to Members:** Marked edits are indicated against the initial revision of the revised chapter rather than the currently adopted text.

## CHAPTER 2.3.9.

### INFECTION WITH SPRING VIRAEMIA OF CARP VIRUS

#### EU comment:

**The EU thanks the OIE and supports the proposed changes to this Chapter.**

**Comments are included in the text below.**

#### 1. Scope

Infection with spring viraemia of carp virus means infection with the pathogenic agent *Carp sprivivirus* (commonly known as spring viraemia of carp virus [SVCV]), of the Genus *Sprivirus* and the Family *Rhabdoviridae*.

#### 2. Disease information

##### 2.1. Agent factors

##### 2.1.1. Aetiological agent

The virus genome is a non-segmented, negative-sense, single strand of RNA. The genome contains 11,019 nucleotides encoding five proteins in the following order: a nucleoprotein (N), a phosphoprotein (P), a matrix protein (M), a glycoprotein (G) and an RNA-dependent, RNA polymerase (L). The genome does not contain a non-virion (NV) gene between the G and L genes as is found in fish rhabdoviruses of the genus *Novirhabdovirus* (Ahne *et al.*, 2002). The type strain of SVCV is available from the American Type Culture Collection (ATCC VR-1390). Two complete genome sequences of the type strain have been submitted to Genbank (Genbank accession U18101 by Bjorklund *et al.* [1996] and Genbank accession AJ318079 by Hoffmann *et al.* [2002]). The complete genome sequence of isolates from China (People's Rep. of) has also been deposited in Genbank (Genbank accession DQ097384 by Teng *et al.* [2007] and Genbank accession EU177782 by Zhang *et al.* [2009]).

Stone *et al.* (2003) used sequence analysis of a 550 nucleotide region of the G-gene to compare 36 isolates from different fish species and geographical locations that were previously identified by serology as SVCV or pike fry rhabdovirus (PFRV) ~~by serology~~. The analysis showed that the isolates could be separated into four distinct genogroups and that all of the SVCV isolates could be assigned to genogroup I, sharing <61% nucleotide identity with viruses in the other three genogroups. Re-analysis of the sequence data generated for viruses assigned to Genogroup I identified four subgroups (Ia–d). Those viruses originating in Asia were assigned to Subgroup Ia, those from Moldova, the Ukraine and Russia to Subgroups Ib and Ic, and those from the UK to Subgroup Id.

##### 2.1.2. Survival and stability in processed or stored samples

There are limited published data on the stability of the pathogen in host tissues. There is also limited information on the stability of the virus in the tissues after death of a diseased animal. Detection of SVCV in the tissues of recently dead animals by either ~~both~~ reverse-transcription polymerase chain reaction (RT-PCR) or ~~and~~ culture may be possible ~~should not be ruled out~~, and therefore, dead fish ~~as well as moribund~~ should ~~may~~ be taken for analysis if moribund fish are not available.

The virus can be stored for several months when frozen in medium containing 2–5% serum. The virus is most stable at lower temperatures, with little loss of titre ~~for~~ when stored for 1 month at –20°C, or for 6 months at –30 or –74°C (Ahne, 1976; Kinkelin & Le Berre, 1974). The virus is stable over four freeze (–30°C)–thaw cycles in medium containing 2% serum (Kinkelin & Le Berre, 1974).

##### 2.1.3. Survival and stability outside the host

The virus has been shown to remain viable outside the host for 5 weeks in river water at 10°C, for more than 6 weeks in pond mud at 4°C, reducing to 4 days in pond mud at 10°C (Ahne, 1976).

## Annex 10 (contd)

For inactivation methods, see Section 2.4.5.

## 2.2. Host factors

### 2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with SVCV according to Chapter 1.5. of the *Aquatic Animal Health Code (Aquatic Code)* are: ~~all varieties and subspecies of common carp (*Cyprinus carpio*), bighead carp (*Aristichthys nobilis*), bream (*Abramis brama*), Caspian white fish (*Rutilus kutum*), fathead minnow (*Pimephales promelas*), golden shiner (*Notemigonus crysoleucas*), goldfish (*Carassius auratus*), grass carp (*Ctenopharyngodon idella*), roach (*Rutilus rutilus*) and sheatfish (also known as European or wels catfish) (*Silurus glanis*).~~

<u>Family</u>	<u>Scientific name</u>	<u>Common name</u>
	<i>Abramis brama</i>	Bream
	<i>Aristichthys nobilis</i>	Bighead carp
	<i>Carassius auratus</i>	Goldfish
<u>Cyprinidae</u>	<i>Ctenopharyngodon idella</i>	Grass carp
	<i>Cyprinus carpio</i>	Common carp (all varieties and subspecies)
	<i>Danio rerio</i>	Zebrafish
	<i>Notemigonus crysoleucas</i>	Golden shiner
	<i>Pimephales promelas</i>	Fathead minnow
	<i>Rutilus kutum</i>	Caspian white fish
	<i>Rutilus rutilus</i>	Roach
<u>Siluridae</u>	<i>Silurus glanis</i>	Sheatfish (also known as European or wels catfish)

### 2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence for susceptibility according to Chapter 1.5. of the *Aquatic Code* are: Crucian carp (*Carassius carassius*), pike (*Esox lucius*), firebelly newt (*Cynops orientalis*), silver carp (*Hypophthalmichthys molitrix*), and Yellow perch (*Perca flavescens*) ~~and zebrafish (*Danio rerio*).~~

Evidence is lacking for these species to either confirm that the identity of the pathogenic agent is SVCV, transmission mimics natural pathways of infection, or presence of the pathogenic agent constitutes an infection.

<u>Family</u>	<u>Scientific name</u>	<u>Common name</u>
<u>Cyprinidae</u>	<i>Carassius carassius</i>	Crucian carp
	<i>Hypophthalmichthys molitrix</i>	Silver carp
<u>Esocidae</u>	<i>Esox lucius</i>	Northern pike
<u>Percidae</u>	<i>Perca flavescens</i>	Yellow perch
<u>Salamandridae</u>	<i>Cynops orientalis</i>	Firebelly newt

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following organisms, but an active infection has not been demonstrated:

<u>Family</u>	<u>Scientific name</u>	<u>Common name</u>
Catostomidae	<i>Catostomus commersonii</i>	White sucker
Cichlidae	<i>Sarotherodon niloticus</i>	Nile tilapia
	<i>Notropis atherinoides</i>	Emerald shiner
<u>Cyprinidae</u>	<i>Cirrhinus mrigala</i>	Mrigal carp
	<i>Labeo rohita</i>	Rohu
	<i>Tinca tinca</i>	Tench
<u>Penaeidae</u>	<i>Litopenaeus vannamei</i>	<u>Pacific white shrimp</u>
	<i>Oncorhynchus tshawytscha</i>	<u>Chinook salmon</u>
<u>Salmonidae</u>	<i>Oncorhynchus nerka</i>	<u> sockeye salmon</u>
	<i>Oncorhynchus mykiss</i>	<u>Rainbow trout</u>
	<i>Oncorhynchus mykiss</i>	<u>Steelhead trout</u>

### 2.2.3. Non-susceptible species

Species that have been found non-susceptible to infection with SVCV according to Chapter 1.5. of the Aquatic Code are:

<u>Family</u>	<u>Scientific name</u>	<u>Common name</u>
Centrarchidae	<i>Micropterus salmoides</i>	Largemouth Bass
Esocidae	<i>Esox masquinongy</i>	Muskellunge
Percidae	<i>Sander vitreus</i>	Walleye

### 2.2.4. Likelihood of infection by species, host life stage, population or sub-populations

Common carp varieties are the principal hosts for SVCV and are considered to be most susceptible to infection with SVCV followed, in order of susceptibility, by other carp species (including hybrids), other susceptible cyprinid species and finally susceptible non-cyprinid fish species. When sampling during surveillance programmes for SVCV, common carp or strains such as koi or ghost (koi x common) carp are preferentially selected, followed by carp hybrids (e.g. common carp x crucian carp), then other carp species such as crucian carp, goldfish, grass carp, bighead carp and silver carp. Should these species not be available then other known susceptible species should be sampled. Cyprinid species may be ~~are increasingly~~ mixed together in polyculture systems and the risk of transmission of SVCV between species during disease outbreaks is high (Billard & Berni, 2004).

Generally, young fish up to 1 year old are most susceptible to clinical disease, but all age groups can be affected. Moreover, there is a high variability in the degree of susceptibility to infection with SVCV among individuals of the same fish species. Apart from the physiological state of the fish, the role of which is poorly understood, age or the age-related status of innate immunity appears to be extremely important: the younger the fish, the higher the susceptibility to overt disease, although even adult broodfish can be susceptible to infection.

Annex 10 (contd)

Fish that have separated from the shoal and found at the water inlet or sides of a pond are more likely to be infected.

**2.2.5. Distribution of the pathogen in the host**

~~The transmission of SVCV is horizontal (Fijan, 1988).~~ SVCV appears to enter via the gills and then spreads to the kidney, liver, heart, spleen and alimentary tract. During disease outbreaks high titres of virus occur in the liver and kidney of infected fish, but much lower titres occur in the spleen, gills and brain (Dixon, 2008). The virus has been detected in ovarian fluid (Bekesi & Csontos, 1985), ~~but vertical transmission has yet to be demonstrated.~~

**2.2.6. Aquatic animal reservoirs of infection**

Liu *et al.* (2004) isolated SVCV in China (People's Rep. of) from common and koi carp exhibiting no external or internal signs of disease, and similarly, the virus was isolated from apparently healthy wild carp in Canada (Garver *et al.*, 2007).

**2.2.7. Vectors**

The parasitic invertebrates *Argulus foliaceus* (Crustacea, Branchiura) and *Piscicola geometra* (Annelida, Hirudinea) have been demonstrated to transfer SVCV from diseased to healthy fish under experimental conditions and the virus has been isolated from *A. foliaceus* removed from infected carp (Ahne *et al.*, 2002; Dixon, 2008). It has been demonstrated experimentally that virus can be isolated from fish tissues regurgitated by herons (*Ardea cinerea*) 120 minutes after being fed with SVCV-infected carp, suggesting a potential route for SVCV transmission, but is not known whether such transmission has occurred in nature (Peters & Neukirch, 1986).

**2.3. Disease pattern****2.3.1. Mortality, morbidity and prevalence**

During an outbreak of infection with SVCV there will be a noticeable increase in mortality in the population. ~~Co-infections with koi herpesvirus or carp oedema virus can increase levels of mortality.~~ Disease patterns are influenced by water temperature, age and condition of the fish, population density and stress factors. The immune status of the fish is also an important factor with both nonspecific (e.g. interferon) and specific immunity (serum antibodies, cellular immunity) having important roles. Poor physiological condition of over-wintered fish may be a contributory factor to disease susceptibility. In European aquaculture, losses can be up to 70% in young carp (Ahne *et al.*, 2002), but are usually from 1 to 40%.

In one survey from Serbia, the virus was isolated by culture in samples collected from 12 of the 38 hatcheries screened over the 10-year period (1992–2002). The virus occurred sporadically in different ponds on one site, and sporadically from year to year at different sites (Svetlana *et al.*, 2004). In another study, 18 of 30 tissue pools (five fish/pool) of wild common carp sampled in Canada in 2006 were positive for SVCV by culture (Garver *et al.*, 2007). The isolation of SVCV in the latter case was from asymptomatic common carp which correlates with observations that SVCV infection can often be clinically inapparent (Fijan, 1999).

**2.3.2. Clinical signs, including behavioural changes**

Fish can become lethargic, separate from the shoal and gather at the water inlet or sides of a pond and some may experience loss of equilibrium. Clinical signs of infection with SVCV are nonspecific and not all fish will exhibit all of the signs. Two of the most obvious and consistent features are abdominal distension and haemorrhages. The latter may occur on the skin, fin bases, eyes and gills, which may be pale. The skin may darken and exophthalmia is often observed. The vent may be swollen, inflamed and trail mucoid casts. ~~During an outbreak of infection with SVCV there will be a noticeable increase in mortality in the population.~~ Diseased fish usually appear darker in colour. There may be no clinical signs in cases with a sudden onset of mortality.



### 2.3.3 Gross pathology

There are no pathognomonic gross lesions. Lesions may be absent in cases of sudden mortality. Gross pathologies are mainly documented for common carp and may include excess ascitic fluid in the abdominal cavity, usually containing blood, degeneration of the gill lamellae and inflammation of the intestine, which contains mucous instead of food. Oedema and haemorrhage of the visceral organs is commonly observed (the spleen is often enlarged), and organs adhere to each other and to the peritoneum. Focal haemorrhages may be seen in the muscle and fat tissue, as well as in the swim bladder (see Dixon, 2008). However, petechial haemorrhages are infrequent uncommon in cases caused by Asian strains of SVCV (Dikkeboom *et al.*, 2004).

### 2.3.4. Modes of transmission and life cycle

The transmission of SVCV is horizontal (Fijan, 1988). Horizontal transmission may be direct, via water, fomites or vectors (Section 2.2.7.) (Fijan, 1988). The virus appears to enter the host via the gill. A viraemia follows and the virus rapidly spreads to the liver, kidney, spleen and alimentary tract. The virus can be detected in faeces and is also shed into the water via faeces and urine (Ahne, 1982).

Vertical or 'egg-associated' transmission cannot be ruled out following one report of isolation of SVCV from carp ovarian fluid, although there have been no further reports (Bekesi & Csontos, 1985).

~~Horizontal transmission may be direct or vectorial, water being the major abiotic vector (Fijan, 1988). Animate vectors (Section 2.2.6.) and fomites may also be involved in transmission of SVCV (Fijan, 1988).~~ Once SVCV is established in populations, it may be very difficult to eradicate without destroying all types of life at the site.

### 2.3.5. Environmental and management factors

Disease outbreaks in carp generally occur between 11 and 17°C. They rarely occur below 10°C, and mortalities, particularly in older fish, decline as the temperature exceeds 22°C (Fijan, 1988). However, the virus was isolated from apparently healthy fish from a lake in Canada that had been sampled over a 13-day period during which the water temperature varied between 24.2°C and 27.3°C (Garver *et al.*, 2007). These fish may have been more susceptible to infection as they were penned and detection was during spawning. Secondary and concomitant bacterial and/or parasitic infections can affect the mortality rate and display of signs. In carp, the disease is often observed in springtime (hence the common name for the disease), particularly in countries having cold winters. It is believed that the poor condition of the over-wintered fish may be a contributory factor in disease occurrence. The disease can occur in fish in quarantine following the stress of transportation, even though there has been no evidence of infection prior to transportation.

### 2.3.6. Geographical distribution

For a long time, the geographical range of SVC was limited to countries of the European continent that experience low water temperatures during winter. ~~Consequently~~ The disease has been recorded from most European countries and from certain of the western Independent States of the former Soviet Union (Belarus, Georgia, Lithuania, Moldova, Russia and the Ukraine) (see Dixon 2008 for references to these and the following locations). However, in 1998, the disease was recorded South America (in goldfish in a lake in Brazil), in 2002 in the USA, and in 2006 in Canada. Detection of the virus in carp in China (People's Rep. of) was confirmed in 2004.

For recent information on distribution at the country level consult the WAHIS interface.

## 2.4. Biosecurity and disease control strategies

### 2.4.1. Vaccination

A safe and effective vaccine is not currently available; however, ~~a number~~ the efficacy of an experimental DNA vaccine has been investigated ~~inactivated preparations, live attenuated vaccines and DNA vaccines have given encouraging results (Dixon, 2008, (Emmenegger & Kurath, 2008). The use of live attenuated vaccines or the DNA vaccines might affect diagnostic performance.~~

## Annex 10 (contd)

**2.4.2. Chemotherapy including blocking agents**

Methisoprinol inhibits the replication of SVCV *in vitro*, but has not been tested under carp culture conditions.

**2.4.3. Immunostimulation**

Injection into carp of single-stranded and double-stranded RNA (which is an interferon inducer) protected carp for longer than 3 weeks, but the treatment is not effective by bath administration (Alikin *et al.*, 1996).

**2.4.4. Breeding resistant strains**

The "Krasnodar" strain of common carp has been bred for increased resistance to SVCV (Kirpichnikov *et al.*, 1993).

**2.4.5. Inactivation methods**

The virus is inactivated at 56°C for 30 minutes, at pH 12 for 10 minutes and pH 3 for 2 hours (Ahne, 1986). Oxidising agents, sodium dodecyl sulphate, non-ionic detergents and lipid solvents are all effective for inactivation of SVCV. The following disinfectants are also effective for inactivation: 3% formalin for 5 minutes, 2% sodium hydroxide for 10 minutes, 540 mg litre<sup>-1</sup> chlorine for 20 minutes, 200–250 ppm (parts per million) iodine compounds for 30 minutes, 100 ppm benzalkonium chloride for 20 minutes, 350 ppm alkyltoluene for 20 minutes, 100 ppm chlorhexidine gluconate for 20 minutes and 200 ppm cresol for 20 minutes (Ahne, 1982; Ahne & Held, 1980; Kiryu *et al.*, 2007).

**2.4.6. Disinfection of eggs and larvae**

Eggs can be disinfected by iodophor treatment (Ahne & Held, 1980).

**2.4.7. General husbandry**

Methods to control of infection with SVCV rely ~~relies~~ on avoiding exposure to the virus coupled with good hygiene practices. This is feasible on small farms supplied by spring or borehole water and a secure system to prevent fish entering the farm via the discharge water. ~~Hygiene measures should include disinfection of eggs by iodophor treatment (Ahne & Held, 1980), until it has been confirmed unequivocally that vertical transmission does not occur, regular disinfection of ponds, chemical disinfection of farm equipment, careful handling of fish to avoid stress and safe disposal of dead fish.~~ Reducing fish stocking density during winter and early spring will reduce the spread of the virus. In rearing facilities with a controlled environment, elevation of water temperature above 19–20°C may stop or prevent outbreaks of infection with SVCV.

**3. Specimen selection, sample collection, transportation and handling**

This Section draws on information in Sections 2.2., 2.3. and 2.4. to identify populations, individuals and samples which are most likely to be infected.

**3.1. Selection of populations and individual specimens**

For disease investigations, moribund fish or fish exhibiting clinical signs of infection with SVCV should be collected. Ideally fish should be alive when collected, however recently dead fish can be collected for diagnostic purposes. It should be noted however, that there will be a significant risk of contamination with environmental bacteria if the animals have been dead for some time. There may be no clinical signs or gross pathognomonic gross lesions and no clinical signs in cases of sudden mortality (see Section 4.1.1.).

**EU Comments:**

**We suggest the paragraph below should start with the words 'For the purposes of disease surveillance, sampling should target...'.**

**This would be clearer in that paragraph 1 sets out the approach when a disease investigation is carried out and paragraph 2 sets out the approach when samples are taken to gain or maintain freedom**

~~Sampling samples should target comprise all susceptible species on the site with each group being represented in the sample. A group is defined as a. The population to be sampled may be stratified into groups. of the same fish species that shares a common water supply and originate from the same broodfish or spawning population. Generally young Moribund fish up to 1 year old are most susceptible to clinical disease, but all age groups can be affected. Any moribund fish present in the fish population to be sampled should be sampled selected first for sample collection and the remainder of the samples should comprise~~

randomly selected live fish from all groups of susceptible species rearing units that represent the lot being examined.

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### 3.2. Selection of organs or tissues

Kidney, spleen, gill and encephalon should be selected from subclinically infected fish (apparently healthy fish).

For clinically affected fish: whole ~~fry~~ ~~alevin~~ (body length  $\leq$  4 cm), entire viscera including kidney and encephalon ( $>$  4 cm body length  $\leq$  6 cm) or, for larger ~~sized~~ fish, liver, kidney, spleen and encephalon should be selected.

### 3.3. Samples or tissues not suitable for pathogen detection

~~Virus isolation may also not be possible from Decomposed clinical samples A number of studies in which attempts were made to isolate virus from reproductive fluids were unsuccessful, although and seminal fluid samples are not suitable. While the virus has been isolated at low frequency from ovarian, but not seminal, fluids, the suitability of these samples has not been substantiated (Bekesi & Csontos, 1985).~~

### 3.4. Non-lethal sampling

Serological assays for antibodies can be undertaken on blood samples; ~~the however, serology can only be used for a presumptive diagnosis given~~ cross reactivity of anti-SVCoV antibodies with viruses of the species pike fry sprivivirus allows for a presumptive indication of infection with SVCoV.

### 3.5. Preservation of samples for submission

For guidance on sample preservation methods for the intended test methods, see Chapter ~~2.2.0 or 2.3.0 or 2.4.0.~~

#### 3.5.1. Samples for pathogen isolation

Samples for virus isolation (Section 3.2.) should be transported to the laboratory at 4°C using refrigerated containers or on ice, preferably in virus transport medium and tested within 24 hours or, in exceptional circumstances, 48 hours. The shipment of organ samples is preferred, but live or whole dead fish can be submitted to the testing laboratory if necessary. If this is not possible, samples can be frozen, but there may be loss of virus viability on thawing the samples. Repeated freeze–thawing of the sample must be avoided.

#### 3.5.2. Preservation of Fixed samples for molecular detection

~~Tissue samples for PCR testing should be preserved in 70–90% (v/v) analytical/reagent grade (absolute) ethanol. The recommended ratio of ethanol to tissue is 10:1 based on studies in terrestrial animal and human health. The use of lower grade (laboratory or industrial grade) ethanol is not recommended. [Alternatives to ethanol can be mentioned if they can be referenced.]~~

The material collected for virus culture is generally used for the molecular diagnostic assays, but additional tissue samples for RT-PCR can be preserved in commercially available RNA preservation solutions according to the manufacturers' recommendations, or, alternatively, samples can be preserved in 80–90% (v/v) analytical grade (absolute) ethanol at the recommended ratio of ethanol to tissue of 10:1.

#### 3.5.3. Fixed samples for histopathology, immunohistochemistry or *in-situ* hybridisation

#### **EU Comment:**

**In-situ hybridisation is not a method which is recommended in Table 4.1. We think that it should therefore be removed for 3.5.3**

Histology samples from each individual fish must be taken into 10% neutral buffered formalin (NBF) immediately after collection to prevent sample deterioration. The recommended ratio of fixative to tissue is 10:1 and each sample should be no thicker than approximately 4 mm to allow the fixative to penetrate the material and should be cut cleanly.

Annex 10 (contd)**3.5.4. Fixed samples for electron microscopy**

~~EM sampling is~~ Samples for electron microscopy are not routinely required as ~~standard, and the material is and are~~ collected only when ~~where~~ it is considered beneficial to facilitate ~~potential~~ further diagnostic investigation work. From each fish sampled, a 2 mm cubed (approximately) sample section from each of the appropriate organs described in section 3.2 should be fixed in glutaraldehyde; the recommended ratio of fixative to tissue is 10:1.

**3.5.5. Samples for other tests**

Tubes for the separation of serum are available commercially. After collection, the blood is allowed to clot by leaving it undisturbed at room temperature. This usually takes 15–30 minutes. Serum is clarified by centrifuging at 1000–2000 **g** for 10 minutes in a refrigerated centrifuge.

It is important to immediately transfer the liquid component (serum) into a clean polypropylene tube using a Pasteur pipette and maintain the samples at 2–8°C while handling. If the serum is not analysed immediately, it should be apportioned into 0.5 ml aliquots, stored, and transported at –20°C or lower. It is important to avoid freeze–thaw cycles because this is detrimental to many serum components. Samples that are haemolysed, icteric or lipaemic can invalidate certain tests.

**3.6. Pooling of samples**

~~Traditionally pools of five animals have been used and more recently this has been increased to pools of ten animals for virus culture. However, no published data on the effect of pooling on test characteristics has been published.~~

Pooling of samples from more than one individual animal for a given purpose should only be recommended where supporting data on diagnostic sensitivity and diagnostic specificity are available. However, smaller life stages (e.g. fry) can be pooled to provide a minimum amount of material for testing.

**4. Diagnostic methods**

The methods currently available for identifying infection that can be used in i) surveillance of apparently healthy populations), ii) presumptive and iii) confirmatory diagnostic purposes are listed in Table 4.1. by life stage. The designations used in the Table indicate:

Key:

- +++ = Recommended method(s) validated for the purpose shown and usually to stage 3 of the OIE Validation Pathway;
- ++ = Suitable method(s) but may need further validation;
- + = May be used in some situations, but cost, reliability, lack of validation or other factors severely limits its application;

Shaded boxes = Not appropriate for this purpose.

The selection of a test for a given purpose depends on the analytical and diagnostic sensitivities and specificities, repeatability and reproducibility. OIE Reference Laboratories welcome feedback on diagnostic performance for assays, in particular PCR methods, for factors affecting assay analytical sensitivity or analytical specificity, such as tissue components inhibiting amplification, presence of nonspecific or uncertain bands, etc., and any assays that are in the +++ category.

**Table 4.1.** OIE recommended diagnostic methods and their level of validation for surveillance of healthy animals and investigation of clinically affected animals

**EU Comment:**

**Table 4.1 indicates that viral RNA cannot be detected in infected early life stages. We do not believe this to be the case.**

**Cell culture alone cannot be used to identify SVCV. It can only be used in combination with molecular or immunological methods.**

Method	A. Surveillance of apparently healthy animals				B. Presumptive diagnosis of clinically affected animals				C. Confirmatory diagnosis <sup>1</sup> of a suspect result from surveillance or presumptive diagnosis			
	Early life stages <sup>2</sup>	Juveniles <sup>2</sup>	Adults	LV	Early life stages <sup>2</sup>	Juvenile s <sup>2</sup>	Adults	LV	Early life stages <sup>2</sup>	Juvenile s <sup>2</sup>	Adults	LV
Wet mounts												
Histopathology <sup>3</sup>												
Cytopathology <sup>3</sup>												
Cell or artificial media culture		++	++	1		++	++	1		++	++	1
Real-time PCR												
Conventional PCR		++	++	1		++	++	1		++	++	1
Amplicon sequencing <sup>4</sup>										+++	+++	1
<i>In-situ</i> hybridisation												
<u>Immunohistochemistry</u>						++	++	1				
Bioassay												
LAMP												

Method	A. Surveillance of apparently healthy animals				B. Presumptive diagnosis of clinically affected animals				C. Confirmatory diagnosis <sup>1</sup> of a suspect result from surveillance or presumptive diagnosis			
	Early life stages <sup>2</sup>	Juveniles <sup>2</sup>	Adults	LV	Early life stages <sup>2</sup>	Juveniles <sup>2</sup>	Adults	LV	Early life stages <sup>2</sup>	Juveniles <sup>2</sup>	Adults	LV
Ab ELISA												
Ag ELISA						++	++	1				
<del>IFAT</del> Other antigen detection methods						++	++	1				

LV = level of validation, refers to the stage of validation in the OIE Pathway (chapter 1.1.2); PCR = polymerase chain reaction; ELISA = enzyme-linked immunosorbent assay; LAMP = loop-mediated isothermal amplification.<sup>1</sup>For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). <sup>2</sup>Early and juvenile life stages have been defined in Section 2.2.4.

<sup>3</sup>Histopathology and cytopathology can be validated if the results from different operators have been statistically compared. <sup>4</sup>Sequencing of the PCR product. Shading indicates the test is inappropriate or should not be used for this purpose.

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Annex 10 (contd)**4.1. Wet mounts**

Not applicable.

**4.2. Histo- and cytopathology**

Histopathological changes can be observed in all major organs. In the liver, blood vessels show oedematous perivascularitis progressing to necrosis. Liver parenchyma shows hyperaemia with multiple focal necrosis and degeneration. The heart shows pericarditis and infiltration of the myocardium progressing to focal degeneration and necrosis. The spleen shows hyperaemia with hyperplasia of the reticuloendothelium and enlarged melanomacrophage centres, and the pancreas is inflamed with multifocal necrosis. In the kidney, damage is seen to excretory and haematopoietic tissue. Renal tubules are clogged with casts and the cells undergo hyaline degeneration and vacuolation. The intestine shows perivascular inflammation, desquamation of the epithelium and atrophy of the villi. The peritoneum is inflamed, and lymph vessels are filled with detritus and macrophages. In the swim bladder, the epithelial lamina changes from a monolayer to a discontinuous multi-layer and vessels in the submucosa are dilated with nearby lymphocyte infiltration.

As the histopathological presentation picture is not specific for the disease, and not all fish will exhibit each feature (Misk *et al.*, 2016), microscopic methods by themselves are not recommended for diagnosis of SVC ~~as the histopathological picture is not specific for the disease~~. They may, however, provide supporting evidence, particularly, when immunohistochemistry immunohistological (IHC) or nucleic acid DNA-based *in-situ* hybridisation methods are used (see the relevant Sections below).

~~Fixed sections can also be used for histoimmunohistochemistry (but see caveats in Section 4.6.).~~

**4.3. Cell or artificial media culture for isolation**

~~If culturing viruses~~ Cell lines should be monitored to ensure that susceptibility to targeted pathogens has not changed.

*Cell culture*

Cell line to be used: EPC, FHM or GCO.

Virus extraction: Use the procedure described in Section A-2.2.2 of Chapter 2.3.0.

Inoculation of cell monolayers: make two serial tenfold dilutions of the 1/10 organ homogenate supernatants in cell culture medium (i.e. the homogenate supernatants will be 1/100 and 1/1000 dilutions of the original organ material) and transfer an appropriate volume of each of these two dilutions on to 24-hour-old cell monolayers drained of their culture medium. Alternatively, make a single tenfold dilution of the 1/10 organ homogenate (i.e. a 1/100 dilution of the original organ material) and add an appropriate volume of both the 1/10 and 1/100 dilutions directly to undrained 24 hour-old cell monolayers, to effect 1/100 and 1/1000 final dilutions of the organ homogenate. Should toxicity of the sample be a problem, make two serial tenfold dilutions of the 1/10 organ homogenate supernatants in cell culture medium as described above and inoculate at least 2 cm<sup>2</sup> of drained cell monolayer with 100 µl of each dilution. Allow to adsorb for 0.5–1 hour at 10–15°C, withdraw the inoculum and add cell culture medium buffered at pH 7.6 and supplemented with 2% fetal calf serum (FCS) (1 ml well<sup>-1</sup> for 24-well cell culture plates). Incubate at 20°C.

Monitoring incubation: Follow the course of infection in positive controls and other inoculated cell cultures by microscopic examination at ×40–100 magnification for 7 days. The use of a phase-contrast microscope is recommended.



Maintain the pH of the cell culture medium at between 7.3. and 7.6. during incubation. This can be achieved by the addition to the inoculated medium of sterile bicarbonate buffer (for tightly closed cell culture flasks) or HEPES-buffered medium (HEPES = N-2-hydroxyethyl-piperazine-N-2-ethanesulfonic acid) or 2 M Tris (Tris [hydroxymethyl] aminomethane)/HCl buffer solution (for cell culture plates).

If a cytopathic effect (CPE) appears in those cell cultures inoculated with the dilutions of the tested homogenate supernatants, identification procedures must be undertaken immediately ~~(see Section 4.6.2.)~~.

Subcultivation procedures: Using a pipette, try to dislodge cells from the cell culture vessels and collect aliquots of cell culture medium plus cells from all inoculated monolayers, keeping different groups separate. The aliquots of the 1/100 and 1/1000 dilutions are pooled and inoculated on to fresh 24 hour-old cell cultures to effect 1/10 and 1/100 final dilutions of the pooled aliquots. Incubate and monitor as described above. ~~If no CPE occurs, the test may be declared negative.~~

If no CPE occurs the test may be declared negative. However, if undertaking surveillance to demonstrate freedom from SVCV it would be advisable to screen the cells at the end of the 14 days using an SVCV-specific RT-PCR ~~or real-time RT-PCR~~ (Section 4.4.). Following a positive result culture should be re-attempted.

Following isolation, the virus must be identified, and this can be achieved by antigen detection methods, virus neutralisation or nucleic acid identification methods. The former two methods are generally regarded as presumptive unless fully validated monoclonal or polyclonal antibodies are used, as cross reactions with other viruses occur. Commercially available kits using polyclonal antibodies may also lack specificity, and those using monoclonal antibodies may not detect all subgenogroups of SVCV (Dixon & Longshaw, 2005). Nucleic acid detection methods must always be followed up by sequencing or use of a method such as reverse hybridisation (Sheppard *et al.*, 2007) to confirm the identity of the virus.

#### 4.4. Nucleic acid amplification

##### EU Comment:

**In this Section and throughout the text there is reference to Real-time PCR. This terminology is not used nowadays as it has been replaced by quantitative PCR of qPCR (see MIQE Guidelines 2009)**

##### 4.4.1. Real-time PCR

The following controls should be run with each assay: negative extraction control; positive control; no template control; internal PCR control.

Real-time RT-PCR assays are available to detect and confirm infection with SVCV (Yue *et al.*, 2008; Zhang *et al.*, 2009), however, they are not currently recommended as they have not been sufficiently validated.

##### 4.4.2. Conventional PCR

The following controls should be run with each assay: negative extraction control; positive control; no template control; internal PCR control.

Nested reverse-transcription polymerase chain reaction (RT-PCR) (confirmation of virus identity from cell culture isolation or directly from fish tissue extracts)

The genome of SVCV consists of a single strand of RNA of approximately 11 kb, with negative polarity. Amplification of a 714 bp fragment of SVCV cDNA is performed using primers derived from sequences of the region coding for the glycoprotein gene: 5'-TCT-TGG-AGC-CAA-ATA-GCT-CAR\*-R\*TC-3' (SVCV F1) and 5'-AGA-TGG-TAT-GGA-CCC-CAA-TAC-ATH\*-ACN\*-CAY\*-3' SVCV R2), using a modification of the method of Stone *et al.* (2003).

- i) Total RNA is extracted from 100 µl of supernatant from cell cultures exhibiting CPE or 50 µl of fish tissue extract and dissolved in 40 µl molecular biology grade DNase- and RNase-free water.

Annex 10 (contd)

A number of total RNA extraction kits are available commercially that will produce high quality RNA suitable for RT-PCR. ~~Examples are Trizol Reagent<sup>®</sup> (RL, Life Technologies, Paisley, UK), SV Total RNA isolation system (Promega) and Nucleospin<sup>®</sup> RNA (AB gene), EZ virus mini kit, Ez RNA tissue mini kit (Qiagen).~~

- ii) For cDNA synthesis, a reverse transcription reaction is performed at 37°C for 1 hour in a 20 µl volume consisting of 1 × M-MLV RT reaction buffer (50 mM Tris, pH 8.3, 75 mM KCl, 10 mM DTT, 3 mM MgCl<sub>2</sub>) containing 1 mM dNTP, 100 pmol SVCV R2 primer, 20 units M-MLV reverse transcriptase (Promega, Southampton, UK) or an equivalent reverse transcriptase system and 1/10 of the total RNA extracted above.
- iii) PCR is performed in a 50 µl reaction volume 1 × PCR buffer (50 mM KCl, 10 mM Tris/HCl, pH 9.0, and 0.1% Triton X-100) containing 2.5 mM MgCl<sub>2</sub>, 200 µM dNTPs, 50 pmol each of the SVCV R2 and SVCV F1 primers, 1.25 units of Taq DNA polymerase, and 2.5 µl reverse transcription reaction mix. The reaction mix is subjected to 35 temperature cycles of: 1 minute at 95°C, 1 minute at 55°C and 1 minute at 72°C followed by a final extension step of 10 minutes at 72°C. Amplified DNA (714 bp) is analysed by agarose gel electrophoresis.
- iv) If the CPE in culture is not extensive it is possible that a visible product will not be generated using a single round of amplification. To avoid such problems, use the semi-nested assay using primers: 5'-TCT-TGG-AGC-CAA-ATA-GCT-CAR\*-R\*TC-3' (SVCV F1) and 5'-CTG-GGG-TTT-CCN\*-CCT-CAA-AGY\*-TGY\*-3' (SVCV R4) according to Stone *et al.* (2003).
- v) The second round of PCR is performed in a 50 µl reaction volume 1 × PCR buffer (50 mM KCl, 10 mM Tris/HCl, pH 9.0, and 0.1% Triton X-100) containing 2.5 mM MgCl<sub>2</sub>, 200 µM dNTPs, 50 pmol each of the SVCV R4 and SVCV F1 primers, 1.25 units Taq DNA polymerase, and 2.5 µl of the first round product. The reaction mix is subjected to 35 temperature cycles of: 1 minute at 95°C, 1 minute at 55°C and 1 minute at 72°C followed by a final extension step of 10 minutes at 72°C. Amplified DNA (606 bp) is analysed by agarose gel electrophoresis.
- vi) All amplified products are confirmed as SVCV in origin by sequencing, and the SVCV subtype (Ia-I<sub>d</sub>) is identified using a BLAST search (<http://www.ebi.ac.uk/blastall/index.html>) or by phylogenetic analysis using the SVCV sequences available in public sequence databases. Phylogenetic analysis is undertaken using a 426 bp region corresponding to nucleotides 429–855 of the glycoprotein gene.
- vii) In cases where the CPE is extensive and the virus replicates to a high titre, or where a semi-nested RT-PCR assay was used, sufficient PCR amplicon will be available for direct sequencing. Where the amplified product is weak it is recommended that the product be inserted into an appropriate sequencing vector (e.g. pGEM-T, pCR<sup>®</sup> 4-TOPO<sup>®</sup>) prior to undertaking the sequencing. At least two independent amplification and sequencing events should be undertaken to eliminate potential sequence errors introduced by the Taq polymerase.

~~The following controls should be run with each assay: negative extraction control; positive control; no template control; internal PCR control.~~

**NOTE:** The appropriate IUB codes have been used where appropriate and are indicated by an asterisk (\*).

Reverse-transcription polymerase chain reaction (RT-PCR) (confirmation of virus identity)

Additional conventional RT-PCR assays are available to detect and confirm SVCV infections (Koutna *et al.*, 2003; Shimahara *et al.*, 2016). A generic primer set based on the polymerase gene also identifies viruses from both the *Sprivirus* and *Perhabdovirus* genera and can be used to screen a virus culture (Ruane *et al.*, 2014). With the exception of the conventional PCR assay developed by Shimahara *et al.* (2016) the other assays were not sufficiently fully-validated against representatives from each of the recognised SVCV genogroups and they may fail to detect the full range of SVCV genotypes.

A summary of the Shimahara *et al.* (2016) RT-PCR method follows. Amplification of a 369 bp fragment of SVCV glycoprotein gene is performed using primers as follows: SVCV-G1: 5'-TGA-AGA-YTG-TGT-CAA-TCA-AGTC-3' and SVCV-G2: 5'-GCG-ART-GCA-GAG-AAA-AAG-TG-3'. Preparation of RNA template is the same as nested RT-PCR above. Reverse transcription of SVCV RNA and amplification of cDNA are carried out using SuperScript III one-step RT-PCR with PlatinumR Taq (Invitrogen) according to the manufacturer's instructions. The RT-PCR reaction mixture contained 10 pmol of each primer, 12.5 µl of 2x reaction mix, 1 µl of SuperScript III RT/Platinum Taq Mix and 2.5 µl template. After reverse transcription at 50°C for 30 minutes and 94°C for 2 minutes, 40 amplification cycles of 94°C for 15 seconds, 56°C for 30 seconds and 68°C for 1 minute followed by a final extension step at 68°C for 7 minutes is performed. All amplified products are confirmed as SVCV in origin by sequencing.

**4.4.3. Other nucleic acid amplification methods**

Loop-mediated isothermal amplification assays are available to detect and confirm SVCV infections (Shivappa *et al.*, 2008), however, they are currently not recommended as they are not sufficiently validated.

Infection with SVCV has also been confirmed using RT-PCR and hybridisation with non-radioactive probes (Oreshkova *et al.*, 1999; Sheppard *et al.*, 2007).

**4.5. Amplicon sequencing**

~~See above (Section 4.4.2). All Nucleotide sequencing of all RT-PCR amplicons should be sequenced to confirm that they are SVCV in origin. (Section 4.4.2) is recommended as one of the final steps for confirmatory diagnosis. SVCV-specific products sequences will share a higher degree of nucleotide identity-similarity to one of the published reference sequences for SVCV (Genbank accession U18101, AJ318079, DQ097384 and EU177782) compared to the published reference sequences for the Pike spriviviruses (GenBank FJ872827, KC113518 and KC113517).~~

**4.6. *In-situ* hybridisation (and histoimmunochemistry)**

~~Although *In-situ* hybridisation can be used to locate SVCV the virus in different tissues on known positive animals, but this assay is currently not recommended as it has not been well validated for SVCV as a diagnostic tool.~~

**4.7. Immunohistochemistry**

SVCV can be detected by immunohistochemistry, however, care must be taken with interpreting the results of serological tests for SVCV, and positive results ~~from antibody-based assays~~ should be confirmed by RT-PCR and sequencing ~~(see Section 4.8.)~~.

- i) Bleed the fish thoroughly.
- ii) Make kidney imprints on cleaned glass slides or at the bottom of the wells of a plastic cell culture plate.

Annex 10 (contd)

- iii) Store and transport the kidney pieces as indicated in Section 2.2.1. of Chapter 2.3.0. together with the other organs required for virus isolation.
- iv) Allow the imprint to air-dry for 20 minutes.
- v) Fix with cold acetone (stored at  $-20^{\circ}\text{C}$ ) for glass slides or 80% acetone in water or 30% acetone in ethanol, also at  $-20^{\circ}\text{C}$ , for plastic wells. Let the fixative act for 15 minutes. Allow the imprints to air-dry for at least 30 minutes and process immediately or freeze at  $-20^{\circ}\text{C}$ .
- vi) Rehydrate the imprints if they have been stored frozen by four rinsing steps with PBS containing 0.05% Tween 20 (PBST), and remove this buffer completely after the last rinse. Block with 5% skim milk or 1% bovine serum albumin, in PBST for 30 minutes at  $37^{\circ}\text{C}$ .
- vii) Rinse four times with PBST, 5 minutes for each rinse. The slides or plastic culture plates can be gently agitated during the rinses.
- viii) Prepare a solution of purified antibody or serum to SVCV in PBST, at the appropriate dilution (which has been established previously or as given by the reagent supplier).
- ix) Incubate the imprints with the antibody solution for 1 hour at  $37^{\circ}\text{C}$  in a humid chamber and do not allow evaporation to occur.
- x) Rinse four times with PBST.
- xi) Incubate the imprints with a solution of fluorescein isothiocyanate (FITC)-conjugated antibody to the immunoglobulin used in the first layer and prepared according to the instructions of the supplier. These FITC antibodies are most often rabbit or goat antibodies.
- xii) Rinse four times with PBST.
- xiii) View the treated imprints on plastic plates immediately, or mount the slides with cover-slips using glycerol saline at pH 8.5, or a commercially-available mountant.
- xiv) Examine under incident ultraviolet (UV) light using a microscope with  $\times 10$  eye pieces and  $\times 20$  or  $\times 40$  objective lenses having numerical aperture of  $>0.65$  and  $>1.3$ , respectively. Positive and negative controls must be found to give the expected results prior to any other observation.

**4.8. Bioassay**

Not available.

**4.9. Antibody-based or antigen detection methods (ELISA, etc.)**

Serological methods must be regarded as presumptive unless fully validated monoclonal or polyclonal antibodies are used, as cross reactions with other viruses closely related spriviviruses (PFRV, GrCRV and TenRV) may occur. Commercially available kits using polyclonal antibodies may lack specificity, and those using monoclonal antibodies may not detect all subgenogroups of SVCV (Dixon & Longshaw, 2005).

*Virus identification by enzyme-linked immunosorbent assay (ELISA)*

- i) Coat the wells of microplates designed for ELISAs with appropriate dilutions of purified immunoglobulins (Ig) specific for SVCV, in 0.02 M carbonate buffer, pH 9.5 ( $200\ \mu\text{l well}^{-1}$ ). Ig may be polyclonal or monoclonal Ig originating most often from rabbit or mouse, respectively. For the identification of SVCV, monoclonal antibodies (MAbs) specific for certain domains of the nucleocapsid (N) protein are suitable.

Annex 10 (contd)

- ii) Incubate overnight at 4°C.
- iii) Rinse four times with PBST.
- iv) Block with skim milk (5% in carbonate buffer) or other blocking solution for 1 hour at 37°C (300 µl well<sup>-1</sup>).
- v) Rinse four times with PBST.
- vi) Add 2% non-ionic detergent (Triton X-100 or Nonidet P-40) to the virus suspension to be identified.
- vii) Dispense 100 µl well<sup>-1</sup> of two- or four-step dilutions of the virus to be identified, and of the non-infected cell culture harvest (negative control). Also include SVCV positive control virus. Incubate for 1 hour at 37°C.
- viii) Rinse four times with PBST.
- ix) Add to the wells, 200 µl of horseradish peroxidase (HRPO)-conjugated MAb or polyclonal antibody to SVCV; or polyclonal IgG to SVCV. An MAb to N protein specific for a domain different from the one of the coating MAb and previously conjugated with biotin can also be used. Incubate for 1 hour at 37°C.
- x) Rinse four times with PBST.
- xi) If HRPO-conjugated antibody has been used, go to step xiii. Otherwise, add 200 µl of HRPO-conjugated streptavidin or ExtrAvidin (Sigma) to those wells that have received the biotin-conjugated antibody and incubate for 1 hour at 37°C.
- xii) Rinse four times with PBST.
- xiii) Add 200 µl of a suitable substrate and chromogen, such as tetramethylbenzidine dihydrochloride. Stop the course of the test when positive controls react, and read the results.

*Enzyme-linked immunosorbent assay (ELISA) using tissue homogenates*

See Section A.2.2.2 of Chapter 2.3.0, for obtaining organ homogenates.

- i) Coat the wells of microplates designed for ELISAs with appropriate dilutions of purified immunoglobulins (Ig) specific for SVCV, in 0.02 M carbonate buffer, pH 9.5 (200 µl well<sup>-1</sup>). Ig may be polyclonal or monoclonal Ig originating most often from rabbit or mouse, respectively. For the identification of SVCV, monoclonal antibodies (MAbs) specific for certain domains of the nucleocapsid (N) protein are suitable.
- ii) Incubate overnight at 4°C.
- iii) Rinse four times with PBST.
- iv) Block with skim milk (5% in carbonate buffer) or other blocking solution for 1 hour at 37°C (300 µl well<sup>-1</sup>).
- v) Rinse four times with PBST.
- vi) Store a 1/4 aliquot of each homogenate at 4°C, in case the test is negative and virus isolation in cell culture is required.
- vii) Treat the remaining part of the homogenate with 2% Triton X-100 or Nonidet P-40 and 2 mM of phenyl methyl sulphonide fluoride; mix gently.
- viii) Dispense 100 µl well<sup>-1</sup> of two- or four-step dilutions of the sample to be identified, and of negative control tissues. Also include an SVCV positive control virus. Incubate for 1 hour at 37°C.

Annex 10 (contd)

- ix) Rinse four times with PBST.
- x) Add to the wells, 200 µl of horseradish peroxidase (HRPO)-conjugated MAb or polyclonal antibody to SVCV; or polyclonal IgG to SVCV. A MAb to N protein specific for a domain different from the one of the coating MAb and previously conjugated with biotin can also be used. Incubate for 1 hour at 37°C.
- xi) Rinse four times with PBST.
- xii) If HRPO-conjugated antibody has been used, go to step xiv. Otherwise, add 200 µl of HRPO-conjugated streptavidin or ExtrAvidin (Sigma) to those wells that have received the biotin-conjugated antibody and incubate for 1 hour at 37°C.
- xiii) Rinse four times with PBST.
- xiv) Add 200 µl of a suitable substrate and chromogen, such as tetramethylbenzidine dihydrochloride. Stop the course of the test when positive controls react, and read the results.
- xv) If the test is negative, process the organ samples stored at 4°C, for virus isolation in cell culture as described in Section 4.3.

Virus identification Confirmation of virus identity by the indirect fluorescent antibody test (IFAT)

- i) Prepare monolayers of cells in 2 cm<sup>2</sup> wells of plastic cell culture plates, flasks or on cover-slips or glass slides in order to reach approximately 80% confluency within 24 hours of incubation at 25°C (seed six cell monolayers per virus isolate to be identified, plus two for positive and two for negative controls). The FCS content of the cell culture medium can be reduced to 2–4%. If numerous virus isolates have to be identified, the use of Terasaki plates is strongly recommended.
- ii) When the cell monolayers are ready for infection, i.e. on the same day or on the day after seeding, inoculate the virus suspensions to be identified by making tenfold dilution steps directly in the cell culture wells or flasks. For tests using cells cultured on glass cover-slips or slides, the dilutions are made in sterile containers and then used to inoculate the cells.
- iii) Dilute the control virus suspension of SVCV in a similar way, in order to obtain a virus titre of about 5000–10,000 PFU ml<sup>-1</sup> in the cell culture medium.
- iv) Incubate at 20°C for 24 hours.
- v) Remove the cell culture medium, rinse once with 0.01 M phosphate-buffered saline (PBS), pH 7.2, then three times briefly with cold acetone (stored at –20°C) for slides or cover-slips or 80% acetone in water or 30% acetone in ethanol, also at –20°C, for cells on plastic substrates. Let the fixative act for 15 minutes. A volume of 0.5 ml is adequate for 2 cm<sup>2</sup> of cell monolayer.
- vi) Allow the cell monolayers to air-dry for at least 30 minutes and process immediately or freeze at –20°C.
- vii) Rehydrate the dried cell monolayers, if they have been stored frozen, by four rinsing steps with PBS containing 0.05% Tween 20–PBST and remove this buffer completely after the last rinse. Block with 5% skim milk or 1% bovine serum albumin, in PBST for 30 minutes at 37°C.
- viii) Rinse four times with PBST, 5 minutes for each rinse. The slides or plastic culture plates can be gently agitated during the rinses.
- ix) Prepare a solution of purified antibody or serum to SVCV in PBST, at the appropriate dilution (which has been established previously or as given by the reagent supplier).

- x) Incubate the cell monolayers with the antibody solution for 1 hour at 37°C in a humid chamber and do not allow evaporation to occur.
- xi) Rinse four times with PBST.
- xii) Incubate the cell monolayers with a solution of fluorescein isothiocyanate (FITC)-conjugated antibody to the immunoglobulin used in the first layer and prepared according to the instructions of the supplier. These FITC antibodies are most often rabbit or goat antibodies.
- xiii) Rinse four times with PBST.
- xiv) View the treated cell monolayers on plastic substrates immediately, or mount the slides or cover-slips using glycerol saline at pH 8.5, or a commercially available mountant.
- xv) Examine under incident ultraviolet (UV) light using a microscope with ×10 eye pieces and ×20 or ×40 objective lenses having numerical apertures of >0.65 and >1.3, respectively. Positive and negative controls must be found to give the expected results prior to any other observation.

#### 4.10. Other serological methods

Not applicable

#### 5. Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations

The method for surveillance of susceptible fish populations for declaration of freedom from infection with SVCV is inoculation of cell culture with tissue homogenates ~~extracts~~ (as described in Section 4.3-4.5) ~~to demonstrate absence of the virus.~~

#### 6. Corroborative diagnostic criteria

This Section only addresses the diagnostic test results for detection of infection in the ~~presence~~ absence (Section 6.1.) or in the presence ~~absence~~ of clinical signs (Section 6.2.) but does not evaluate whether the infectious agent is the cause of the clinical event.

The case definitions for a suspect and confirmed case have been developed to support decision making related to trade and confirmation of disease status at the country, zone or compartment level. Case definitions for disease confirmation in endemically affected areas may be less stringent. It is recommended that all samples that yield suspect positive test results in an otherwise pathogen-free country or zone or compartment should be referred immediately to the OIE Reference Laboratory for confirmation, whether or not clinical signs are associated with the case. If a laboratory does not have the capacity to undertake the necessary diagnostic tests it should seek advice from the appropriate OIE Reference Laboratory.

##### 6.1. Apparently healthy animals or animals of unknown health status<sup>8</sup>

Apparently healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link(s) to an infected population. Geographical proximity to, or movement of animals or animal products or equipment, etc., from a known infected population equate to an epidemiological link. Alternatively, healthy populations are sampled in surveys to demonstrate disease freedom.

<sup>8</sup> For example transboundary commodities.

Annex 10 (contd)**6.1.1. Definition of suspect case in apparently healthy animals**

The presence of infection shall be suspected if: ~~a positive result has been obtained on at least one animal from~~ at least one of the following ~~diagnostic tests~~ criteria is met:

- i) Positive result by conventional RT-PCR ~~a recommended molecular or antigen or antibody detection test~~
- ii) Cytopathic effect in cell culture ~~(viruses)~~

**6.1.2. Definition of confirmed case in apparently healthy animals**

The presence of infection shall be confirmed if ~~positive results has been obtained on at least one animal from two test used in the following combination~~ the following criterion is met:

- i) Pathogen isolation ~~AND Conventional~~ in cell culture, virus identification by conventional RT-PCR test followed by ~~and~~ amplicon sequencing

Reference Laboratories should be contacted for specimen referral when testing laboratories cannot undertake any of the recommended test methods and testing is being undertaken that will result in notification to the OIE.

**6.2. Clinically affected animals**

Clinical signs are not pathognomonic for infection with SVCV ~~a single disease~~; however they may narrow the range of possible diagnoses. ~~[For many diseases, especially those affecting mollusc, 'clinical signs' are extremely limited and mortality may be the only or most dominant observation.]~~

**6.2.1. Definition of suspect case in clinically affected animals**

The presence of infection shall be suspected if at least one of the following criteria ~~is~~ are met:

- i) Gross pathology or clinical signs associated with the disease as described in this chapter, with or without elevated mortality
- ii) Positive result by conventional PCR ~~a recommended molecular or antigen or antibody detection test on at least one animal~~
- iii) Positives result by antigen ELISA or IFAT or immunohistochemistry
- iv) Cytopathic effect in cell culture.

**6.2.2. Definition of confirmed case in clinically affected animals**

The presence of infection shall be confirmed if ~~positive results has been obtained on at least one animal from two test used in the following combination~~ the following criterion is met:

- i) Pathogen isolation ~~AND Conventional~~ in cell culture, virus identification by conventional RT-PCR test followed by ~~and~~ amplicon sequencing

Reference Laboratories should be contacted for specimen referral when testing laboratories cannot undertake any of the recommended test methods and testing is being undertaken that will result in notification to the OIE.



### 6.3. Diagnostic sensitivity and specificity for diagnostic tests

Test type	Test purpose	Source population	Tissue/sample type	Species	DSe (n)	DSp (n)	Reference test	Citation
<u>Cell culture</u>	<u>Surveillance, diagnosis</u>	=	<u>Tissue homogenates</u>	=	<u>Not available</u>	<u>Not available</u>	=	=
<u>RT-PCR</u>	<u>Surveillance, diagnosis</u>	=	<u>Tissue homogenates</u>	=	<u>Not available</u>	<u>Not available</u>	=	=
<u>RT-PCR</u>	<u>Surveillance, diagnosis</u>	=	<u>Cell culture</u>	=	<u>Not available</u>	<u>Not available</u>	=	=
<u>RT-LAMP*</u>	Surveillance	Live-imported fish	Spleen, kidney and brain homogenate	Common carp, koi, goldfish	92.6 (27)	98.2 (445)	Virus isolation	Liu <i>et al.</i> , 2008

DSe: = diagnostic sensitivity, DSp = diagnostic specificity,  
 RT-LAMP: = real-time loop mediated isothermal amplification. \*Listed as suitable test

### 7. References

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\* \*

**NB:** There are OIE Reference Laboratories for Spring viraemia of carp (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/scientific-expertise/reference-laboratories/list-of-laboratories/>).

Please contact the OIE Reference Laboratories for any further information on Spring viraemia of carp

**NB:** First adopted in 1995 as spring viraemia of carp. Most recent updates adopted in 2012.



## INFECTION WITH *BATRACHOCHYTRIUM SALAMANDRIVORANS*

### EU comment:

**The EU thanks the OIE and supports the proposed changes to this Chapter.**

### 1. Scope

Infection with *Batrachochytrium salamandrivorans* (Bsal) means infection of amphibians with the pathogenic agent *Batrachochytrium salamandrivorans*, of the Division Chytridiomycota and Order Rhizophydiales ~~Genus *Batrachochytrium* and Family Incertae sedis.~~

### 2. Disease information

#### 2.1. Agent factors

##### 2.1.1. Aetiological agent

The type strain of the pathogenic chytrid fungal agent *Batrachochytrium salamandrivorans* (Bsal) ~~type strain~~ is AMFP13/1. Three more isolates have been described (Martel *et al.*, 2014) but no information is available on genetic structuring or phenotypic variation. Phylogenetic analyses show that Bsal forms a clade with its sister species *B. dendrobatidis* (Martel *et al.*, 2013). The genome size of the type strain was determined at 32.6 Mb with 10,138 protein-coding genes predicted (Farrer *et al.*, 2017). The contribution of these proteins to virulence is currently not clear.

##### 2.1.2. Survival and stability inside the host tissues ~~in processed or stored samples~~

Bsal is an intracellular pathogen that develops inside epidermal cells. The presence of Bsal could be demonstrated using real-time polymerase chain reaction (qPCR) on dorsal skin swabs up to 7 days on average post-mortem and using histopathology of dorsal skin tissue up to 3 days on average post-mortem (Thomas *et al.*, 2018). It is not clear how long Bsal can survive inside tissues of a dead host and how long a dead host remains infectious. ~~Storage of tissues or skin swabs in 70% ethanol or at 20°C allows detection of Bsal using qPCR for more than 150 years as demonstrated by analysis of museum specimens (Martel *et al.*, 2014).~~

##### 2.1.3. Survival and stability outside the host

Encysted spores have been shown to remain infectious in pond water up to at least 31 days (Stegen *et al.*, 2017) and are considered more ~~environmentally~~ resistant in the environment compared with zoospores. Experimentally inoculated forest soil was demonstrated to remain infectious to fire salamanders for 48 hours (Stegen *et al.*, 2017). However, Bsal DNA was detected up to 28 weeks in contaminated forest soil (Stegen *et al.*, 2017). However, whether this reflects the presence of viable Bsal organisms is not clear. The effect of desiccation on Bsal survival has not been studied.

For inactivation methods, see Section 2.4.5.

### 2.2. Host factors

#### 2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with Bsal according to Chapter 1.5. of the *Aquatic Animal Health Code (Aquatic Code)* include: [alpine newt (*Ichthyosaura alpestris*), blue-tailed fire-bellied newt (*Cynops cyanurus*), fire salamander (*Salamandra salamandra*), eastern newt (*Nothophthalmus viridescens*), French cave salamander (*Hydromantes strinatii*), Italian newt (*Lissotriton italicus*), yellow spotted newt (*Neurergus crocatus*), Japanese fire-bellied newt (*Cynops pyrrhogaster*), northern spectacle salamander (*Salamandrina perspicillata*), Tam Dao salamander (*Paramesotriton deloustali*), rough-skinned newt (*Taricha granulosa*), sardinian brook salamander (*Euproctus platycephalus*) and Spanish ribbed newt (*Pleurodeles waltfi*)] (under study).

UNOFFICIAL VERSION

## Annex 11 (contd)

## 2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence for susceptibility according to Chapter 1.5. of the Aquatic Code are: [under study]

2.2.3. Non-susceptible species

Species that have been found non-susceptible to infection with Bsal according to Chapter 1.5. of the Aquatic Code are: [under study]

## 2.2.4. Likelihood of infection by species, host life stage, population or sub-populations

Bsal is a pathogenic agent that mainly affects urodeles. Evidence from experimental infections and disease outbreaks in the wild and in captivity show that at least most, if not all, species of the family Salamandridae, as well as species of the family Hynobiidae are likely to become infected when exposed to Bsal. However, differences in susceptibility to infection between species do exist: for example, for fire salamanders (*Salamandra salamandra*), the infectious dose of Bsal was determined to be a theoretical one zoospore, whereas a significantly higher dose was necessary to infect Alpine newts (*Ichthyosaura alpestris*; Stegen *et al.*, 2017) and one western Palearctic species (*Lissotriton helveticus*) may be more resistant to infection (Martel *et al.*, 2014). For the largest family of salamanders (Plethodontidae), little information is currently available; at least one European species (*Speleomantes strinatii*) can be infected but other, North American species (*Gyrinophilus porphyriticus*, *Plethodon glutinosus*, Ambystomatidae) seem less susceptible to infection (Martel *et al.*, 2014). Susceptibility of the family of Cryptobranchidae is not clear, with a single infection found in a farmed Chinese giant salamander (*Andrias davidianus*, Zhiyong *et al.*, 2018). No information is available on the urodele families *Proteidae*, *Rhyacotritonidae* and *Amphiumidae*. Bsal infection in anurans has only been detected in two species, in captivity, the wild and in lab trials (Nguyen *et al.*, 2017; Stegen *et al.*, 2017).

Thus far, infections with Bsal have been demonstrated only in amphibians post-metamorphosis. In one experimental infection trial, larvae of fire salamanders were exposed to Bsal, but did not become ~~were not~~ infected (Van Rooij *et al.*, 2015). The extent to which factors such as like-age and sex affect susceptibility to infection post-metamorphosis is unknown.

In Europe, Bsal has been detected in captive collections of urodeles (Fitzpatrick *et al.*, 2018, Sabino-Pinto *et al.*, 2015) and the pet trade in salamanders and newts has been hypothesised to play a central role in the distribution of this fungus (Fitzpatrick *et al.*, 2018; Yap *et al.*, 2015; Zhiyong *et al.*, 2018). Hence, urodeles that directly (by co-housing, contact of wild animals with released or captive animals) or indirectly (via materials, contaminated water or soil) come in contact with traded urodeles, may have a high likelihood of exposure to ~~be more likely to contract~~ Bsal infection.

## 2.2.5. Distribution of the pathogen in the host

Bsal only infects the skin, where it remains limited to the epidermis.

2.2.6. Aquatic animal reservoirs of ~~Persistent~~ infection

A large number of salamanders, mainly belonging to the families Salamandridae and Hynobiidae, may survive episodes of infection (for example Alpine newts) or be considered tolerant, resulting in persistent subclinical infections. Although persistent infection has not been demonstrated for all species, in the native Bsal range in east Asia, Bsal infection and disease dynamics appear to be consistent for all species examined and appear capable of long-term persistent infections (Laking *et al.*, 2017; Martel *et al.*, 2014; Zhiyong *et al.*, 2018).

In its invasive range, persistent infections (e.g. in Alpine newts) have been implicated in the extirpation of a highly susceptible species (fire salamanders). It is currently not clear which of the species, mentioned in 2.2.1 may sustain persistent infections in the invasive Bsal range. At least some species (the best-known example is the fire salamander) are highly susceptible and invariably die shortly ~~briefly~~ after exposure (Martel *et al.*, 2014; Stegen *et al.*, 2017), making ~~which would make~~ them unlikely to sustain persistent infections.

It is not known whether other, biotic reservoirs of Bsal exist.

### 2.2.7. Vectors

There is evidence that birds may carry zoospores attached to their ~~the feet of birds~~ (Stegen *et al.*, 2017), ~~which may~~ and thus may act as vectors for Bsal.

## 2.3. Disease pattern

### 2.3.1. Mortality, morbidity and prevalence

In its native range in east Asia, Bsal has been demonstrated to be present in the wild at a prevalence of between 2 and 4% on average (data from China [People's Rep. of], Japan, Thailand, and Vietnam; Laking *et al.*, 2017; Martel *et al.*, 2014; Zhiyong *et al.*, 2018), but in the absence of any observed morbidity or mortality under natural conditions. In some populations (*Paramesotriton hongkongensis*), prevalence may reach 50% (Zhiyong *et al.*, 2018). In its invasive range in Europe, Bsal was present in a population of fire salamanders at a prevalence of between 25 and 63% (Stegen *et al.*, 2017). In captive collections of urodeles in Europe, Bsal occurrence and associated mortality were detected in Germany (1), the United Kingdom (4), Belgium (1), the Netherlands (2) and Spain (1) (number in brackets indicates number of collections). When left untreated, morbidity and mortality can reach 100%, at least in members of the genus *Salamandra*.

Morbidity, mortality and minimum infectious dose vary considerably between species (Martel *et al.*, 2014; Stegen *et al.*, 2017). Based on natural outbreaks in captivity and in the wild and ~~in an~~ infection trials, the case morbidity and case mortality rate in fire salamanders can reach 100%, independent of the initial level of Bsal exposure. This has resulted in the loss of over 99.9% of the fire salamander population at the Bsal index outbreak site in the Netherlands (Spitzen-van der Sluijs *et al.*, 2016). All tested western Palearctic urodeles, except for *Lissotriton helveticus* and *Salamandrella keyserlingii*, showed 100% morbidity and mortality when exposed to a single, high dose of Bsal (Martel *et al.*, 2014). However, at least for Alpine newts, the case morbidity and case fatality rates depend on the Bsal dose that the animal is exposed to: a high dose resulting in the highest mortality, while a low dose does not necessarily result in morbidity or mortality.

~~It is important to mention that~~ Morbidity and mortality also depend on environmental temperature. For the Bsal type strain, temperatures above 20°C reduces the level of ~~tempore~~ infection and temperatures above 25°C eventually result in killing of Bsal and elimination of infection (Bloo *et al.*, 2015b-2015a). Exposure of infected animals to conditions that inhibit Bsal growth may thus result in non-clinical or sub-clinical infections in susceptible species.

### 2.3.2. Clinical signs, including behavioural changes

Chytridiomycosis caused by Bsal may be accompanied by a combination of the following signs: epidermal ulcerations (ranging from discrete ~~tiny~~ to extensive), excessive skin shedding, skin haemorrhages and/or fluid loss, anorexia, apathy, abnormal body postures and convulsions ~~and death~~ (Martel *et al.*, 2013).

### 2.3.3 Gross pathology

Skin anomalies (haemorrhages, ulcerations, presence of sloughed skin) are the main pathological findings (Martel *et al.*, 2013).

### 2.3.4. Modes of transmission and life cycle

Colonial or monocentric thalli of this fungus develop inside host epidermal cells and produce motile zoospores or walled, encysted spores, both of which are infectious stages. Zoospores are released through one or several discharge tubes. While motile spores actively swim towards a suitable substrate (e.g. a host), the encysted spores float at the water-air interface and passively adhere to a passing host (Stegen *et al.*, 2017). *In vitro*, developing thalli form fine rhizoids. Mature thalli *in vitro* are between 16 and 50 µm in diameter, *in vivo* between 7 and 17 µm; zoospores are approximately 5 µm in diameter. Motile zoospores are roughly spherical, the nucleus is located outside of the ribosomal mass, with aggregated ribosomes, multiple mitochondria and numerous lipid globules. The position of the non-flagellated centriole in free swimming zoospores varies from angled to parallel to the kinetosome (Martel *et al.*, 2013).



## Annex 11 (contd)

There are no indications of vertical transmission. However, this cannot be excluded in species giving birth to metamorphosed offspring (e.g. *Salamandra atra*, *Salamandra lanzai*, *Lyciasalamandra helverseni*). Horizontal transmission occurs through direct contact or contact with contaminated soil or water (Stegen *et al.*, 2017). Infectious stages include the motile zoospore and the environmentally resistant encysted spores (Stegen *et al.*, 2017). Infections can be reproduced under experimental conditions by topically applying a Bsal inoculum on the dorsum of amphibians and housing the exposed animals at 15°C (Martel *et al.*, 2013; 2014; Stegen *et al.*, 2017). This inoculum can either contain motile zoospores or the immobile, encysted spores.

Pathways of Bsal dispersal within Europe are poorly understood but may be anthropogenic (e.g. through contaminated material). Zoospores attach to bird feet, suggesting birds may spread Bsal over larger distances (Stegen *et al.*, 2017). Direct animal-to-animal contact is necessary for transmission of Bsal: salamanders only separated by 1 cm from infected conspecifics were not infected in laboratory trials, in contrast to co-housed animals (Spitzen-van der Sluijs *et al.*, 2018). Overall, dispersal ability of Bsal in Europe currently seems limited: Bsal was found not to be transmitted to a neighbouring site in the Netherlands, despite being downstream of a small stream, and the current distribution of Bsal in Europe is probably not continuous (Spitzen-van der Sluijs *et al.*, 2018).

Although Bsal dispersal between populations is now hypothesised to be mainly human mediated, other factors (e.g. wildlife, water) may play key roles and critical knowledge about Bsal dispersal is currently lacking.

### 2.3.5. Environmental and management factors

The Bsal type strain AMFP13/1 tolerates temperatures up to 25°C but is killed at higher temperatures (Bloo *et al.*, 2015b–2015a). As Bsal infections have been demonstrated in aquatic newts at water temperatures above 25°C (Laking *et al.*, 2017; Zhiyong *et al.*, 2018), it is likely, however, that thermal tolerance may be Bsal lineage dependent. A temperature of 4°C results in slower progression build-up of infection but does not reduce morbidity or mortality (Stegen *et al.*, 2017). Desiccation is likely to be poorly tolerated by Bsal, although data are currently lacking, and the encysted spore may be resistant to drying (Stegen *et al.*, 2017; Van Rooij *et al.*, 2015). It is not known to what extent Bsal tolerates freezing.

Co-occurrence of highly susceptible species such as fire salamanders with less susceptible species, such as Alpine newts may facilitate density independent disease dynamics that lead to the extirpation of the highly susceptible species (Stegen *et al.*, 2017).

Barriers to pathogen dispersal, for example those preventing migration of infected hosts such as amphibian fences or roads, or those preventing transmission by potential Bsal vectors including humans, fomites and wildlife, may prevent transmission at small spatial scales (Spitzen-van der Sluijs *et al.*, 2018).

### 2.3.6. Geographical distribution

Asia is currently considered the region of origin of Bsal (Martel *et al.*, 2014), where the infection appears to be endemic in amphibian communities across a wide taxonomic, geographical and environmental range, albeit at a low prevalence between 2 and 4% (Zhiyong *et al.*, 2018). In Asia, Bsal was shown to be widely present in urodele populations in China (People's Rep. of), Japan, Thailand and Vietnam. East Asia is presumed to be the native range of the fungus (Laking *et al.*, 2017; Martel *et al.*, 2014; Zhiyong *et al.*, 2018).

Europe is considered the invasive range of the fungus where Bsal was first identified during a mortality event in fire salamanders (*Salamandra salamandra*) in Bunderbos, the Netherlands (Martel *et al.*, 2013). In Europe, Bsal was detected by surveys of wild susceptible species in Belgium, Germany and the Netherlands (Martel *et al.*, 2014; Spitzen-van der Sluijs *et al.*, 2016), and in captive urodele populations in Belgium, Germany, the Netherlands, Spain, and the United Kingdom (Fitzpatrick *et al.*, 2018; Sabino-Pinto *et al.*, 2015).

Bsal has not been reported in Africa or the Americas.

## 2.4. Biosecurity and disease control strategies

### 2.4.1. Vaccination

Not available.

#### 2.4.2. Chemotherapy including blocking agents

A combined treatment using Polymyxin E, voriconazole and a temperature regime of 20°C has been shown to be effective in eradicating Bsal from infected hosts (Bloo *et al.*, [2015c-2015b](#)). If the treatment is not performed properly and does not achieve eradication, low level carriers are created and the likelihood of Bsal detection, is reduced.

#### 2.4.3. Immunostimulation

Not available.

#### 2.4.4. Breeding resistant strains

~~Breeding resistant strains is one of the few options for long term sustainable disease mitigation.~~

No information available.

#### 2.4.5. Inactivation methods

Bsal is sensitive to a wide variety of disinfectants (Van Rooij *et al.*, 2015). Inactivation using formalin has been shown to hamper DNA detection using real-time PCR-qPCR. Bsal is killed within 30 seconds in 70% ethanol (Van Rooij *et al.*, 2017). Inactivation in 70% ethanol allows for subsequent molecular tests yet is less suitable for histopathology. The Bsal type strain AMFP 13/1 is killed at temperatures exceeding 25°C; consequently, inactivation of this fungus can be achieved through heat treatment by autoclaving ([Martel \*et al.\*, 2013](#)).

#### 2.4.6. Disinfection of eggs and larvae

No information available.

#### 2.4.7. General husbandry

In captivity, pathogen detection is difficult due to low prevalence in sub-clinically infected animals that often carry Bsal at low intensities (Martel *et al.*, 2014; Zhiyong *et al.*, 2018). These subclinically infected animals often belong to (but are not restricted to) taxa of Asian urodeles. Highly susceptible species (such as fire salamanders *Salamandra salamandra*) may serve a sentinel function. Temperature regimes in captivity may strongly interfere with pathogen detection. Temperatures higher than 20°C (and below 25°C) severely impair pathogen proliferation in the host skin (Bloo *et al.*, [2015b-2015a](#)) and may result in infections that cannot be detected.

Heat treatment can be used to clear infection with Bsal in thermo-tolerant salamander species (Bloo *et al.*, 2015a).

### 3. Specimen selection, sample collection, transportation and handling

This Section draws on information from Sections 2.2., 2.3. and 2.4. to identify populations, individuals and samples which are most likely to be infected.

#### 3.1. Selection of populations and individual specimens

In case of disease or mortality in urodeles in captivity, sampling should be focused primarily on diseased or moribund animals (i.e. those showing skin lesions and abnormal behaviour). In a population with ongoing disease and mortality, live but diseased animals are preferentially sampled. The second choice is dead animals. Only freshly dead animals should be sampled as detectability of Bsal deteriorates post-mortem (Thomas *et al.*, 2018). However, in the absence of diseased or freshly dead animals, apparently healthy animals can be sampled.

Similarly, in wild populations, samples should be taken preferentially from diseased or moribund or freshly dead animals ~~should preferentially be sampled, but;~~ however, as these may quickly be removed (i.e. through predation, scavenging) only healthy animals may ~~only~~ be available. Populations which have declined or where dead animals have been observed should be targeted.

## Annex 11 (contd)

### 3.2. Selection of organs or tissues

The only relevant tissue is skin tissue and probably only from amphibians post metamorphosis. Both invasive (skin biopsies) and non-invasive (cotton tipped swabs) ~~samples sampling~~ are appropriate, given the apical shedding of Bsal spores. In dead animals, dorsal skin is the preferred tissue, given its slower post mortem decay (Thomas *et al.*, 2018).

### 3.3. Samples or tissues not suitable for pathogen detection

Any other tissue than skin is not suitable for the detection of Bsal in amphibians.

### 3.4. Non-lethal sampling

Non-lethal sampling is possible, either by collecting skin biopsies (toeclips or tailclips) or by non-invasively collecting samples using cotton tipped swabs. The latter is preferred given its minimal impact on animal ~~welfare well-being~~. As Bsal is limited to the superficial skin layers of the amphibian host, non-lethal sampling results are equivalent to lethal sampling results. ~~In the absence of other, Bsal specific diagnostic tests (other than the laborious isolation of the fungus),~~ Large numbers of animals can be sampled using skin swabs with minimal effects on animal welfare. Cotton tipped swabs should be rubbed firmly over the abdomen (10 times), the underside of a foot (10 times) and the ventral tail (10 times) using the tip of the swab. The use of disposable gloves for manipulating amphibians is highly recommended.

### 3.5. Preservation of samples for submission

#### 3.5.1. Samples for pathogen isolation

Bsal isolation is a ~~very~~ laborious procedure, requiring up to two months ~~to obtain for obtaining~~ a pure culture from a clinical sample. Isolation from animals that died due to Bsal infection is hampered by bacterial overgrowth. The best sample for Bsal isolation is a diseased, living animal, which is euthanised just prior to an isolation attempt. Before sampling diseased animals should be kept at temperatures between 5 and 15°C to avoid clearance of infection (Bloo *et al.*, ~~2015b~~ 2015a).

#### 3.5.2. Preservation of Fixed ~~samples~~ for molecular detection

Tissue samples for PCR testing should be preserved in 70–90% (v/v) analytical/reagent-grade (undenatured) ethanol. The recommended ratio of ethanol to tissue is 10:1. The use of lower grade (laboratory or industrial grade) ethanol is not recommended. If material cannot be fixed it may be frozen.

Skin swabs should be stored dry and preferably frozen.

#### 3.5.3. Fixed samples for histopathology, immunohistochemistry or *in-situ* hybridisation

Skin samples for histopathology should be fixed immediately after collection. The recommended ratio of formalin (10%) to tissue is 10:1.

#### 3.5.4. Fixed samples for electron microscopy

For transmission electron microscopy, skin samples can be fixed in glutaraldehyde in 0.05 M sodium cacodylate buffer and 1% osmium tetroxide post-fixation (Martel *et al.*, 2013).

#### 3.5.5. Samples for other tests

Not applicable.

### 3.6. Pooling of samples

Pooling of up to ~~four~~ five skin swab samples appears to allow reliable detection of Bsal in clinically affected animals (Sabino-Pinto *et al.*, 2018) but estimates ~~of~~ on the impact on diagnostic performance of the test characteristics have not been determined. Given low infection intensities in subclinically infected animals, sampling of individual animals is recommended.

#### 4. Diagnostic methods

The methods currently available for identifying infection that can be used in i) surveillance of apparently healthy populations), ii) presumptive and iii) confirmatory diagnostic purposes are listed in Table 4.1. by life stage. The designations used in the Table indicate:

Key:

+++ = Recommended method(s) validated for the purpose shown and usually to stage 3 of the OIE Validation Pathway;

++ = Suitable method(s) but may need further validation;

+ = May be used in some situations, but cost, reliability, lack of validation or other factors severely limits its application;

Shaded boxes = Not appropriate for this purpose.

The selection of a test for a given purpose depends on the analytical and diagnostic sensitivities and specificities repeatability and reproducibility. OIE Reference Laboratories welcome feedback on diagnostic performance for assays, in particular PCR methods, for factors affecting assay analytical sensitivity or analytical specificity, such as tissue components inhibiting amplification, presence of nonspecific or uncertain bands, etc., and any assays that are in the +++ category.

## Annex 11 (contd)

**Table 4.1. OIE recommended diagnostic methods and their level of validation for surveillance of healthy animals and investigation of clinically affected animals**

Method [amend or delete as relevant]	A. Surveillance of apparently healthy animals				B. Presumptive diagnosis of clinically affected animals				C. Confirmatory diagnosis <sup>1</sup> of a suspect result from surveillance or presumptive diagnosis			
	Early life stages <sup>2</sup>	Juveniles <sup>2</sup>	Adults	LV	Early life stages <sup>2</sup>	Juveniles <sup>2</sup>	Adults	LV	Early life stages <sup>2</sup>	Juveniles <sup>2</sup>	Adults	LV
Wet mounts	+	+	+	1	+	+	+	1				1
Histopathology <sup>3</sup>	+	+	+	1	++	++	++	1				1
Cell or artificial media culture									+	+	+	
Real-time PCR	+++	+++	+++	2	+++	+++	+++	2	+++	+++	+++	2
Conventional PCR												
Amplicon sequencing <sup>4</sup>												
<i>In-situ</i> hybridisation												
LAMP												
Lateral flow assay	+	+	+	1	++	++	++	1				1
Immunohistochemistry												

LV = level of validation, refers to the stage of validation in the OIE Pathway (Chapter 1.1.2.); PCR = polymerase chain reaction.

<sup>1</sup>For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). <sup>2</sup>Early and juvenile life stages have been defined in Section 2.2.4.

<sup>3</sup>Cytopathology and histopathology can be validated if the results from different operators has been statistically compared. <sup>4</sup>Sequencing of the PCR product.

Shading indicates the test is inappropriate or should not be used for this purpose.

#### 4.1. Wet mounts

Wet mounts of skin scraping or pieces of shed skin can be examined at magnification 10x using light microscopy. The presence of motile spores of approximately 5 µm are indicative of amphibian chytrid infection.

#### 4.2. Histo- and cytopathology

~~No reports are available on the use of cytology.~~ Histopathology of skin in amphibian post-metamorphosis may provide strong indications of Bsal infection. In ~~a haematoxylin/eosin staining of skin~~ stained sections, histopathological evidence suggestive of Bsal infections of skin, ~~is~~ multifocal epidermal necrosis with loss of distinction between layers of keratinocytes associated with myriad intracellular and extracellular chytrid-type fungal thalli provides histopathological evidence of Bsal infection (Martel *et al.*, 2013; White *et al.*, 2016). Using immunohistochemistry, Bsal thalli can be stained, which aids in detecting low level infections (Thomas *et al.*, 2018). Histopathology is highly indicative, yet does not allow specific-definitive identification of Bsal, which needs further confirmation. In randomly collected skin samples from experimentally infected salamanders, histopathology was capable of detecting Bsal in only a minority of the samples (Thomas *et al.*, 2018). In dead animals, post-mortem decay of the epidermis may mask the lesions (Thomas *et al.*, 2018). Lesions can be so extensive, that the epidermis is entirely eroded and no fungal thalli can be observed. Mild infections can be missed due to the multifocal and small lesions (Thomas *et al.*, 2018). ~~For asymptotically~~ In subclinically infected animals, sensitivity should be rated low. ~~Sensitivity. In clinically affected animals, sensitivity~~ and specificity of histopathology and immunohistochemistry have not been quantified.

No reports are available on the use of cytopathology.

#### 4.3. Cell or artificial media culture for isolation

Bsal can be isolated and cultured on artificial media, yet this is a laborious and difficult procedure, typically requiring between 4 weeks and 2 months. There is a significant probability of bacterial overgrowth, which hampers fungal isolation, resulting in poor sensitivity. The protocol of Fisher *et al.* (2018) can be used. Small (approximately 1 mm<sup>2</sup>) pieces of skin from an infected, diseased animal should first be thoroughly cleaned by wiping through agar plates. The cleaned pieces of skin can then each be transferred to a well of a 96-well plate, containing tryptone-gelatin hydrolysate lactose broth (TGH) containing penicillin/streptomycin (200 mg/litre) and incubated at 15°C. Wells showing chytrid growth without bacterial contamination can be used for subculturing (Martel *et al.*, 2013). Chytrid growth can be visualised by examining the wells under an inverted microscope (10–40 x magnification).

Given the difficulties to isolate Bsal from infected animals and the high uncertainty to obtain a viable culture, this method is not appropriate as first diagnostic approach, but (in rare cases) to confirm infection and for obtaining isolates for research (for example for epidemiological tracing).

#### 4.4. Nucleic acid amplification

##### 4.4.1. Real-time PCR

The following information is derived from Blooi *et al.* (2013), Thomas *et al.* (2018) and Sabino Pinto *et al.* (2018). DNA from skin swabs can be extracted in 100 µl Prepman Ultra Reagent (Applied Biosystems, Foster City, CA) or by using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). The latter follows the animal tissues protocol (Qiagen DNeasy Blood and Tissue kit) with pre-treatment for Gram-positive bacteria and expanded initial incubation for 1 hour. DNA from skin tissue can be extracted using proteinase K digestion or DNA Easy Tissue Kit. Extracted DNA is diluted tenfold to minimise possible PCR inhibition. Controls should be run with each assay: at least a negative extraction control and a positive control; preferably, an internal PCR control is included. Positive control consists of DNA extracts of a tenfold dilution series of Bsal zoospores from 1 to 100,000 to allow quantification.

## Annex 11 (contd)

A TaqMan PCR has been partially validated to level 2 without however, stating its intended purpose (Thomas *et al.*, 2018). SYBR green real-time PCR, may be used as well but needs further validation to determine specificity and sensitivity (Martel *et al.*, 2013). The TaqMan PCR can either be used as simplex PCR or in combination with primers to detect *B. dendrobatidis* in a duplex PCR (Bloom *et al.*, 2013) and uses the forward primer STerF (5'-TGC-TCC-ATC-TCC-CCC-TCT-TCA-3'), reverse primer STerR (5'-TGA-ACG-CAC-ATT-GCA-CTC-TAC-3') and Cy5 labelled probe STerC (5'-ACA-AGA-AAA-TAC-TAT-TGA-TTC-TCA-AAC-AGG-CA-3') to detect the presence of the 5.8S rRNA gene of Bsal. Intra- and interassay efficiency were 94 and 99%, respectively (Bloom *et al.*, 2013). This TaqMan duplex PCR does not decrease detectability of both Bd and Bsal, except in case of mixed infections (Thomas *et al.*, 2018). The use of simplex Bsal-specific PCR is therefore recommended in case Bd has been detected in the sample. The sensitivity of this real-time qPCR is between 96 and 100% and diagnostic specificity 100% (95% CI: 73–100%; Thomas *et al.*, 2018) when used in clinically affected animals. Although DNA quantities as low as 0.1 genomic equivalent can be detected (Bloom *et al.*, 2013), Thomas *et al.* (2018) recommend a threshold of 1 genomic equivalent per reaction to reduce the likelihood of false positive results. Borderline results ( $\leq 1$  GE per reaction) should be classified as suspect and need confirmation by sequencing (or isolation).

Samples are preferably run in duplicate. A sample is considered positive based on the combination of (1) the shape of the amplification curves (2) positive results in both duplications, (3) returning GE values above the detection threshold (1 GE per reaction) (4) low variability between duplicates ( $< 0.3$  Ct value).

### 4.4.2. Conventional PCR (PCR)

~~The use of real-time PCR is recommended.~~ No conventional PCR protocol has been validated.

### 4.4.3. Other nucleic acid amplification methods

None validated.

## 4.5. Amplicon sequencing

~~For confirmation of suspect samples, amplified products can be sequenced with the primers as described in 4.4.1.~~

No conventional PCR protocol has been validated.

## 4.6. *In-situ* hybridisation (and histoimmunochemistry)

No *In-situ* hybridisation: ~~no~~ validated protocols are available.

## 4.7. Immunohistochemistry

Immunohistochemistry is currently not Bsal specific, due to the lack of Bsal specific antibodies (Dillon *et al.*, 2017; Thomas *et al.*, 2018). ~~Sensitivity of immunohistochemistry in diseased or dead animals can be estimated to be high if clinically affected skin regions have been selected.~~

## 4.8. Bioassay

Not available.

#### 4.9. Antibody-based or antigen detection methods (ELISA, etc.)

A lateral flow assay (LFA) using an IgM monoclonal antibody (MAb) was developed to detect infection in amphibian skin samples. This MAb does not discriminate between *B. salamandrivorans*, *B. dendrobatidis* and *Homolaphlyctis polyrhiza* (Dillon *et al.*, 2017-2016). The sensitivity of this test is likely to be lower than that of the real-time qPCR (Dillon *et al.*, 2017): in experimentally Bd inoculated frogs, 1/5 animals tested positive in LFA compared to 4/5 using real-time qPCR. This would make this technique most useful in animals with high infection loads. Such techniques may be useful for point-of-care testing if specificity is increased and provided thorough validation.

#### 4.10. Other serological methods

Not available.

#### 5. Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations

The use of real-time PCR on skin swabs is recommended for surveillance.

#### 6. Corroborative diagnostic criteria

This Section only addresses the diagnostic test results for detection of infection in the ~~presence~~ absence (Section 6.1.) or in the presence ~~absence~~ of clinical signs (Section 6.2.) but does not evaluate whether the infectious agent is the cause of the clinical event.

The case definitions for a suspect and confirmed case have been developed to support decision making related to trade and confirmation of disease status at the country, zone or compartment level. Case definitions for disease confirmation in endemically affected areas may be less stringent.

#### 6.1. Apparently healthy animals or animals of unknown health status<sup>9</sup>

Apparently healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link(s) to an infected population. Geographic proximity to, or movement of animals or animal products or equipment, etc., from a known infected population equate to an epidemiological link. Alternatively, healthy populations are sampled in surveys to demonstrate disease freedom.

Such surveys typically consist of non-invasive sampling using skin swabs that are examined for the presence of Bsal using real-time PCR. When applied to animals in the wild, confirmation by using a complementary technique, other than sequencing the PCR product, is often not feasible.

##### 6.1.1. Definition of suspect case in apparently healthy animals

The presence of infection shall be suspected if ~~a positive result has been obtained on at least one animal from at least one of the following diagnostic tests~~ criteria is met:

- i) Positive result by real-time PCR.
- ii) Histopathological changes ~~(including immunohistochemistry)~~ consistent with the presence of the pathogen or the disease.

<sup>9</sup> For example transboundary commodities.



Annex 11 (contd)

- iii) The presence of motile spores, compatible with chytrid zoospores, in wet mount of urodele skin.
- iv) Positive result from lateral flow assay (LFA).

**6.1.2. Definition of confirmed case in apparently healthy animals**

The presence of infection is confirmed if ~~positive results have been obtained on at least, in addition to the criteria in Section 6.1.1., one animal from two tests used in~~ of the following combination criteria is met:

- i) Positive result by real-time PCR on skin swab or skin tissue, ~~and by histopathology or immunohistochemistry on skin tissue.~~
- ii) ~~Positive result by real-time PCR on skin swab or skin tissue, and~~ Pathogenic agent isolation from the skin in culture and confirmation-identification by real-time PCR.

**6.2. Clinically affected animals**

Clinical signs are not pathognomonic for a single disease; however, they may narrow the range of possible diagnoses.

**6.2.1. Definition of suspect case in clinically affected animals**

The presence of infection shall be suspected if at least one of the following criteria are met:

- i) Clinical signs (haemorrhages, ulcerations, presence of sloughed skin, see Section 2.3.2.), notably the presence of skin ulcers and/or disecdysis.
- ii) Positive result by real-time PCR on at least one swab or skin tissue.
- iii) Histopathological changes consistent with the presence of the pathogenic agent or the disease.
- iv) Visual observation (by microscopy) of motile spores, compatible with amphibian chytrid zoospores, in a wet mount of the skin of at least one diseased urodele.
- v) Positive result of antigen detection ~~technique such as~~ by LFA.
- vi) ~~Positive result from immunohistochemistry.~~

**6.2.2. Definition of confirmed case in clinically affected animals**

The presence of infection is confirmed if positive results have been obtained on at least one animal from ~~two tests used in~~ one of the following combination diagnostic tests:

- i) Positive result by real-time PCR on skin swab or skin tissue ~~and by histopathology.~~
- ii) ~~Positive result by real-time PCR on skin swab or skin tissue, and~~ Pathogenic agent isolation from the skin in culture and identification by real-time PCR ~~and confirmation by real-time PCR.~~

~~Reference Laboratories should be contacted for specimen referral when testing laboratories cannot undertake any of the recommended test methods and testing is being undertaken that will result in notification to the OIE.~~

## 6.3. Diagnostic sensitivity and specificity for diagnostic tests

<u>Test type</u>	<u>Test purpose</u>	<u>Source populations</u>	<u>Tissue or sample types</u>	<u>Species</u>	<u>DSe (n)</u>	<u>DSp (n)</u>	<u>Reference test</u>	<u>Citation</u>
<u>Real-time PCR</u>	<u>Diagnosis</u>	<u>Experimentally infected salamanders (clinical and subclinical infection)</u>	<u>Skin swabs</u>	<u><i>Salamandra salamandra</i></u>	<u>96–100 (26)</u>	<u>100 (12)</u>	<u>Droplet digital PCR</u>	<u>Thomas <i>et al.</i> (2018)</u>

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\*  
\* \*

NB: There are currently no OIE Reference Laboratories for infection with *Batrachochytrium salamandrivorans*

**NB:** First adopted in 20XX.

UNOFFICIAL VERSION

## CHAPTER 2.3.4.

**INFECTION WITH INFECTIOUS  
HAEMATOPOIETIC NECROSIS VIRUS**

**EU Comment:**

**The EU thanks the OIE and supports the proposed changes to this Chapter.**

**Comments are included in the body of the text.**

**We consider that because of the similarities of this annex and annex 13 we suggest a revision of both annexes together to standardise the stylistic differences between the two chapters.**

**1. Scope**

Infection with infectious haematopoietic necrosis virus means infection with the pathogenic agent Salmonid novirhabdovirus (commonly known as infectious haematopoietic necrosis virus [IHNV]) of the Genus *Novirhabdovirus* and Family *Rhabdoviridae*.

**2. Disease information****2.1. Agent factors****2.1.1. Aetiological agent**

IHNV consists of a bullet-shaped particle of approximately 150–190 nm in length and 65–75 nm in diameter that encapsulates a non-segmented, negative-sense, single-stranded RNA genome of approximately 11,000 nucleotides. The viral genome codes for six proteins in the following order: a nucleoprotein (N), a phosphoprotein (P), a matrix protein (M), a glycoprotein (G), a non-virion protein (NV), and a polymerase (L). Due to the primary position of the nucleoprotein gene on the IHNV genome, nucleoprotein transcripts and protein are the first and most abundant during viral infection and is typically the preferred target of diagnostic tests. The glycoprotein forms spike-like projections on the surface of the mature virion and is the primary antigenic component of the virus such that anti glycoprotein serum is sufficient to neutralise infections.

The type strain of IHNV is the Western Regional Aquaculture Center (WRAC) strain available from the American Type Culture Collection (ATCC VR-1392). The GenBank accession number of the genomic sequence of the WRAC strain is L40883 (Morzunov *et al.*, 1995; Winton & Einer-Jensen, 2002).

**EU Comment:**

**We suggest that geographical range for rainbow trout (*Oncorhynchus mykiss*) which currently includes Europe, Asia and Idaho, USA, be extended to include Africa. A supporting scientific paper is providing details of an outbreak of IHNV of genotype J in farmed rainbow trout was published in 2018 by Mulei *et al.***

Phylogenetic analyses based on G-gene nucleotide sequences have classified IHNV isolates into five major genogroups denoted U, M, L, E, and J that correspond to geographical location rather than host

species (Cieslak *et al.*, 2017; Enzmann *et al.*, 2005; 2010; Johansson *et al.*, 2009; Kim *et al.*, 1999; Kolodziejek *et al.*, 2008; Kurath *et al.*, 2003; Nishizawa *et al.*, 2006). Nevertheless, IHNV displays a strong phylogeographic signature reflecting the host species from which the virus is most commonly isolated in various geographical areas (e.g. sockeye salmon [*Oncorhynchus nerka*] in the Northeast Pacific – U genogroup; Chinook salmon [*Oncorhynchus tshawytscha*] in California, USA – L genogroup; and rainbow trout [*Oncorhynchus mykiss*] in Europe, Asia and Idaho, USA – E, J and M genogroups, respectively). Additionally, experimental infections demonstrating that U and M genogroup viruses had higher virulence in sockeye salmon and rainbow trout, respectively (Garver *et al.*, 2006), supports the observation that IHNV strains isolated from its historical phylogeographic host tends to be more virulent for the same species in comparison to other species.

#### 2.1.2. Survival and stability in processed or stored samples

IHNV stability in host tissues during storage and processing is largely influenced by temperature. The virus is more stable at lower temperature and remained infectious for at least 3 days at 4°C in naturally infected or IHNV-seeded tissue (Burke & Mulcahy, 1983; Gosting & Gould, 1981; Hostnik *et al.*, 2002; Pietsch *et al.*, 1977). For long-term survival of infectious virus, tissues should be stored at temperatures below –20°C (Burke & Mulcahy, 1983; McClure *et al.*, 2008). The preferred method for retaining infectious virus is to maintain the IHNV sample on ice with rapid processing and inoculation of cell cultures as soon as possible due to the progressive reduction in titre with increasing temperature (Barlic-Maganja *et al.*, 2002; Gosting & Gould, 1981).

## Annex 12 (contd)

## 2.1.3. Survival and stability outside the host

IHNV can survive outside the host tissue in freshwater and seawater, but is impacted by temperature, UV exposure, microbial community and suspended sediments. At 4°C–15°C, 10<sup>5</sup> pfu/ml of IHNV remained detectable via cell culture after 1 week in either fresh or salt water (Kell *et al.*, 2014). However, when exposed to sunlight (UV-A and UV-B), IHNV at the water surface is rapidly inactivated with six orders of magnitude of virus rendered non-infectious within 3 hours (Garver *et al.*, 2013). In addition, infectious virus is inactivated by the microbial community within the water source and with increased amounts of suspended sediments (Garver *et al.*, 2013; Kamei *et al.*, 1987).

For inactivation methods, see Section 2.4.6.

## 2.2. Host factors

## 2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with IHNV according to Chapter 1.5 of *Aquatic Animal Health Code (Aquatic Code)* are:

Family	Scientific name	Common name
Esocidae	<i>Esox lucius</i>	Northern pike
	<i>Salmo marmoratus</i>	Marble trout
	<i>Salmo salar</i>	Atlantic salmon
	<i>Salmo trutta</i>	Brown trout
	<i>Salvelinus alpinus</i>	Arctic char
	<i>Salvelinus fontinalis</i>	Brook trout
Salmonidae	<i>Salvelinus namaycush</i>	Lake trout
	<i>Oncorhynchus clarki</i>	Cutthroat trout
	<i>Oncorhynchus tshawytscha</i>	Chinook salmon
	<i>Oncorhynchus keta</i>	Chum salmon
	<i>Oncorhynchus kisutch</i>	Coho salmon
	<i>Oncorhynchus masou</i>	Masou salmon
	<i>Oncorhynchus mykiss</i>	Rainbow trout
	<i>Oncorhynchus nerka</i>	Sockeye salmon

## 2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to infection with IHNV according to Chapter 1.5 of the *Aquatic Code* are:

Family	Scientific name	Common name
Acipenseridae	<i>Acipenser transmontanus</i>	White sturgeon
Anguillidae	<i>Anguilla anguilla</i>	European eel
Aulorhynchidae	<i>Aulorhynchus flavidus</i>	Tube-snout
Clupeidae	<i>Clupea pallasii</i>	Pacific herring
Embiotocidae	<i>Cymatogaster aggregate</i>	Shiner perch
Schophthalmidae	<i>Scophthalmus maxima</i>	Turbot

Annex 12 (contd)

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following species, but an active infection has not been demonstrated:

Family	Scientific name	Common name
Cyprinidae	<i>Cyprinus carpio</i>	Common carp
Percidae	<i>Perca flavescens</i>	American yellow perch

### 2.2.3. Non-susceptible species

None known.

### 2.2.4. Likelihood of infection by species, host stage population or sub-populations

IHNV predominantly infects salmon and trout species with fry being the most highly susceptible stage (LaPatra, 1998). Resistance to infection typically increases with fish age until the spawning stage. Returning adult spawning salmon, can be highly infected and shed large amounts of virus in ovarian fluid and milt despite a lack of clinical disease (Dixon *et al.*, 2016).

### 2.2.5. Distribution of the pathogen in the host

IHNV targets haematopoietic tissue and is most commonly isolated from kidney and spleen tissues. The virus has also been isolated from gill, oesophagus, intestine, stomach, pyloric caeca, liver, brain, heart, thymus, adipose tissue, muscle, skin, fin and mucous (Drolet *et al.*, 1994; Harmache *et al.*, 2006; LaPatra *et al.*, 1989; Yamamoto *et al.*, 1990). In spawning fish IHNV has also been isolated in ovarian fluid and milt (Mulcahy *et al.*, 1982).

### 2.2.6. Aquatic animal reservoirs of infection

Field surveillance programmes and experimental infection trials have documented subclinical IHNV infections in various salmon and trout species (Knusel *et al.*, 2007; Mulcahy *et al.*, 1984; Pascoli *et al.*, 2015; St-Hilaire *et al.*, 2001; Traxler *et al.*, 1997). Survivors of laboratory exposures have demonstrated IHNV persistence for months to over 1 year post-exposure (Drolet *et al.*, 1995; Foott *et al.*, 2006; Kim *et al.*, 1999; Muller *et al.*, 2015). With the exception of high viral load occurring in subclinically infected spawning adult salmon, the IHNV levels associated with subclinical infections tend to be lower than in fish undergoing clinical disease.

### 2.2.7. Vectors

A single study has demonstrated that adult salmon lice, *Lepeophtheirus salmonis* are capable of acquiring and transmitting IHNV to naïve Atlantic salmon through parasitism (Jakob *et al.*, 2011). Regardless of whether salmon lice acquired IHNV through water bath exposure or after parasitising IHNV-infected fish, the duration of virus association with salmon lice diminished rapidly with infectious virus levels falling below cell culture detection limits within hours. IHNV has also been isolated from freshwater invertebrates (e.g. leeches, copepods, and mayflies) however their capacity to transmit virus is unknown (Dixon *et al.*, 2016; Garver & Wade, 2017).

## 2.3. Disease pattern

### 2.3.1. Mortality, morbidity and prevalence

Depending on the species of fish, rearing conditions, temperature, and virus strain, outbreaks of infection with IHNV may range from acute to chronic. An outbreak of infection with IHNV in farmed Atlantic salmon in British Columbia resulted in cumulative losses on affected farms between 20 and 94% (Saksida, 2006). In chronic cases, losses are protracted and fish in various stages of disease can be observed in the pond. The prevalence of infection in chronic cases remains unknown. The limited available data indicated that prevalence of infection with IHNV can be high (59%) in endemically infected rainbow trout farms in Europe (reviewed by Dixon *et al.*, 2016).

IHNV is endemic among populations of free-ranging salmonids throughout much of its historical range along the west coast of North America. Sockeye salmon have incurred losses of up to 99% at the fry stage (Kurath *et al.*, 2003). As the fish ages, the prevalence of infection decreases with marine phase sockeye salmon smolts, and the prevalence of infection in adults is generally low (<15%) to undetectable. However, the prevalence of infection can again reach high levels in mature adult spawning sockeye salmon, with long term studies revealing greater than 50% prevalence in wild populations (Meyers *et al.*, 2003).



Annex 12 (contd)**2.3.2. Clinical signs, including behavioural changes**

Fish with acute infection with IHNV can exhibit lethargy interspersed with bouts of frenzied, abnormal activity. During outbreaks, fish can display spiral swimming, flashing, and have trailing faecal casts. Fish may also show darkening of the skin, exophthalmia, distended abdomen and external haemorrhaging. In instances where fish survive an outbreak, spinal deformities may become evident (Bootland & Leong, 1999).

**2.3.3 Gross pathology**

Gross observations are non-pathognomonic and can involve ascites, pale gills, liver, kidney and spleen, petechial haemorrhaging, yellow mucous in the intestine and a lack of food in the stomach (Bootland & Leong, 1999; Traxler, 1986).

**2.3.4. Modes of transmission and life cycle**

The transmission of IHNV between fish is primarily horizontal through direct contact with virus contaminated water or via cohabitation with IHNV infected fish (Bootland & Leong, 1999). However, cases of vertical or egg-associated transmission have been recorded (Mulcahy & Pascho, 1985). While egg-associated transmission is significantly reduced by the now common practice of surface disinfection of eggs with an iodophor solution, it is the only mechanism accounting for the appearance of infection with IHNV in new geographical locations among fry originating from eggs that were incubated and hatched in virus-free water (Dixon *et al.*, 2016; Winton, 1991).

**2.3.5. Environmental and management factors**

The most important environmental factor affecting the disease progression is water temperature. Experimental trials have demonstrated that IHNV can produce mortality from 3°C to 18°C; however clinical disease typically occurs below 15°C under natural conditions (LaPatra, 1998).

**2.3.6. Geographical distribution**

Cases of infection with IHNV have been reported from Europe, Asia-Pacific and the Americas. For recent information on distribution at the country level consult the WAHIS interface.

**EU comment:**

**We suggest adding Africa to the list. We have already provided references in the Code.**

**2.4. Biosecurity and disease control strategies****2.4.1. Vaccination**

Plasmid DNA vaccines containing the gene for the IHNV glycoprotein have proven highly efficacious against infection with IHNV resulting in the licensing of one for commercial use in Atlantic salmon net-pen aquaculture on the west coast of North America (Alonso & Leong, 2013; Salenius *et al.*, 2007). Administered via intramuscular injection, an IHNV DNA vaccine was rapidly disseminated systemically followed by plasmid persistence in muscle at the injection site; consequently, caution should be employed when testing fish vaccinated with the IHNV DNA vaccine as diagnostic methods targeting viral G-gene nucleotide sequence or protein have the potential to cross react with the vaccine.

**2.4.2. Chemotherapy including blocking agents**

Chemotherapeutics, including natural compounds, have been identified to have anti-IHNV properties; however, these have not found commercial use in aquaculture against IHNV (Winton, 1991). Direct application of anti-IHNV compounds to cell cultures has caused growth inhibition and toxicity that could affect the sensitivity of detecting IHNV in affected cultures (Balmer *et al.*, 2017; Hasobe & Saneyoshi, 1985).

**2.4.3. Immunostimulation**

Immunostimulants are not used commercially in aquaculture for IHNV (Ooi *et al.*, 2008).

#### 2.4.4. Breeding resistant strains

Experimental trials of triploid or inter-species hybrids have been conducted (Barroso *et al.*, 2008; Winton, 1991) with resistance typically determined early in the infection process and associated with lower early viral replication (Purcell *et al.*, 2010). However, no resistant strains are commercially available.

#### 2.4.5. Inactivation methods

IHNV is readily inactivated by common disinfectants with active ingredients such as sodium hypochlorite, iodophor, benzalkonium chloride, saponated cresol, formaldehyde and potassium permanganate solution (Yoshimizu *et al.*, 2005). As these substances have virucidal properties any carry-over on sampling equipment or contact with samples may result in reduced viral titres.

#### 2.4.6. Disinfection of eggs and larvae

Iodophor disinfection of eggs is a common practice to effectively mitigate egg-associated transmission of IHNV (Bovo *et al.*, 2005). Chapter 4.4. of the *Aquatic Code* provides recommendations for surface disinfection of salmonid eggs. Iodine has been shown to inhibit PCRs (Auinger *et al.*, 2008) and could affect PCR testing results of disinfected eggs.

#### 2.4.7. General husbandry

In addition to disinfection of eggs, use of a virus-free water supply and decreasing rearing densities have significant positive effects in the management of IHNV. Transmission of IHNV increases with host density (Ogut & Reno, 2004).

### 3. Specimen selection, sample collection, transportation and handling

This section draws on information in Sections 2.2, 2.3 and 2.4 to identify populations, individuals and samples which are most likely to be infected.

#### 3.1. Selection of populations and individual specimens

Clinical inspections are best carried out during a period whenever the water temperature is below 14°C, or whenever the water temperature is likely to reach its lowest annual point. All production units (ponds, tanks, net-cages, etc.) must be inspected for the presence of dead, weak or abnormally behaving fish of any susceptible species, and if they are present, such fish should be selected. Particular attention should be paid to the water outlet area, where weak fish tend to accumulate due to the water current. If additional fish are required for the sample, healthy individuals should be selected as follows:

- i) In farms with salmonids, if rainbow trout are present, only fish of that species should be selected for sampling. If rainbow trout are not present, the sample has to be obtained from fish of all other IHNV-susceptible species.
- ii) Susceptible species should be sampled following risk-based criteria for targeted selection of populations with a history of abnormal mortality or potential exposure events (e.g. via untreated surface water, wild harvest or replacement with stocks of unknown risk status).
- iii) If more than one water source is used for fish production, fish from all water sources should be included in the sample.

#### 3.2. Selection of organs or tissues

The optimal tissue material to be examined is spleen, anterior kidney, and either heart or brain. In the case of spawning fish, ovarian fluid and milt may be examined.

In the case of small fry, whole fish less than 4 cm long can be homogenised (using, for example, sterile scissors or a scapel) after removal of the body behind the gut opening. If a sample consists of whole fish with a body length between 4 cm and 6 cm, the viscera including kidney should be collected. For larger size fish, kidney, spleen, heart, encephalon, and ovarian fluid from brood fish at the time of spawning, should be the tissues to be sampled. When possible, samples should be taken in duplicate to permit retesting if needed.

### 3.3. Samples or tissues not suitable for pathogen detection

IHNV is very sensitive to enzymic degradation, therefore sampling tissues with high enzymatic activities or large numbers of contaminating bacteria, such as the intestine or skin, should be avoided when possible. Given the haematopoietic nature of IHNV, muscle tissue should be avoided as a target tissue. The yolk sac of fry has also shown toxicity to cell lines and should be removed before inoculating cells for virus isolation. Preservatives and fixatives, such as RNAlater and formaldehyde can be toxic to tissue culture cells such as epithelioma papulosum cyprini (EPC) and fathead minnow (FHM), and can impact molecular detection methods (Auinger *et al.*, 2008; Pham *et al.*, 2018).

### 3.4. Non-lethal sampling

Ovarian fluid and milt are suitable samples for detection of IHNV in spawning adult salmon and trout (Dixon *et al.*, 2016; Meyers *et al.*, 2003). There is evidence that IHNV may be isolated from gill, fin and mucous samples but detection may be impacted by the state of infection, time since exposure and sample size (Burbank *et al.*, 2017; LaPatra *et al.*, 1989).

### 3.5. Preservation of samples for submission

For guidance on sample preservation methods for the intended test methods, see Chapter 2.3.0.

#### 3.5.1. Samples for pathogen isolation

The success of pathogen isolation and results of bioassay depend strongly on the quality of samples (time since collection and time in storage). Fresh specimens should be kept on ice and preferably sent to the laboratory within 24 hours of collection. Alternate storage methods should only be used after consultation with the receiving laboratory.

Before shipment or transfer to the laboratory, pieces of the organs to be examined should be removed from the fish with sterile dissection tools and transferred to sterile plastic tubes containing transport medium, i.e. cell culture medium with 10% fetal calf serum (FCS) and antibiotics. The combination of 200 International Units (IU) penicillin, 200 µg streptomycin, and 200 µg kanamycin per ml are recommended, although other antibiotics of proven efficacy may also be used. The tissue in each sample should be larger than the analytical unit size required for initial laboratory testing (e.g. between 0.5 and 2 g) and taken in duplicate if retesting may be required.

Tubes containing fish tissues in transport medium for cell cultivation should be placed in insulated containers, such as thick-walled polystyrene boxes, together with sufficient ice or an alternative cooling medium with the similar cooling effect to ensure chilling of the samples during transportation to the laboratory. However, freezing of the samples should be avoided. The temperature of a sample during transit must never exceed 10°C, and ice must still be present in the transport box at receipt or one or more freeze blocks must still be partly or completely frozen.

Whole fish may be sent to the laboratory if the temperature requirements referred to in the first paragraph during transportation can be fulfilled. Whole fish should be wrapped up in paper with absorptive capacity and enclosed in a plastic bag. Live fish may also be transported to the laboratory. All packaging and labelling must be performed in accordance with present national and international transport regulations, as appropriate.

The virological examination on cell culture should be started as soon as possible, and no later than 48 hours after the collection of the samples. In exceptional cases, the virological examination may be started at the latest within 72 hours after the collection of the material, provided that the material to be examined is protected by a transport medium, and that the temperature requirements during transportation can be fulfilled.

#### 3.5.2. Preservation of samples for molecular detection

Samples can be taken from the fish in accordance with the procedure described in Section 3.5.1., using a sterile instrument, and transferred to a sterile plastic tube containing transport medium.

Alternatively, samples may be placed in at least five volumes of RNA stabilisation reagents, according to the recommendation from the manufacturers. Samples in RNA stabilising reagents can be shipped on ice or at room temperature if transport time does not exceed 24 hours.

Whole fish may also be sent to the laboratory (see Section 3.5.1).

### 3.5.3. Fixed samples for histopathology, immunohistochemistry or *in-situ* hybridisation

Tissue samples for histopathology should be immediately fixed at a fixative to tissue ratio of 10:1. A suitable fixative is 10% buffered formalin. To avoid excessive cross-linking, tissue should be transferred to ethanol after 24hrs if methods other than histopathology are used e.g. *in-situ* hybridisation.

### 3.5.4. Fixed samples for electron microscopy

Not relevant.

### 3.5.5. Samples for other tests

Not relevant.

## 3.6. Pooling of samples

No data are currently available concerning the effect of pooling samples on the detection of IHNV. However, small life stages such as fry can be pooled to provide the minimum amount of material needed for testing.

## 4. Diagnostic methods

The methods currently available for identifying infection that can be used in i) surveillance of apparently healthy populations, ii) presumptive and iii) confirmatory diagnostic purposes are listed in Table 4.1. by life stage. The designations used in the Table indicate:

Key:

+++ = Recommended method(s) validated for the purpose shown and usually to stage 3 of the OIE Validation Pathway;

++ = Suitable method(s) but may need further validation;

+ = May be used in some situations, but cost, reliability, lack of validation or other factors severely limits its application;

Shaded boxes = Not appropriate for this purpose.

The selection of a test for a given purpose depends on the analytical and diagnostic sensitivities and specificities repeatability and reproducibility. OIE Reference Laboratories welcome feedback on diagnostic performance for assays, in particular PCR methods, for factors affecting assay analytical sensitivity or analytical specificity, such as tissue components inhibiting amplification, presence of nonspecific or uncertain bands, etc., and any assays that are in the +++ category.

## Annex 12 (contd)

Table 4.1. OIE recommended diagnostic methods and their level of validation for surveillance of healthy animals and investigation of clinically affected animals

**EU comment:**

For the method IFAT, we suggest to be named as 'cell culture + IFAT', and to score it across columns A, B and C as +++

Method	A. Surveillance of apparently healthy animals				B. Presumptive diagnosis of clinically affected animals				C. Confirmatory diagnosis <sup>1</sup> of a suspect result from surveillance or presumptive diagnosis			
	Early life stages <sup>2</sup>	Juveniles <sup>2</sup>	Adults	LV	Early life stages <sup>2</sup>	Juveniles <sup>2</sup>	Adults	LV	Early life stages <sup>2</sup>	Juveniles <sup>2</sup>	Adults	LV
Wet mounts												
Histopathology <sup>3</sup>						++	++	1				
Cytopathology <sup>3</sup>												
Cell or artificial media culture	+++	+++	+++	3	+++	+++	+++	3	+++	+++	+++	3
Real-time PCR	+++	+++	+++	3	+++	+++	+++	3	+++	+++	+++	3
Conventional PCR						++	++	++	++	++	++	
Amplicon sequencing <sup>4</sup>									+++	+++	+++	
<i>In-situ</i> hybridisation												
Bioassay												
LAMP												
IFAT									++	++	++	2
Ag-ELISA									++	++	++	2
Neutralisation test (antibody or antiserum) <sup>5</sup>									++	++	++	2

LV = level of validation, refers to the stage of validation in the OIE Pathway (Chapter 1.1.2); PCR = polymerase chain reaction; LAMP = loop-mediated isothermal amplification.

IFAT = indirect fluorescent antibody test; Ag-ELISA = antigen enzyme-linked immunosorbent assay. <sup>1</sup>For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). <sup>2</sup>Early and juvenile life stages have been defined in Section 2.2.4.

<sup>3</sup>Cytopathology and histopathology can be validated if the results from different operators has been statistically compared. <sup>4</sup>Sequencing of the PCR product.

<sup>5</sup>Specify the test used. Shading indicates the test is inappropriate or should not be used for this purpose.

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#### 4.1. Wet mounts

Not relevant

#### 4.2. Histopathology and cytopathology

Histopathological findings reveal degenerative necrosis in haematopoietic tissues, kidney, spleen, liver, pancreas, and digestive tract. Necrosis of eosinophilic granular cells in the intestinal wall is pathognomonic of IHNV infection (Bootland & Leong, 1999).

The blood of affected fry shows reduced haematocrit, leukopenia, degeneration of leucocytes and thrombocytes, and large amounts of cellular debris. As with other haemorrhagic viraemias of fish, blood chemistry is altered in severe cases (Bootland & Leong, 1999).

Electron microscopy of virus-infected cells reveals bullet-shaped virions of approximately 150–190 nm in length and 65–75 nm in width (Wolf, 1988). The virions are visible at the cell surface or within vacuoles or intracellular spaces after budding through cellular membranes. The virion possesses an outer envelope containing host lipids and the viral glycoprotein spikes that react with immunogold staining to decorate the virion surface

Smears are not appropriate for detection or identification of IHNV.

#### 4.3. Cell or artificial media culture for isolation

##### 4.3.1. Cell lines

The recommended cell lines for IHNV detection are EPC or FHM. Cell lines should be monitored to ensure that susceptibility to targeted pathogens has not changed.

EPC or FHM cells are grown at 20–30°C in suitable medium, e.g. Eagle's minimal essential medium (MEM; or modifications thereof) with a supplement of 10% fetal bovine serum (FBS) and antibiotics in standard concentrations. When the cells are cultivated in closed vials, it is recommended to buffer the medium with bicarbonate. The medium used for cultivation of cells in open units may be buffered with Tris-HCl (23 mM) and Na-bicarbonate (6 mM). The pH must be  $7.6 \pm 0.2$ . Cell culture plates should be seeded 4–48 hours and not 100% confluent prior to inoculation. 15–30 minutes prior to sample inoculation, cells should be pre-treated with 7% (w/v) PEG-20,000 solution (10–15  $\mu\text{l}/\text{cm}^2$ ) (Batts & Winton, 1989).

##### 4.3.2. Sample preparation and inoculation

Note: Tissue and fluid samples should be kept cool throughout sample preparation procedures.

- i) Homogenise tissue samples using mortar and pestle, stomacher, polytron or equivalent. A small volume of media (MEM-4 or Hank's balanced salt solution with antibiotics) may be needed to achieve complete homogenisation.
- ii) Adjust the volume of media to a final ratio of 10:1 (media:tissue) and mix thoroughly. For fluid samples adjust the volume of media to a final ratio of 1:1.
- iii) Centrifuge the homogenate or fluid samples at 2000–4000 **g** for 15 minutes at 2–5°C.
- iv) Remove the supernatant and pass through a 0.45  $\mu\text{m}$  membrane filter (if available).
- v) If the sample cannot be inoculated within 48 hours after collection, the supernatant may be stored at –80°C provided virological examination is carried out within 14 days.
- vi) If samples originate from an area where infectious pancreatic necrosis virus (IPNV) is present, supernatants may be treated with IPNV antiserum. Mix the supernatant with equal parts of a suitably diluted pool of antisera to the indigenous serotypes of IPNV and incubate for a minimum of 1 hour at 15°C or up to 18 hours at 4°C. The titre of the antiserum must be at least 1/2000 in a 50% plaque neutralisation test.

Annex 12 (contd)

- vii) Samples are inoculated into cell cultures in at least two dilutions, i.e. the primary dilution and a 1:10 dilution thereof, resulting in final dilutions of tissue material in cell culture medium of 1:100 and 1:1000, respectively. The ratio between inoculum size and volume of cell culture medium should be about 1:10. For each dilution and each cell line, a minimum of about 2 cm<sup>2</sup> cell area, corresponding to one well in a 24-well cell culture tray, has to be used. Use of cell culture trays is recommended, but other units of similar or with larger growth area are acceptable as well.
- viii) Inoculated cell cultures are incubated at 15°C for 7–10 days. Using a microscope with 40–150× magnification, cultures should be inspected for toxicity the day after inoculation, particularly if supernatant was not filtered in step iv. The use of a phase-contrast microscope is recommended.
- ix) Monitor the cells regularly (2–3 times a week) for the presence of cytopathic effect (CPE).

*Interpretation of results*

If CPE is observed, confirmatory testing is required to identify IHNv.

If no CPE is observed in the primary culture or subcultivation, the sample is negative.

**4.4. Nucleic acid amplification****EU Comment:**

**We would be grateful if consideration could be given to replacing the two-step RT-qPCR method by a one-step method which has been published by Hoferer et al which provides an optimised and validated procedure for genome detection.**

**4.4.1. Real-time PCR**

There are several reverse-transcription real-time PCR assays available for the detection of IHNv.

The assay described is a stage 3 validated two-step real-time TaqMan PCR assay that amplifies a region of the nucleoprotein gene of all known IHNv genogroups with some E-genogroup isolates (D332-92, FV23, and FV91-40) having reduced amplification efficiency due to single nucleotide polymorphism within the probe sequence (Hoferer *et al.*, 2019; Purcell *et al.*, 2013).

Positive and negative controls should be run with each stage of the assay: extraction, reverse transcription and real-time PCR. Due to the sensitive nature of PCR-based assays, it is important to be able to distinguish a true positive from the positive control material. This may be achieved using an artificial positive control as employed by Purcell *et al.* (2013). It is also highly recommended that master mix, template addition and PCR amplification occur in designated hoods or spatially separated areas.

*RNA extraction and reverse-transcription (RT)*

- i) Total RNA from infected cells and/or tissues is extracted using a phase-separation method (e.g. phenol-chloroform or Trizol) or by use of a commercially available RNA isolation kit used according to the manufacturer's instructions.
- ii) Extracted RNA is reverse transcribed non-discriminately into cDNA using random primers. The cDNA synthesis reactions and cycling conditions are best performed using the manufacturer's instructions for commercially available kits which have been extensively tested with a variety of RNA templates, including GC- and AU-rich targets and RNase expressed at low levels.

*Real-time PCR*

The TaqMan real-time PCR assay uses forward primer IHNv N 796F (5'-AGA-GCC-AAG-GCA-CTG-TGC-G-3'), reverse primer IHNv N 875R (5'-TTC-TTT-GCG-GCT-TG-GTT-GA-3') and FAM-labelled probe, IHNv N 818T (5'-6FAM-TGA-GAC-TGA-GCG-GGA-CA-MGBNFQ-3'). Primers are used at a final concentration of 900 nM each and the final probe concentration is 250 nM. 2.5 µl cDNA product is added to each 25 µl rPCR reaction. Thermal cycling conditions are 50°C 2 minutes, 95°C 10 minutes followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute.

The sample is negative if no Ct (threshold cycle) is recorded, while samples with a Ct are considered positive for IHNv.



#### 4.4.2. Conventional PCR

Several conventional PCR assays are available with limited validation data.

The PCR assay described recognises a broad range of genotypes by targeting a central region of the IHNV G gene (Emmenegger *et al.*, 2000), and produces a PCR amplicon that is used for identification of genetic strains and for epidemiological tracing of virus movements (Kurath *et al.*, 2003).

Positive and negative controls should be run with each stage of the assay: extraction, RT-PCR and second round PCR. Due to the sensitive nature of PCR-based assays it is highly recommended that master mix, template addition and PCR amplification occur in designated hoods or spatially separated areas.

##### *RNA extraction*

Total RNA may be prepared as described in section 4.4.1.

##### *Conventional RT-PCR (Round 1)*

The first round RT-PCR combines cDNA synthesis and PCR amplification into one step by using an IHNV-specific primer set that generates the first-strand synthesis of IHNV RNA and subsequent PCR amplification through 30 cycles. The first round PCR produces a 693 bp PCR amplicon using forward primer (5'-AGA-GAT-CCC-TAC-ACC-AGA-GAC-3') and reverse primer (5'-GGT-GGT-GTT-GTT-TCC-GTG-CAA-3') at a final concentration of 200 nM each. The thermal cycling conditions are one cycle of 50°C for 30 minutes; one cycle of 95°C for 2 minutes; 30 cycles of 95°C for 30 seconds, 50°C for 30 seconds, 72°C for 60 seconds; one cycle of 72°C for 7 minutes and 4°C hold.

A sample is IHNV positive if a 693 bp PCR amplicon is observed and no bands were observed in the negative controls. If no band is observed for a sample and the positive controls passed proceed to the second round nested PCR.

##### *Second round (nested PCR)*

Due to the sensitivity of the test along with the need for repetitive handling of tubes, nested PCR is prone to contamination and good sterile technique must be practiced.

The first round positive and negative controls are carried over and included with the nested PCR assay. In addition, a separate negative and positive control specific to the nested assay are required.

The second round PCR produces a 483 bp PCR amplicon using forward primer (5'-TCA-CCC-TGC-CAG-ACT-CAT-TGG-3') and reverse primer (5'-ATA-GAT-GGA-GCC-TTT-GTG-CAT-3') at a final concentration of 200 nM each. The thermal cycling conditions are: 95°C for 2 minutes followed by 30 cycles of 95°C for 30 seconds, 50°C for 30 seconds, 72°C for 60 seconds; one cycle of 72°C for 7 minutes and 4°C hold.

A sample is IHNV positive if a 483 bp PCR amplicon is observed and no band(s) are observed in the negative controls. A sample is negative if no bands are observed and positive controls passed.

#### 4.4.3. Other nucleic acid amplification methods

To date, no other nucleic acid amplification method capable of universal IHNV detection has been sufficiently validated.

#### 4.5. Amplicon sequencing

Nucleotide sequencing of the conventional PCR product (Section 4.4.2) is recommended as one of the final steps for confirmatory diagnosis. This central region of IHNV glycoprotein gene is used for identification of genetic strains and for epidemiological study (Kurath *et al.*, 2003). It is recommended to forward any sequence data obtained to the OIE Reference Laboratory, particularly in the event where isolate sequences differ from any of the target sequences of the recommended molecular assays.

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**4.6. In-situ hybridisation**

Not relevant.

**4.7. Immunohistochemistry**

Not relevant.

**4.8. Bioassay**

Not relevant.

**4.9. Antibody-based or antigen detection methods (ELISA, etc.)**

Antibody-based and antigen detection methods may be used to confirm the presence of IHNV in cell culture. Kits and antibodies are commercially available and should be used according to manufacturer's instructions. Sensitivity, specificity and sample preparation can influence the results; a negative result should be viewed with caution. These techniques should not be used as a screening method.

**EU Comment:**

**If the final sentence in the above paragraph is to be retained, we would question whether it is also relevant for all other chapters where ELISA methods are set out.**

**4.9.1. Neutralisation test (identification in cell culture)**

- i) Collect the culture medium of the cell monolayers exhibiting CPE and centrifuge an aliquot at 2000 *g* for 15 minutes at 4°C, or filter through a 0.45 µm (or 450 nm) pore membrane to remove cell debris.
- ii) Dilute virus-containing medium from 10<sup>2</sup>–10<sup>4</sup>.
- iii) Mix aliquots (for example 200 µl) of each dilution with equal volumes of an IHNV antibody solution. The neutralising antibody solution must have a 50% plaque reduction titre of at least 2000. Likewise, treat a set of aliquots of each virus dilution with cell culture medium to provide a non-neutralised control.
- iv) In parallel, a neutralisation test must be performed against a homologous IHNV strain (positive neutralisation test) to confirm the reactivity of the antiserum.
- v) Incubate all the mixtures at 15°C for 1 hour.
- vi) Transfer aliquots of each of the above mixtures on to 24-hour-old monolayers overlaid with cell culture medium containing 10% FBS (inoculate two wells per dilution) and incubate at 15°C; 24- or 12-well cell culture plates are suitable for this purpose, using a 50 µl inoculum.
- vii) Check the cell cultures for the onset of CPE and read the results for each suspect IHNV sample and compare to the occurrence of CPE of non-neutralised controls. Results are recorded either after a simple microscopic examination (phase contrast preferable) or after discarding the cell culture medium and staining cell monolayers with a solution of 1% crystal violet in 20% ethanol.
- viii) The tested virus is identified as IHNV when CPE is prevented or noticeably delayed in the cell cultures that received the virus suspension treated with the IHNV-specific antibody, whereas CPE is evident in all other cell cultures.

Other neutralisation tests of demonstrated performance may be used instead.

**4.9.2. Indirect fluorescent antibody test (IFAT) (identification in cell culture)**

- i) Prepare monolayers of cells in 2 cm<sup>2</sup> wells of cell culture plastic plates or on cover slips in order to reach around 80% confluency, which is usually achieved within 24 hours of incubation at 22°C (seed six cell monolayers per virus isolate to be identified, plus two for positive and two for negative controls). The FBS content of the cell culture medium can be reduced to 2–4%. If numerous virus isolates have to be identified, the use of black 96-well plates for immunofluorescence is recommended.

**EU comment:**

**We suggest adding more text in the first sentence to read:**

**'Prepare monolayers of cells in 2 cm<sup>2</sup> wells of cell culture plastic plates or on cover slips in order to reach around 80% confluency, which is achieved within 24 hours of incubation at 22°C the optimal temperature of the cell line in question (e.g. 26°C for EPC and 20°C for RTG) (seed six cells...'**

- ii) When the cell monolayers are ready for infection (i.e. on the same day or on the day after seeding) inoculate the virus suspensions to be identified by making tenfold dilution steps directly in the cell culture wells or flasks.

- iii) Dilute the control virus suspension of IHNV in a similar way, in order to obtain a virus titre of about 5,000–10,000 plaque-forming units (PFU)/ml in the cell culture medium.
- iv) Incubate at 15°C for 24 hours.
- v) Remove the cell culture medium, rinse once with 0.01 M phosphate buffered saline (PBS), pH 7.2, then three times briefly with a cold mixture of acetone 30%/ethanol 70% (v/v) (stored at –20°C).
- vi) Let the fixative act for 15 minutes. A volume of 0.5 ml is adequate for 2 cm<sup>2</sup> of cell monolayer.
- vii) Allow the cell monolayers to air-dry for at least 30 minutes and process immediately or freeze at –20°C.
- viii) Prepare a solution of purified IHNV antibody or serum in 0.01 M PBS, pH 7.2, containing 0.05% Tween-80 (PBST), at the appropriate dilution (which has been established previously or is given by the reagent supplier).
- ix) Rehydrate the dried cell monolayers by four rinsing steps with the PBST solution and remove this buffer completely after the last rinsing.
- x) Treat the cell monolayers with the antibody solution for 1 hour at 37°C in a humid chamber and do not allow evaporation to occur (e.g. by adding a piece of wet cotton to the humid chamber). The volume of solution to be used is 0.25 ml/2 cm<sup>2</sup> well.
- xi) Rinse four times with PBST as above.
- xii) Treat the cell monolayers for 1 hour at 37°C with a solution of fluorescein isothiocyanate- or tetramethylrhodamine-5-(and-6-) isothiocyanate-conjugated antibody to the immunoglobulin used in the first layer and prepared according to the instructions of the supplier. These conjugated antibodies are most often rabbit or goat antibodies.
- xiii) Rinse four times with PBST.
- xiv) Examine the treated cell monolayers on plastic plates immediately, or mount the cover slips using, for example, glycerol saline, pH 8.5 prior to microscopic observation.
- xv) Examine under incident UV light using a microscope with × 10 eye pieces and × 20–40 objective lens having numerical aperture >0.65 and >1.3, respectively. Positive and negative controls must be found to give the expected results prior to any other observation.

Other IFAT or immunocytochemical (alkaline phosphatase or peroxidase) techniques of demonstrated performance may be used instead.

#### 4.9.3. Enzyme-linked immunosorbent assay (ELISA)

- i) Coat the wells of microplates designed for ELISAs with appropriate dilutions of purified immunoglobulins (Ig) or serum specific for IHNV, in 0.01 M PBS, pH 7.2 (200 µl/well).
- ii) Incubate overnight at 4°C.
- iii) Rinse four times with 0.01 M PBS containing 0.05% Tween-20 (PBST).
- iv) Block with skim milk (5% in PBST) or other blocking solution for 1 hour at 37°C (200 µl/well).
- v) Rinse four times with PBST.
- vi) Add 2% Triton X-100 to the virus suspension to be identified.
- vii) Dispense 100 µl/well of two- or four-step dilutions of the virus to be identified and of IHNV control virus, and a heterologous virus control (e.g. viral haemorrhagic septicaemia virus). Allow the samples to react with the coated antibody to IHNV for 1 hour at 20°C.
- viii) Rinse four times with PBST.
- ix) Add to the wells either biotinylated polyclonal IHNV antiserum or MAb to N protein specific for a domain different from the one of the coating MAb and previously conjugated with biotin.
- x) Incubate for 1 hour at 37°C.
- xi) Rinse four times with PBST.
- xii) Add streptavidin-conjugated horseradish peroxidase to those wells that have received the biotin-conjugated antibody, and incubate for 1 hour at 20°C.

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- xiii) Rinse four times with PBST. Add the substrate and chromogen. Stop the course of the test when positive controls react and read the results.
- xiv) Interpretation of the results is according to the optical absorbencies achieved by negative and positive controls and must follow the guidelines for each test, e.g. absorbency at 450 nm of positive control must be minimum 5–10 × A450 of negative control.

The above biotin–avidin-based ELISA version is given as an example. Other ELISA versions of demonstrated performance may be used instead.

#### 4.10. Other serological methods

Not relevant

### 5. Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations

Virus isolation in cell culture or real-time RT-PCR are the recommended tests for surveillance to demonstrate freedom from infection with IHNIV.

### 6. Corroborative diagnostic criteria

This section only addresses the diagnostic test results for detection of infection in the absence (Section 6.1.) or in the presence of clinical signs (Section 6.2.) but does not evaluate whether the infectious agent is the cause of the clinical event.

The case definitions for a suspect and confirmed case have been developed to support decision making related to trade and confirmation of disease status at the country, zone or compartment level. Case definitions for disease confirmation in endemically affected areas may be less stringent. It is recommended that all samples that yield suspect positive test results in an otherwise pathogen-free country or zone or compartment should be referred immediately to the OIE Reference Laboratory for confirmation, whether or not clinical signs are associated with the case. If a laboratory does not have the capacity to undertake the necessary diagnostic tests it should seek advice from the appropriate OIE Reference Laboratory.

#### 6.1. Apparently healthy animals or animals of unknown health status<sup>10</sup>

Apparently healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link to an infected population. Geographic proximity to, or movement of animals or animal products or equipment, etc., from a known infected population equate to an epidemiological link. Alternatively, healthy populations are sampled in surveys to demonstrate disease freedom.

##### 6.1.1. Definition of suspect case in apparently healthy animals

The presence of infection shall be suspected if at least one of the following criteria is met:

- i) Positive result by real-time RT-PCR;
- ii) Cytopathic effect in cell culture.

##### 6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection shall be confirmed if positive results have been obtained on at least one animal from two tests used in the following combination:

- i) Positive result by real-time RT PCR followed by a positive result from a conventional PCR targeting a non-overlapping region of the genome and amplicon sequencing;
- ii) Isolation of virus in cell culture confirmed by IFAT or Ag-ELISA or by a neutralisation test and a positive result by real-time RT-PCR;

<sup>10</sup> For example transboundary commodities.

- iii) Isolation of virus in cell culture confirmed by IFAT or Ag-ELISA or by a neutralisation test and conventional PCR and amplicon sequencing;
- iv) Isolation of virus in cell culture confirmed by conventional PCR and amplicon sequencing.

Reference Laboratories should be contacted for specimen referral when testing laboratories cannot undertake any of the recommended test methods and testing is being undertaken that will result in notification to the OIE.

## 6.2 Clinically affected animals

Clinical signs are not pathognomonic for a single disease; however, they may narrow the range of possible diagnoses.

### 6.2.1. Definition of suspect case in clinically affected animals

The presence of infection shall be suspected if at least one of the following criteria is met:

- i) Gross pathology or clinical signs associated with the disease as described in this chapter, with or without elevated mortality;
- ii) Positive result by real-time RT-PCR;
- iii) Cytopathic effect in cell culture.

### 6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection shall be confirmed if: positive results has been obtained on at least one animal from two test used in the following combination:

- i) Positive result by real-time RT-PCR followed by a positive result from a conventional PCR targeting a non-overlapping region of the genome and amplicon sequencing;
- ii) Isolation of virus in cell culture confirmed by IFAT or Ag-ELISA or by a neutralisation test and a positive result by real-time RT-PCR;
- iii) Isolation of virus in cell culture confirmed by IFAT or Ag-ELISA or by a neutralisation test and conventional PCR and amplicon sequencing;
- iv) Isolation of virus in cell culture confirmed by conventional PCR and amplicon sequencing;

Reference Laboratories should be contacted for specimen referral when testing laboratories cannot undertake any of the recommended test methods and testing is being undertaken that will result in notification to the OIE.

## 6.3. Diagnostic sensitivity and specificity for diagnostic tests

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (n)	DSp (n)	Reference test	Citation
Real-time RT-PCR	Diagnosis	Experimentally infected salmon	Kidney	Steelhead ( <i>Oncorhynchus mykiss</i> )	100 (50)	100 (50)	Animals of known infection status	Purcell <i>et al.</i> , 2013
RT-PCR (single step)	Diagnosis	Experimentally infected salmon	Kidney	Steelhead ( <i>Oncorhynchus mykiss</i> )	58 (50)	100 (50)	Animals of known infection status	Purcell <i>et al.</i> , 2013
Virus Isolation	Diagnosis	Experimentally infected salmon	Kidney	Steelhead ( <i>Oncorhynchus mykiss</i> )	84 (50)	100 (50)	Animals of known infection status	Purcell <i>et al.</i> , 2013
		Field samples	Kidney and spleen	Atlantic salmon ( <i>Salmo salar</i> )	80-86 (50)	100 (50)	Clinical signs – history	McClure <i>et al.</i> , 2008

Annex 12 (contd)**7. References**

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\* \*

NB: There are OIE Reference Laboratories for Infection with infectious haematopoietic necrosis 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/scientific-expertise/reference-laboratories/list-of-laboratories/> ).

Please contact the OIE Reference Laboratories for any further information on infection with viral haemorrhagic septicaemia virus

**NB:** FIRST ADOPTED IN 1995 AS INFECTIOUS HAEMATOPOIETIC NECROSIS;  
MOST RECENT UPDATES ADOPTED IN 2019.



## CHAPTER 2.3.10.

## INFECTION WITH VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUS

**EU Comment:**

**The EU thanks the OIE and supports the proposed changes to this Chapter.**

**Other comments are included in the body of the text.**

**As commented in annex 12, we consider that because of the similarities of this annex and annex 12 we suggest a revision of both annexes together to standardise the stylistic differences between the two chapters.**

**1. Scope**

Infection with viral haemorrhagic septicaemia virus (VHSV) means infection with the pathogenic agent viral haemorrhagic septicaemia virus of the Genus *Novirhabdovirus* and Family *Rhabdoviridae*.

**2. Disease information****2.1. Agent factors****2.1.1. Aetiological agent**

VHSV is a bullet-shaped particle, approximately 70 nm in diameter and 180 nm in length, that contains a negative-sense, single-stranded RNA genome of approximately 11,000 nucleotides, and possesses an envelope that contains the membrane glycoprotein, which is the neutralising surface antigen. The genome encodes six proteins: a nucleoprotein N; a phosphoprotein P (formerly designated M1); a matrix protein M (formerly designated M2); a glycoprotein G; a non-virion protein NV and a polymerase L (Walker *et al.*, 2000).

G-gene nucleotide sequences have been used to classify VHSV isolates into four major genotypes (I, II, III and IV) and nine subtypes (Ia–Ie and IVa–IVd) with almost distinct geographical distributions (Einer-Jensen *et al.*, 2004; Elsayed *et al.*, 2006). The host range and the pathogenicity appear, at least to some extent, to be linked to the genotype of VHSV.

## i) Genotype Ia

Almost all VHSV isolates causing outbreaks in European rainbow trout (*Oncorhynchus mykiss*) farms cluster in sub-lineage Ia, of which isolates have been reported from most continental European countries (Einer-Jensen *et al.*, 2004; Kahns *et al.*, 2012; Snow *et al.*, 2004; Toplak *et al.*, 2010). However, genotype Ia isolates have also been detected in other species such as brown trout (*Salmo trutta*), pike (*Esox lucius*) and grayling (*Thymallus thymallus*) (de Kinkelin & Le Berre, 1977; Jonstrup *et al.*, 2009). Genotype Ia isolates have generally caused outbreaks in freshwater farms, but isolates have also been obtained from rainbow trout in seawater net pens and turbot (*Scophthalmus maximus* syn. *Psetta maxima*) (Schlotfeldt *et al.*, 1991; Snow *et al.*, 2004). Genotype Ia can be further subdivided into two major subpopulations, Ia-1 and Ia-2, with a distinct geographic distribution within Europe (Kahns *et al.*, 2012).

## ii) Genotype Ib

Viruses have been isolated from fish in the marine environment in the Baltic Sea, Kattegat, Skagerrak, the North Sea and the English Channel (Einer-Jensen *et al.*, 2004; Skall *et al.*, 2005; Snow *et al.*, 2004) and as far north as latitude 70°N close to Nordkapp in Norway (Sandlund *et al.*, 2014). A single case was observed in Japan (Nishizawa *et al.*, 2002). None of the isolations from wild fish has been associated with clinical disease outbreaks (Johansen *et al.*, 2013). Genotype Ib has been associated with evidence of transfer between wild fish and farmed rainbow trout in only two cases in pen-reared rainbow trout in Sweden in 1998 and 2000 (Nordblom, 1998; Nordblom & Norell, 2000; Skall *et al.*, 2005).

## iii) Genotype Ic

This genotype is a smaller group consisting of Danish farmed rainbow trout isolates from earlier dates. Isolates of this genotype have also been identified in Germany and Austria (Jonstrup *et al.*, 2009).

Annex 13 (contd)

## iv) Genotype Id

This group consists of some old Scandinavian isolates from the 1960s until the first VHS outbreaks occurred in Finland in sea-reared rainbow trout in 2000 at two different areas where all isolates sampled proved to cluster in the Id genotype. In infection trials, it was demonstrated that the isolates were pathogenic to rainbow trout, but less virulent than most Ia isolates (Raja-Halli *et al.*, 2006).

## v) Genotype Ie

These isolates have been obtained from both freshwater and marine (the Black Sea) environments in Georgia and Turkey. Isolations were from both farmed and wild turbot (Jonstrup *et al.*, 2009; Kalayci *et al.*, 2006; Nishizawa *et al.*, 2006) and from rainbow trout (Einer-Jensen *et al.*, 2004). VHSV Ie has also been identified in whiting (*Merlangius merlangus*) and sea bass (*Dicentrarchus labrax*) from the Black Sea (Altuntas & Ogut, 2010).

## vi) Genotype II

The members of this group consist of marine isolates from wild fish in the Baltic Sea, including the Gulf of Bothnia and the Gulf of Finland, especially from Atlantic herring (*Clupea harengus*) (Gadd *et al.*, 2011; Snow *et al.*, 2004) and lamprey (*Lampetra fluviatilis*) from the rivers Kalajoki and Lestijoki having an outlet into the Gulf of Bothnia (Gadd *et al.*, 2010).

## vii) Genotype III

These isolates originate from wild and farmed fish in the North Atlantic Sea from the Flemish Cap (Lopez-Vazquez *et al.*, 2006b) to the Norwegian coast (Dale *et al.*, 2009), the North Sea, around the British Isles, Skagerrak and Kattegat. VHS outbreaks in farmed turbot in the United Kingdom and Ireland in the 1990s were due to genotype III isolates, and in 2007 an outbreak in sea-reared rainbow trout at the Norwegian west coast was due to VHSV genotype III. VHS outbreaks in five species of wrasse used as cleaner fish around the Shetland Islands were also due to this genotype (Munro *et al.*, 2015).

## viii) Genotype IVa

Isolates originate from both the east and west coasts of North America, as well as from the Asian countries of South Korea and Japan. Genotype IVa isolates in North America have caused severe epidemics in numerous wild marine species such as Pacific herring (*Clupea pallasii*) (Meyers & Winton, 1995), which can serve as a reservoir of virus to sympatric net-pen farmed Atlantic salmon (*Salmo salar*) (Garver *et al.*, 2013). In Asia, genotype IVa isolates have caused disease outbreaks in olive flounder (*Paralichthys olivaceus*).

## ix) Genotype IVb

Isolates originate from the North America Laurentian Great Lakes region (Gagne *et al.*, 2007; Thompson *et al.*, 2011; Winton *et al.*, 2008) where they have caused die-off events in numerous fish species and have been detected in a micro-invertebrate (*Diporeia* spp) (Faisal & Winters, 2011).

## x) Genotype IVc

Isolates originate from the estuarine waters of New Brunswick and Nova Scotia, Canada (Gagne *et al.*, 2007; Pierce & Stepien, 2012; Stepien *et al.*, 2015).

## xi) Genotype IVd

Isolates originate from Iceland where they were identified in wild and farmed lumpfish (*Cyclopterus lumpus*) (Gudmundsdottir *et al.*, 2019).

### 2.1.2. Survival and stability in processed or stored samples

VHSV survival in host tissue is dependent on the conditions for storage. VHSV remains infectious for long time periods while stored frozen in fish tissue. However, VHSV-infected fish at commercial freezing temperatures had a 90% reduction in viral titre after the tissue was thawed (Arkush *et al.*, 2006). VHSV is sensitive to enzymatic degradation, environments with high bacterial load and high temperatures (above 28°C). Fresh (unfrozen) muscle tissue from VHSV-infected rainbow trout could transmit VHS to naïve fish (Oidtmann *et al.*, 2011). VHSV is also tolerant of high salt concentrations such as in brine-treated fish (Skall *et al.*, 2015) or while stored in concentrated ammonium sulphate solution (Pham *et al.*, 2018). For optimal retention of VHSV in fish tissue, the sample should be placed in transport medium with antibiotics and kept on ice without freezing and processed within 24 hours after sampling.

### 2.1.3. Survival and stability outside the host

VHSV survival outside the host is dependent on the physico-chemical conditions of the aqueous medium (Ahne, 1982) and on temperature: the virus survives for longer periods at 4°C compared with 20°C (Parry & Dixon, 1997).

VHSV is significantly more stable in freshwater than saltwater. The virus has been documented to persist in freshwater for 28–35 days at 4°C (Parry & Dixon, 1997) and has been found to be infective for 1 year at 4°C in filtered freshwater (Hawley & Garver, 2008). In raw freshwater at 15°C, the 99.9% inactivation time was 13 days, but in seawater the virus was inactivated within 4 days (Hawley & Garver, 2008). In another study using seawater at 15°C, the infectivity of the virus was reduced by 50% after 10 hours, but could still be recovered after 40 hours (Kocan *et al.*, 2001). There appears to be no consistent correlation between the origin and stability of the virus isolates: freshwater isolates are not always the most stable in freshwater and seawater isolates are not consistently more stable in seawater (Hawley & Garver, 2008).

The virus remains stable for a longer time if sterile organic materials are added to the water, such as ovarian fluids or blood products, such as bovine serum (Kocan *et al.*, 2001). When the sea water was sterilised by autoclaving, or when passed through a 0.22 µm membrane, virus survival was prolonged significantly (60 days at 15°C and 32 days at 20°C), suggesting the bacterial load in the water is an important factor of viral decay.

## 2.2. Host factors

### 2.2.1. Susceptible host species

#### EU Comment:

**We would request that *Salvelinus fontinalis* and *Lota lota* should be considered for listing as species which are susceptible to VHS. Mortalities in *S. fontinalis* have been reported under natural conditions in a hatchery within the EU and the cause of infection was confirmed to be VHS genotype Ia. This virus was subsequently used to infect *Lota lota* using experimental bath challenge and the virus was subsequently isolated from all dead or euthanised fish.**

Species that fulfil the criteria for listing as susceptible to infection with VHSV according to Chapter 1.5. of the *Aquatic Animal Health Code (Aquatic Code)* include:

Family	Scientific name	Common name	Genotype
Ammodytidae	<i>Ammodytes hexapterus</i>	Pacific sand lance	IVa
Aralichthyidae	<i>Paralichthys olivaceus</i>	Bastard halibut	IVa
Carangidae	<i>Trachurus mediterraneus</i>	Mediterranean horse mackerel	Ie
	<i>Ambloplites rupestris</i>	Rock bass	IVb
	<i>Lepomis gibbosus</i>	Pumpkinseed	IVb
Centrarchidae	<i>Lepomis macrochirus</i>	Bluegill	IV, IVb
	<i>Micropterus dolomieu</i>	Smallmouth bass	IVb
	<i>Micropterus salmoides</i>	Largemouth bass	IVb
	<i>Pomoxis nigromaculatus</i>	Black crappie	IVb

## Annex 13 (contd)

Family	Scientific name	Common name	Genotype
	<i>Alosa immaculata</i>	Pontic shad	Ie
	<i>Sardina pilchardus</i>	Pilchard	
Clupeidae	<i>Clupea harengus</i>	Atlantic herring	Ib, III
	<i>Clupea pallasii pallasii</i>	Pacific herring	IVa
	<i>Dorosoma cepedianum</i>	American gizzard shad	IVb
	<i>Sardinops sagax</i>	South American pilchard	IVa
	<i>Sprattus sprattus</i>	European sprat	Ib
Cyclopteridae	<i>Cyclopterus lumpus</i>	Lumpfish	IVd
	<i>Danio rerio</i>	Zebra fish	IVa
Cyprinidae	<i>Notropis hudsonius</i>	Spottail shiner	IVb
	<i>Notropis atherinoides</i>	Emerald shiner	IVb
	<i>Pimephales notatus</i>	Bluntnose minnow	IVb
	<i>Pimephales promelas</i>	Fathead minnow	IVb
Embiotocidae	<i>Cymatogaster aggregata</i>	Shiner perch	IVa
Engraulidae	<i>Engraulis encrasicolus</i>	European anchovy	Ie
Esocidae	<i>Esox lucius</i>	Northern pike	IVb
	<i>Esox masquinongy</i>	Muskellunge	IVb
Fundulidae	<i>Fundulus heteroclitus</i>	Mummichog	IVc
	<i>Gadus macrocephalus</i>	Pacific cod	IVa
Gadidae	<i>Gadus morhua</i>	Atlantic cod	Ib, III
	<i>Merlangius merlangus</i>	Whiting	Ie
	<i>Micromesistius poutassou</i>	Blue whiting	Ib, III
	<i>Trisopterus esmarkii</i>	Norway pout	Ib, III
Gasterosteidae	<i>Gasterosteus aculeatus</i>	Three-spine stickleback	IVc
Gobiidae	<i>Neogobius melanostomus</i>	Round goby	IVb
	<i>Pomatoschistus minutus</i>	Sand goby	Ib
Ictaluridae	<i>Ictalurus nebulosus</i>	Brown bullhead	IVb
	<i>Centrolabrus exoletus</i>	Rock cook wrasse	III
Labridae	<i>Ctenolabrus rupestris</i>	Goldsinny wrasse	III
	<i>Labrus bergylta</i>	Ballan wrasse	III
	<i>Labrus mixtus</i>	Cuckoo wrasse	III
	<i>Symphodus melops</i>	Corkwing wrasse	III
Lotidae	<i>Gaidropsarus vulgaris</i>	Three-bearded rockling	Ie
	<i>Morone americana</i>	White perch	IVb
Moronidae	<i>Morone chrysops</i>	White bass	IVb
	<i>Morone saxatilis</i>	Striped bass	IVb, IVc
Mullidae	<i>Mullus barbatus</i>	Red mullet	Ie
Osmeridae	<i>Thaleichthys pacificus</i>	Eulachon	IVa
Percidae	<i>Sander vitreus</i>	Walleye	IVb
	<i>Perca flavescens</i>	Yellow perch	IVb

## Annex 13 (contd)

Family	Scientific name	Common name	Genotype
Petromyzontidae	<i>Lampetra fluviatilis</i>	River lamprey	II
	<i>Limanda limanda</i>	Common dab	Ib
Pleuronectidae	<i>Platichthys flesus</i>	European flounder	Ib
	<i>Pleuronectes platessus</i>	European plaice	III
Rajidae	<i>Raja clavata</i>	Thornback ray	Ie
	<i>Coregonus artedii</i>	Lake cisco	IVb
	<i>Coregonus clupeaformis</i>	Lake whitefish	IVb
	<i>Coregonus lavaretus</i>	Common whitefish	Ia
	<i>Oncorhynchus kisutch</i>	Coho salmon	IVa
Salmonidae	<i>Oncorhynchus mykiss</i>	Rainbow trout	Ia-e, III, IVb
	<i>Oncorhynchus mykiss</i> <i>Oncorhynchus kisutch</i>	Rainbow trout X coho salmon hybrids	Ia
	<i>Oncorhynchus tshawytscha</i>	Chinook salmon	IVa, IVb
	<i>Salmo marmoratus</i>	Marble trout	Ia
	<i>Salmo salar</i>	Atlantic salmon	Ia, Ib, II, III, IVa
	<i>Salmo trutta</i>	Brown trout	Ia, Ib
	<i>Salvelinus namaycush</i>	Lake trout	Ia, IVa, IVb
	<i>Thymallus thymallus</i>	Grayling	I
Scophthalmidae	<i>Scophthalmus maxima</i>	Turbot	Ib, III
Sciaenidae	<i>Aplodinotus grunniens</i>	Freshwater drum	IVb
Scombridae	<i>Scomber japonicus</i>	Pacific chub mackerel	IVa
Soleidae	<i>Solea senegalensis</i>	Senegalese sole	III
Uranoscopidae	<i>Uranoscopus scaber</i>	Atlantic stargazer	Ie

ND: Not determined.

#### 2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to infection with VHSV according to Chapter 1.5 of the *Aquatic Code* include:

Family	Scientific name	Common name	Genotype
Adrianichthyidae	<i>Oryzias latipes</i>	Japanese rice fish	IVb
	<i>Oryzias dancena</i>	Marine medaka	IVa
Ammodytidae	<i>Ammodytes personatus</i>	Sandeel	Ib
Anguillidae	<i>Anguilla anguilla</i>	European eel	III
Argentinidae	<i>Argentina sphyraena</i>	Lesser Argentine	Ib
Belonidae	<i>Belone belone</i>	Garfish	Ie
Carangidae	<i>Seriola dumerili</i>	Greater amberjack	IVa
	<i>Catostomus commersonii</i>	White sucker	IVb
Catostomidae	<i>Moxostoma anisurum</i>	Silver redhorse	IVb
	<i>Moxostoma macrolepidotum</i>	Shorthead redhorse	IVb

## Annex 13 (contd)

Family	Scientific name	Common name	Genotype	
Centrarchidae	<i>Pomoxi annularis</i>	White crappie	IVb	
Clupeidae	<i>Alosa pseudoharengus</i>	Alewife	IVb	
Cottidae	<i>Cottus pollux</i>	Japanese fluvial sculpin	IVb	
Cyprinidae	<i>Semotilus corporalis</i>	Fallfish	IVb	
	<i>Notemigonus crysoleucas</i>	Golden shiner	IVb	
Esocidae	<i>Esox lucius</i> X <i>E. masquinongy</i> hybrids	Tiger muskellunge ( <i>Esox masquinongy</i> X <i>E. lucius</i> or <i>E. lucius</i> X <i>E. masquinongy</i> )	IVb	
Fundulidae	<i>Fundulus diaphanus</i>	Banded killifish	IVb	
	<i>Gadiculus argenteus</i>	Silvery pout	Ib	
Gadidae	<i>Melanogrammus aeglefinus</i>	Haddock	III	
	<i>Theragra chalcogramma</i>	Alaska pollock	IVa	
	<i>Trisopterus minutus</i>	Poor cod	III	
Ictaluridae	<i>Ictalurus punctatus</i>	Channel catfish	IVb	
Liparidae	<i>Liparis tessellatus</i>	Cubed snailfish	IV	
Lotidae	<i>Lota lota</i>	Burbot	IVb	
	<i>Enchelyopus cimbrius</i>	Fourbeard rockling	Ib	
Merlucciidae	<i>Merluccius productus</i>	North Pacific hake	IVa	
Moronidae	<i>Dicentrarchus labrax</i>	European sea bass	Ia	
Mugilidae	<i>Mugil cephalus</i>	Flathead grey mullet	IV	
Ophidiidae	<i>Hoplobrotula armata</i>	Armoured cusk	IV	
Osmeridae	<i>Hypomesus pretiosus</i>	Surf smelt	ND	
Oxudercidae	<i>Rhinogobius</i> sp. (undescribed species)	Yoshinobori	IVb	
Percopsidae	<i>Percopsis omiscomaycus</i>	Trout perch	IVb	
	<i>Glyptocephalus stelleri</i>	Blackfin flounder	IVa	
Pleuronectidae	<i>Hippoglossus hippoglossus</i>	Atlantic halibut	III	
	<i>Reinhardtius hippoglossoides</i>	Greenland halibut	III	
Salmonidae	<i>Oncorhynchus mykiss</i> X <i>Salvelinus alpinus</i> hybrids	Rainbow trout X Arctic charr hybrids	Ia	
	<i>Oncorhynchus mykiss</i> X <i>Salvelinus namaycush</i> hybrids	Rainbow trout X lake trout hybrids	Ia	
	<i>Oncorhynchus mykiss</i> X <i>Salmo trutta</i> hybrids	Rainbow trout X brown trout hybrids	Ia	
	<i>Salvelinus alpinus</i>	Arctic charr	Ia	
	<i>Salvelinus fontinalis</i>	Brook trout	Ie	
	Sciaenidae	<i>Larimichthys polyactis</i>	Yellow croaker	IV
	Scorpaenidae	<i>Scorpaena porcus</i>	Black scorpionfish	Ie
<i>Scorpaena izensis</i>		Izu scorpionfish	IV	
Scyliorhinidae	<i>Scyliorhinus torazame</i>	Claudy catshark	IV	
Stromateidae	<i>Pampus argenteus</i>	Silver pomfret	IV	
Trichiuridae	<i>Trichiurus lepturus</i>	Largehead hairtail	IV	
Triglidae	<i>Eutrigla gurnardus</i>	Gray gurnard	III	

ND: Not determined.



In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following organisms, but an active infection has not been demonstrated: Sablefish (*Anoplopoma fimbria*).

#### 2.2.3. Non-susceptible species

None known.

#### 2.2.4. Likelihood of infection by species, host life stage, population or sub-populations

Rainbow trout is the most susceptible species to VHSV infection with genotype Ia. For VHSV genotypes Ib, II and III, shoaling wild-living species such as Atlantic herring and European sprat (*Sprattus sprattus*) are likely to be the natural hosts, while for genotype IV, Pacific herring is the natural host. VHSV genotype III has caused disease in farmed turbot and wrasse and genotype IVa in farmed Atlantic salmon, turbot, and olive flounder.

Infection with VHSV may cause disease and mortality in all life stages of susceptible fish. VHSV does not infect fish eggs (Munro & Gregory, 2010).

In surveys of wild marine fish, VHSV has been isolated from most year classes. Few fry have been tested however, as they are usually not caught during the surveys. The highest prevalence of virus was found in shoaling fish, such as Atlantic herring, European sprat and Norway pout (*Sprattus sprattus*) (Skall *et al.*, 2005).

#### 2.2.5. Distribution of the pathogen in the host

In fish showing clinical signs, the virus is abundant in all tissues including gill, skin and muscles (Sandlund *et al.*, 2014). Target organs are anterior kidney, heart and spleen, as these are the sites in which virus is most abundant. In chronic stages, virus titres can become high in the brain (Smail & Snow, 2011; Wolf, 1988).

#### 2.2.6. Aquatic animal reservoirs of infection

Some survivors of epizootics will become long-term carriers of the virus. Pacific herring surviving infection with VHSV genotype IVa have transmitted disease to naïve cohabitants (Gross *et al.*, 2019). Almost all isolations of VHSV genotype Ib, II and III from free-living fish species are from individuals with no clinical signs of infection with VHSV and with low virus titres (Skall *et al.*, 2005).

#### 2.2.7. Vectors

VHSV has been isolated from common snapping turtle (*Chelra serpentina*), leech (*Myzobdella lugubris*), northern map turtle (*Grapeternys geographica*) and water flea (*Moina macrocopa*) and these species are considered to be vectors for transmission of VHSV rather than true susceptible species (Faisal & Schultz, 2009; Goodwin & Merry, 2011; Ito & Olesen, 2017). VHSV has also been isolated from the amphipods *Hyalella* spp. and *Diporeia* spp., suggesting that benthic macroinvertebrates may be vectors for VHSV IVb in endemically affected systems. In contrast VHSV was not detected in mussels or sediments in the same water environment (Faisal & Winters 2011; Throckmorton *et al.*, 2017). VHSV has also been isolated from leech, *Myzobdella lugubris*, in the Great Lakes but whether the leech or amphipods can transmit VHSV from one fish to another is unknown (Faisal & Schulz, 2009; Faisal & Winters, 2011).

Piscivorous birds may act as VHSV vectors by carrying the virus, for example, on their feet (Olesen & Jorgensen, 1982), or through regurgitation of infected fish (Peters & Neukirch, 1986).

### 2.3. Disease pattern

#### 2.3.1. Mortality, morbidity and prevalence

Mortality varies, depending on many environmental and physiological conditions, most of which have not been fully determined. The disease is, in general, a cool or cold water disease with highest mortality at temperatures around 9–12°C. Small rainbow trout fry (0.3–3 g) are most susceptible to genotype Ia with mortalities close to 100%, but all sizes of rainbow trout can be affected with mortalities ranging from 5 to 90% (Skall *et al.*, 2004). Immersion infection trials also induced up to 100% mortality in Pacific herring when challenged with genotype IVa (Hershberger *et al.*, 2010). Mortality in free living fish also varies from no observable deaths to severe die-offs. The prevalence of VHSV genotype Ib, II and III varies from 0 to 16.7% in Northern European waters (Skall *et al.*, 2005b).

Annex 13 (contd)**2.3.2. Clinical signs, including behavioural changes**

The occurrence of the following clinical signs is characteristic of infection with VHSV: rapid onset of mortality, lethargy, darkening of the skin, exophthalmia, anaemia (pale gills), haemorrhages at the base of the fins or in the gills, eyes or skin, abnormal swimming such as flashing and spiralling, and a distended abdomen due to oedema in the peritoneal cavity. In rainbow trout, the clinical appearance is typically lethargic dark fish with exophthalmia at the pond shores and the outlet. Characteristically, diseased fish will not attempt to escape when netted.

**2.3.3 Gross pathology**

Gross pathology includes generalised petechial haemorrhaging in the skin, muscle tissue (especially in dorsal muscles) and internal organs. It is important to examine the dorsal musculature for the presence of petechial bleeding, which is a very common sign of infection with VHSV. The kidney is dark red in the acute phase and can demonstrate severe necrosis in moribund fish. The spleen is moderately swollen. The liver is often pale and mottled. The gastrointestinal tract, especially the hind gut, is pale and devoid of food.

**2.3.4. Modes of transmission and life cycle**

Transmission primarily occurs horizontally through water, with excretion of virus in the urine, and directly from the skin (Smail & Snow, 2011). Oral transmission was also demonstrated indicating that preying on infected fish and vectors may transfer the disease (Schonherz *et al.* 2012).

Experimentally it has been demonstrated that feeding fresh (unfrozen) muscle tissue from VHSV-infected rainbow trout can transmit VHSV to naïve fish (Oidtmann *et al.*, 2011).

There are no indications or evidence of true vertical transmission of VHSV (Bovo *et al.*, 2005a; Munro & Gregory, 2010).

**2.3.5. Environmental and management factors**

Disease generally occurs at temperatures between 4°C and 14°C. At water temperatures between 15°C and 18°C, the disease generally takes a short course with low levels of mortality.

Low water temperatures (1–5°C) generally result in an extended disease course with low daily mortality but high accumulated mortality. VHS outbreaks occur during all seasons but are most common in spring when water temperatures are rising or fluctuating. For more detailed reviews, see Wolf (1988) and Smail & Snow (2011).

**2.3.6. Geographical distribution****EU Comment:**

**We would request that as they are updated, chapters would move from listing individual countries as being infected, to listing at the continent level instead. This comment applies horizontally.**

Until the late 1980s, VHSV was considered to be restricted to farmed rainbow trout in continental Europe, with the occasional isolation from a restricted number of other freshwater fish species (e.g. brown trout, pike [Meier & Jorgensen, 1980; Schlotfeldt & Ahne, 1988]). With the detection and isolation of VHSV from Pacific salmon off the Pacific North American coast in the late 1980s, subsequent studies have demonstrated that infection with VHSV occurs in numerous farmed and wild fish species along the Pacific and Atlantic North American coast (Skall *et al.*, 2005), in the Great Lakes area of North America (Thompson *et al.*, 2011), the seas around the UK (Skall *et al.*, 2005), the Baltic Sea, Skagerrak and Kattegat (Skall *et al.*, 2005), in the waters around Japan (Skall *et al.*, 2005), and in the Black Sea area, with the distinct genotype 1e (Nishizawa *et al.*, 2006).

Infection with VHSV in farmed rainbow trout has been reported from almost all European and Middle East countries and from China (People's Rep. of) and Russia. However, a number of countries in Europe, such as Denmark, Ireland, Norway, Sweden and UK, are officially declared free of infection with VHSV. Infection with VHSV has never been reported from the Southern Hemisphere.

For recent information on distribution at the country level consult the WAHIS interface.

## **2.4. Biosecurity and disease control strategies**

### **2.4.1. Vaccination**

Although research on vaccine development for VHSV has been ongoing for more than four decades, a commercial vaccine is not yet available. Candidate vaccines have included killed vaccines, attenuated live vaccines, a recombinant vaccine in prokaryotic and eukaryotic expression systems, and DNA-based vaccines. For a review see Lorenzen & LaPatra (2005). No vaccines currently affect the sensitivity and specificity of infection with VHSV diagnostics.

### **2.4.2. Chemotherapy including blocking agents**

No therapies are currently available.

### **2.4.3. Immunostimulation**

Several immunostimulants, such as yeast-derived beta-glucans, IL-1 $\beta$ -derived peptides, and probiotics have been assessed for enhancing protection against infection with VHSV (Peddie *et al.*, 2003). Several researchers report positive effects, but no immunostimulant directed specifically at enhanced resistance to infection with VHSV is available. Furthermore, it remains unknown as to whether their use can affect sensitivity and specificity of infection with VHSV diagnostics.

### **2.4.4. Breeding resistant strains**

Additive genetic variation in rainbow trout has been detected for resistance to VHSV (Dorson *et al.*, 1995; Henryon *et al.*, 2002a; 2002b). In a study by Henryon *et al.* (2005), the heritability of resistance to VHS was 0.11 for time to death on a logarithmic timescale. Identification of a major quantitative trait loci (QTL) for VHSV resistance in rainbow trout may pave the way for genetic selection for VHSV resistant fish (Verrier *et al.*, 2013), however, no resistant rainbow trout strains are yet commercially available.

### **2.4.5. Inactivation methods**

VHSV is sensitive to a number of common disinfectants (e.g. UV, chlorine, iodophore, sodium hypochlorite), to temperatures above 30°C, to bacterial degradation in sediments and enzymatic activity in decomposing fish. For a review see Bovo *et al.*, 2005b.

### **2.4.6. Disinfection of eggs and larvae**

Disinfection of eyed and green eggs is an efficient and cost-effective preventative measure for stopping the spread of the disease in salmonids (for the recommended protocol see Chapter 4.4. of the *Aquatic Code*).

### **2.4.7. General husbandry**

Poor water quality, high fish density, high feeding rate, other diseases such as proliferative kidney disease, ichthyophthiriasis, bacterial kidney disease, etc. can influence the course and severity of infection with VHSV. In general, an increase in temperature, restricted feeding, reduced fish density and restricted handling may reduce mortality. In endemically infected farms, stocking with naïve fry is usually done at as high water temperatures as possible.

## **3. Specimen selection, sample collection, transportation and handling**

### **3.1. Selection of populations and individual specimens**

Clinical inspections should be carried out during a period when the water temperature is below 14°C or whenever the water temperature is likely to reach its lowest annual point. All production units (ponds, tanks, net-cages, etc.) should be inspected for the presence of dead, weak or abnormally behaving fish. Particular attention should be paid to the water outlet area where weak fish tend to accumulate due to the water current.

Annex 13 (contd)

Fish to be sampled are selected as follows:

- i) For genotype I, in farms where rainbow trout are present, only fish of that species should be selected for sampling. If rainbow trout are not present, the sample should be obtained from fish of all other VHSV-susceptible species present, as listed in Tables 2.1. and 2.2. However, the species should be proportionally represented in the sample. For other genotypes (II, III, and IV), species of known susceptibility to the genotype in question should be sampled.
- ii) Susceptible species should be sampled following risk-based criteria for targeted selection of populations with a history of abnormal mortality or potential exposure events (e.g. via untreated surface water, wild harvest or introduction of stocks of unknown risk status).
- iii) If more than one water source is used for fish production, fish from all water sources should be included in the sample.

### 3.2. Selection of organs or tissues

The optimal tissue material to be examined is spleen, anterior kidney, heart and encephalon. In some cases, ovarian fluid and milt must be examined.

In case of small fry, whole fish less than 4 cm long can be minced with sterile scissors or a scalpel after removal of the body behind the gut opening. If a sample consists of whole fish with a body length between 4 cm and 6 cm, the viscera including kidney should be collected. For larger size fish, kidney, spleen, heart and encephalon, and ovarian fluid from brood fish at the time of spawning should be the tissues to be sampled.

### 3.3. Samples or tissues not suitable for pathogen detection

VHSV is very sensitive to enzymatic degradation, therefore sampling tissues with high enzymatic activities, such as viscera and liver, or large numbers of contaminating bacteria, such as the intestine or skin, should be avoided. Preservatives and fixatives, such as RNAlater and formaldehyde can be toxic to tissue culture cells such as epithelioma papulosum cyprini (EPC) and fathead minnow (FHM), and can impact molecular detection methods (Aunger *et al.*, 2008; Pham *et al.*, 2018).

### 3.4. Non-lethal sampling

Fin and gill biopsies were shown to be effective nonlethal samples for detection of VHSV genotype IVb (Cornwell *et al.*, 2013) and nested reverse-transcription polymerase chain reaction (RT-PCR) on blood samples from infected fish was shown to be efficient for VHSV detection (Lopez-Vazquez *et al.*, 2006a). In the case of brood fish, ovarian fluid and milt can be used for testing as alternative to lethal testing. However, no non-lethal samplings have been fully validated for detection of all VHSV genotypes and are therefore not prescribed in this chapter.

### 3.5. Preservation of samples for submission

For guidance on sample preservation methods for the intended test methods, see Chapter 2.3.0.

#### 3.5.1. Samples for pathogen isolation

The success of pathogen isolation and results of bioassay depend strongly on the quality of samples (time since collection and time in storage). Fresh specimens should be kept on ice and preferably sent to the laboratory within 24 hours of collection. Alternate storage methods should be used only after consultation with the receiving laboratory.

Before transfer to the laboratory, pieces of the organs to be examined should be removed from the fish with sterile dissection tools and transferred to sterile plastic tubes containing at least 4 ml transport medium, i.e. cell culture medium with 10% fetal calf serum (FCS) and antibiotics. The combination of 200 International Units (IU) penicillin, 200 µg streptomycin, and 200 µg kanamycin per ml are recommended, although other antibiotics of proven efficiency may also be used. The tissue in each sample should be larger than the analytical unit size required for initial laboratory testing (e.g. between 0.5 and 2 g) and taken in duplicate if retesting may be required.

Tubes containing fish tissues in transport medium for cell cultivation should be placed in insulated containers, such as thick-walled polystyrene boxes, together with sufficient ice or an alternative cooling medium with the similar cooling effect to ensure chilling of the samples during transportation to the laboratory. However, freezing of the samples should be avoided. The temperature of a sample during transit must never exceed 10°C and ice must still be present in the transport box at receipt or one or more freeze blocks must still be partly or completely frozen.

Whole fish may be sent to the laboratory if the temperature requirements referred to in the first paragraph during transportation can be fulfilled. Whole fish should be wrapped up in paper with absorptive capacity and enclosed in a plastic bag. Live fish may also be transported to the laboratory. All packaging and labelling must be performed in accordance with present national and international transport regulations, as appropriate.

The virological examination for isolation in cell culture should be started as soon as possible and no later than 48 hours after the collection of the samples. In exceptional cases, the virological examination may be started at the latest within 72 hours after the collection of the material, provided that the material to be examined is protected by a transport medium and that the temperature requirements during transportation can be fulfilled.

### 3.5.2. Preservation of samples for molecular detection

Samples can be taken from the fish in accordance with the procedure described in Section 3.5.1., using a sterile instrument, and transferred to a sterile plastic tube containing transport medium.

Alternatively, samples may be placed in at least five volumes of RNA stabilisation reagents according to the recommendation from the manufacturers. Samples in RNA stabilising reagents can be shipped on ice or at room temperature if transport time does not exceed 24 hours.

Whole fish may also be sent to the laboratory (see Section 3.5.1.).

Samples may also be frozen and kept frozen until assayed.

### 3.5.3. Samples for histopathology, immunohistochemistry or *in-situ* hybridisation

Tissue samples for histopathology should be fixed in 10% neutral buffered formalin immediately after collection. The recommended ratio of fixative to tissue is 10:1. To avoid excessive cross-linking, tissue should be transferred to ethanol after 24 hours if methods other than histopathology are used e.g. *in-situ* hybridisation.

### 3.5.4. Fixed samples for electron microscopy

Sampling for electron microscopy should be done according to standard procedures (for an example, see Chapter 2.2.9 *Infection with yellow head virus genotype 1*). Sampling for electron microscopy is not relevant for diagnostic purposes.

### 3.5.5. Samples for other tests

If samples are processed for ELISA or other immunochemical assays, the procedures described in Section 3.5.1. for pathogen isolation should be followed.

## 3.6. Pooling of samples

The effect of pooling on diagnostic sensitivity has not been evaluated, therefore larger fish should be processed and tested individually. However, samples, especially fry or specimens up to 0.5 g, can be pooled to obtain enough material for virus isolation or molecular detection.

## 4. Diagnostic methods

The methods currently available for identifying infection that can be used in i) surveillance of apparently healthy populations), ii) presumptive and iii) confirmatory diagnostic purposes are listed in Table 4.1. by life stage. The designations used in the Table indicate:

Key:

+++ = Recommended method(s) validated for the purpose shown and usually to stage 3 of the OIE Validation Pathway;

++ = Suitable method(s) but may need further validation;

+ = May be used in some situations, but cost, reliability, lack of validation or other factors severely limits its application;

Shaded boxes = Not appropriate for this purpose.

The selection of a test for a given purpose depends on the analytical and diagnostic sensitivities and specificities, and repeatability and reproducibility. OIE Reference Laboratories welcome feedback on diagnostic performance for assays, in particular PCR methods, for factors affecting assay analytical sensitivity or analytical specificity, such as tissue components inhibiting amplification, presence of nonspecific or uncertain bands, etc., and any assays that are in the +++ category.

## Annex 13 (contd)

**Table 4.1.** OIE recommended diagnostic methods and their level of validation for surveillance of healthy animals and investigation of clinically affected animals

Method	D. Surveillance of apparently healthy animals				E. Presumptive diagnosis of clinically affected animals				F. Confirmatory diagnosis <sup>1</sup> of a suspect result from surveillance or presumptive diagnosis			
	Early life stages <sup>2</sup>	Juvenile s <sup>2</sup>	Adults	LV	Early life stages <sup>2</sup>	Juvenile s <sup>2</sup>	Adults	LV	Early life stages <sup>2</sup>	Juvenile s <sup>2</sup>	Adults	LV
Wet mounts												
Immunohistopathology <sup>3</sup>									++	2	2	2
Histopathology <sup>3</sup>						++	++					
Cell culture	+++	+++	+++	3	+++	+++	+++	3	+++	+++	+++	3
Real-time PCR	+++	+++	+++	3	+++	+++	+++	3	+++	+++	+++	3
Conventional RT-PCR	++	++	++	3	+++	++	+++	3	+++	++	+++	3
Amplicon sequencing <sup>4</sup>									+++	+++	+++	
<i>In-situ</i> hybridisation												
Bioassay												
LAMP												
Ab-ELISA		+	++	2								
Ag-ELISA									+ <sup>5</sup>	++ <sup>5</sup>	++ <sup>5</sup>	1
IFAT					++	++	++	2	++ <sup>5</sup>	++ <sup>5</sup>	++ <sup>5</sup>	2
Serum neutralisation for Ab detection		+	++	2								

LV = level of validation, refers to the stage of validation in the OIE Pathway (chapter 1.1.2); RT-PCR = reverse-transcription polymerase chain reaction; Ab- or Ag-ELISA = antibody or antigen enzyme-linked immunosorbent assay, respectively; IFAT = indirect fluorescent antibody test; LAMP = loop-mediated isothermal amplification <sup>1</sup>For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). <sup>2</sup>Early and juvenile life stages have been defined in Section 2.2.4. <sup>3</sup>Histopathology and cytopathology can be validated if the results from different operators has been statistically compared. <sup>4</sup>Sequencing of the PCR product. <sup>5</sup>only for identification of cultured pathogen. Shading indicates the test is inappropriate or should not be used for this purpose.

#### 4.1. Wet mounts

Not relevant.

#### 4.2. Histopathology and cytopathology

The kidney, liver and spleen show extensive focal necrosis and degeneration – cytoplasmic vacuoles, pyknosis, karyolysis, and lymphocytic invasion. While the skeletal muscle does not appear to be a site of infection, erythrocytes can accumulate in the skeletal muscle bundles and fibres without causing damage to the muscle *per se* (Evensen *et al.*, 1994).

#### 4.3. Cell or artificial media culture for isolation

The recommended cell lines for VHSV detection are bluegill fry (BF-2), epithelioma papulosum cyprini (EPC) or fathead minnow (FHM). Susceptibility of a cell line to VHSV infection will depend on a range of parameters, including cell-line lineage or viral strain differences. Generally, VHSV isolates belonging to either genotypes I, II, or III culture best on BF-2 (Lorenzen *et al.*, 1999), while genotype IV isolates culture best on the EPC cell line (US Department of the Interior, 2007).

##### 4.3.1. Cell lines

Cell lines should be monitored regularly (e.g. every 6 months) to ensure that susceptibility to targeted pathogens has not changed.

Cells are grown at 20–24°C in a suitable medium, e.g. Eagle's minimal essential medium (MEM) (or modifications thereof) with a supplement of 10% fetal bovine serum (FBS) and antibiotics in standard concentrations. When the cells are cultivated in closed vials, it is recommended to buffer the medium with bicarbonate. The medium used for cultivation of cells in open units may be buffered with Tris/HCl (23 mM) and Na-bicarbonate (6 mM), or with HEPES-buffered medium (HEPES=N-2-hydroxyethyl-piperazine-N-2-ethanesulphonic acid). The pH must be maintained at  $7.6 \pm 0.2$ . Cell cultures to be used for inoculation with tissue material should be young (4–48 hours old) and actively growing (not confluent) at inoculation. Cell susceptibility can be enhanced by reducing the amount of FBS to 2%. Pre-treatment of cells with 7% (w/v) PEG-20,000 solution (10–15  $\mu\text{l}/\text{cm}^2$ ) 15–30 minutes prior to sample inoculation has also been shown to increase detection of VHSV in culture (Batts *et al.*, 1991).

##### 4.3.2. Sample preparation and inoculation

- i) **Note:** Tissue and fluid samples should be kept cool throughout sample preparation procedures. Homogenise tissue samples using mortar and pestle, stomacher, polytron or equivalent. A small volume of media (MEM-4 or HBSS [Hank's balanced salt solution] + antibiotics) may be needed to achieve complete homogenisation.
- ii) Adjust the volume of media to a final ratio of 10:1 (media:tissue) and mix thoroughly. For fluid samples adjust the volume of media to a final ratio of 1:1.
- iii) Centrifuge the homogenate or fluid samples at 2000–4000 **g** for 15 minutes at 2–5°C.
- iv) Remove the supernatant and pass through a 0.45  $\mu\text{m}$  membrane filter (if available) or treat for either 4 hours at 15°C or overnight at 4°C with antibiotics, e.g. gentamicin 1 mg ml<sup>-1</sup>.

If the sample cannot be inoculated within 48 hours after collection, the supernatant may be stored at –80°C provided virological examination is carried out within 14 days.

- v) If samples originate from an area where infectious pancreatic necrosis virus (IPNV) is present, supernatants may be treated with IPNV antiserum. Mix the supernatant with equal parts of a suitably diluted pool of antisera to the indigenous serotypes of IPNV and incubate for a minimum of one hour at 15°C or up to 18 hours at 4°C. The titre of the antiserum must be at least 1/2000 in a 50% plaque neutralisation test.

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Treatment of all inocula with antiserum to IPNV (a virus that in some parts of Europe occurs in 50% of fish samples) aims at preventing cytopathic effect (CPE) caused by IPNV from developing in inoculated cell cultures. This will reduce the duration of the virological examination as well as the number of cases in which occurrence of CPE would have to be considered potentially indicative of VHSV. When samples come from production units that are considered free from infection with IPNV, treatment of inocula with antiserum to IPNV may be omitted.

- vi) Samples are inoculated into cell cultures in at least two dilutions, i.e. the primary dilution and a 1:10 dilution thereof, resulting in final dilutions of tissue material in cell culture medium of 1:100 and 1:1000, respectively. The ratio between inoculum size and volume of cell culture medium should be about 1:10. For each dilution and each cell line, a minimum of about 2 cm<sup>2</sup> cell area, corresponding to one well in a 24-well cell culture tray, has to be used. Use of cell culture trays is recommended, but other units of similar or with larger growth area are also acceptable.
- vii) Inoculated cell cultures are incubated at 15°C for 7–10 days. Using a microscope with 40–150x magnification, cultures should be inspected for toxicity the day after inoculation, particularly if supernatant was not filtered in step iv. The use of a phase-contrast microscope is recommended.
- viii) Monitor the cells regularly (2–3 times a week) for the presence of CPE.

If CPE is observed, virus identification is required using tests recommended in Section 6. If no CPE is observed after the primary incubation period, subcultivation is performed.

*Subcultivation*

- i) Remove cell culture supernatant from the primary culture and inoculate a newly (<48 hours) seeded cell culture plate.
- ii) Incubate inoculated plates at 15°C and monitor for 7–10 days as described above.

If CPE is observed, virus identification is required using tests recommended in Section 6. If no CPE is observed after the primary incubation period or subcultivation, the sample is negative.

**4.4. Nucleic acid amplification**

Use of molecular tests (RT-PCR and real-time RT-PCR) is common because of their rapidity, sensitivity and specificity. Real-time RT-PCR tests are generally more sensitive than conventional RT-PCR tests. These tests for virus detection and identification during the acute stage of disease has been justified for a number of years. At the acute stage, the sensitivity of some RT-PCR (Kim *et al.*, 2018) and real-time RT-PCR tests (Garver *et al.*, 2011; Jonstrup *et al.*, 2013) is comparable to detection by cell culture and subsequent identification. The molecular methods described in this chapter are all targeting the Nucleoprotein gene, as it is the highest transcribed gene in the VHSV genome (Chico *et al.*, 2006).

Recently, a novel one-step RT-PCR test was developed and validated (Kim *et al.*, 2018) to be used instead of the previously recommended conventional RT-PCR for detecting VHSV. This novel assay has a higher sensitivity detecting all VHSV genotypes, and outperforms the old method, particularly in detecting genotype IV.

For detecting VHSV with real-time RT-PCR, the one-step method of Jonstrup *et al.* (2013) has been stage 3 validated, showing a sensitivity similar to detection by cell culture. This method, having high analytical and diagnostic sensitivity and specificity, has been shown to be highly robust across laboratories (Jonstrup *et al.*, 2013; Warg *et al.*, 2014a; 2014b).

The following controls should be run with each assay: negative extraction control; positive control; no template control; internal PCR control.

**4.4.1. Real-time RT-PCR**

Total RNA can be purified from: aliquots of cell culture medium from infected monolayer cells; or tissue/organs homogenised in MEM specified in Section 4.3.1, tissue samples in RNA stabilising reagent, fresh or frozen tissue samples, ovarian fluid.

In the case of culture medium from infected monolayer cells, or in tissue homogenised in MEM, aliquots should be centrifuged at 1000 **g** for 5 minutes to remove cell debris.



One-step (Jonstrup *et al.*, 2013) and two-step (Garver *et al.*, 2011) real-time RT-PCR assays targeting the nucleoprotein gene of VHSV have been stage 3 validated and are described herein.

Positive and negative controls should be included with each stage of the assay: extraction, reverse-transcription (two-step assay only) and real-time PCR. An internal (endogenous) PCR control can be included however given the large number of fish species susceptible to infection with VHSV, the selection of an internal control is not trivial. If an endogenous control is to be used, primers and probes have to be designed, optimised and validated for each fish species to be tested.

Total RNA from infected cells and/or tissues is extracted using a phase-separation method (e.g. phenol-chloroform or Trizol) or by use of a commercially-available RNA isolation kit used according to the manufacturer's instructions.

#### *One-step real-time RT-PCR*

In one-step RT-PCR gene-specific primers are used both to generate a cDNA transcript and for real-time PCR. Both reactions occur in the same tube, which minimises the probability of contamination. The one-step real-time RT-PCR amplification can be performed using forward primer 5'-AAA-CTC-GCA-GGA-TGT-GTG-CGT-CC-3', reverse primer: 5'-TCT-GCG-ATC-TCA-GTC-AGG-ATG-AA-3', and FAM-labelled probe: 6'-FAM-TAG-AGG-GCC-TTG-GTG-ATC-TTC-TG-BHQ1. Primers are used at a final concentration of 900 nM and the final probe concentration is 250 nM. 5 µl of extracted RNA (50 ng–2 µg) is added to each 25 µl PCR reaction. The assay was validated using Quantitect Probe RT-PCR kit (Qiagen, Germany) following the manufacturer's instructions and is recommended as other one-step kits have demonstrated reduced sensitivity (Jonstrup *et al.*, 2013). Thermal cycling conditions are 50°C for 30 minutes, 95°C for 15 minutes, 40 cycles of 94°C for 15 seconds, 60°C for 40 seconds, 72°C for 20 seconds.

#### *Two-step real-time RT-PCR*

##### i) Step 1: Reverse-transcription

Extracted RNA is reverse transcribed non-discriminately into cDNA using random primers. The cDNA synthesis reactions and cycling conditions are best performed using manufacturer's instructions for commercially available kits which have been extensively tested with a variety of RNA templates, including GC- and AU-rich targets and RNase expressed at low levels.

##### ii) Step 2: Real-time PCR

The TaqMan real-time PCR assay uses forward primer 5'-ATG-AGG-CAG-GTG-TCG-GAG-G-3', reverse primer 5'-TGT-AGT-AGG-ACT-CTC-CCA-GCA-TCC and FAM-labelled probe 5'-6FAM-TAC-GCC-ATC-ATG-ATG-AGT-MGBNFQ-3'. Primers are used at a final concentration of 600 nM, and the final concentration of the probe is 200 nM. 2.5 µl of cDNA product is added to each 25 µl PCR reaction. Thermal cycling conditions are 50°C for 2 minutes, 95°C for 10 minutes followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute.

A sample is negative if no Ct (threshold cycle) is recorded, while samples with a Ct are considered positive for VHSV. Cut-off value depends on the set-up in each laboratory, but is usually set at Ct  $\geq$  40).

#### **4.4.2. Conventional PCR (PCR)**

RNA isolation is done as in Section 4.4.1.

A one-step RT-PCR should be performed as described by Kim *et al.* (2018) with 3F2R primer set: forward primers (3F, 5'-(GGG-ACA-GGA-ATG-ACC-ATG-AT-3') and reverse primer (2R, (5'-TCT-GTC-ACC-TTG-ATC-CCC-TCC-AG-3') targeting a 319 nt region in the nucleoprotein gene (positions 658–977).

The RT-PCR can be performed using, e.g. Qiagen OneStep RT-PCR System (Qiagen, Germany) or similar kit, according to the manufacturer's instructions. Briefly, the reaction mixture is adjusted to a final volume of 25 µl including 5 µl of extracted viral RNA, 5 µl 5 × One Step RT-PCR Buffer containing 12.5 mM MgCl<sub>2</sub> (final concentration 2.5 mM), 10 pM of each primer, and 1 µl of enzyme mix.

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The following cycles are recommended: 50°C for 30 minutes, 95°C for 15 minutes, 35 cycles at 94°C for 30 seconds, 60°C for 30 seconds, and 68°C for 60 seconds. Subsequently, the reaction is held at 68°C for 7 minutes.

#### 4.4.3. Other nucleic acid amplification methods

To date, no other nucleic acid amplification method capable of universal VHSV detection has been sufficiently validated.

#### 4.5. Amplicon sequencing

Nucleotide sequencing of the glycoprotein gene is commonly used for identification of genetic strains and for epidemiological study and is recommended as one of the final steps for confirmatory diagnosis. There are several conventional PCR assays available that amplify the central (669 nt) or full (1524 nt) glycoprotein gene coding sequence, but there are limited validation data. The glycoprotein gene can be amplified by conventional PCR using the primer sets and concentrations listed in Table 4.2. The reverse transcription and subsequent PCR amplification can be done using a kit designed for that purpose according to manufacturing instructions.

**Table 4.2.** Primer sets for the conventional PCR

Primer	Sequence (5'–3')	Product size (bp)	Final primer concentration	Reference
GB+	GTC-GAA-GAA-GAG-ATA-GGC		0.6 µM	Einer-Jensen <i>et al.</i> , 2004
GB-	GTT-GGG-TCG-CCA-TGT-TTC-T	1757	0.6 µM	Gudmundsdottir <i>et al.</i> , 2019
G330+	ACT-ACC-TAC-ACA-GAG-TGA-C	914	0.2 µM	Garver <i>et al.</i> , 2013
G1243-	CAA-TTT-GTC-CCC-GAA-TAT-CAT		0.2 µM	
G422+	TCC-CGT-CAA-GAG-GCC-AC	669	0.2 µM	
G1179-	TTC-CAG-GTG-TTG-TTT-ACC-G		0.2 µM	

#### 4.6. In-situ hybridisation

Not relevant in relation to primary diagnosis and surveillance of infection with VHSV.

#### 4.7. Immunohistochemistry

Immunohistochemistry reveals VHSV-positive endothelial cells, primarily in the vascular system (Evensen *et al.*, 1994).

Specific polyclonal and monoclonal antibodies for immunohistochemistry are commercially available.

#### 4.8. Bioassay

Not relevant in relation to primary diagnostics and surveillance of infection with VHSV.

#### 4.9. Antibody or antigen detection methods

##### 4.9.1. Antigen enzyme-linked immunosorbent assay (ELISA)

- i) Coat the wells of microplates designed for enzyme-linked immunosorbent assays (ELISAs) with appropriate dilutions of protein-A purified immunoglobulins (Ig) from rabbit anti sera against VHSV in carbonate buffer, pH 9.6 (50 µl well<sup>-1</sup>).
- ii) Incubate overnight at 4°C.

- iii) Rinse in phosphate-buffered saline (PBS) containing 0.05% Tween-20 (PBST).
- iv) Add 1% Triton X-100 to the virus suspension to be identified.
- v) Dispense 50  $\mu\text{l}$  well<sup>-1</sup> of two- or four-step dilutions (in PBST containing 1% bovine serum albumin) of the virus to be identified and of VHSV control virus, as well as a negative control (e.g. infectious haematopoietic necrosis virus [IHNV]), and allow to react with the coated antibody to VHSV for 1 hour at 37°C.
- vi) Rinse in PBST.
- vii) Add to the wells monoclonal antibodies to VHSV N protein (IP5B11) 50  $\mu\text{l}$  well<sup>-1</sup>.
- viii) Incubate for 1 hour at 37°C.
- ix) Rinse in PBST.
- x) Add to the wells (50  $\mu\text{l}$  well<sup>-1</sup>) horseradish peroxidase (HRP)-conjugated monoclonal anti-mouse antibodies.
- xi) Incubate for 1 hour at 37°C.
- xii) Rinse in PBST.
- xiii) Visualise the reaction using TMB (3,3',5,5'-tetramethylbenzidine) and measure the absorbance at a wavelength of 450 nm.

The above ELISA version is given as an example. Other ELISA versions of demonstrated performance may be used instead.

For positive controls, use cell culture supernatant from cultures inoculated with known VHSV isolate.

For negative controls, use cell culture supernatant from same cell line inoculated with heterologous virus (e.g. IHNV) or from non-infected culture.

#### 4.9.2. Indirect fluorescent antibody test (IFAT)

- i) Prepare monolayers of cells in 2 cm<sup>2</sup> wells of cell culture plastic plates or on cover-slips to reach around 80% confluence, which is usually achieved within 24 hours of incubation at 22°C (seed six cell monolayers per virus isolate to be identified, plus two for positive and two for negative controls). The FCS content of the cell culture medium can be reduced to 2–4%. If numerous virus isolates have to be identified, the use of Terasaki plates is strongly recommended.
- ii) When the cell monolayers are ready for infection, i.e. on the same day or on the day after seeding, inoculate the virus suspensions to be identified by making tenfold dilution steps directly in the cell culture wells or flasks.
- iii) Dilute the control virus suspension of VHSV in a similar way, in order to obtain a virus titre of about 5000–10,000 plaque-forming units (PFU) ml<sup>-1</sup> in the cell culture medium.
- iv) Incubate at 15°C for 24 hours.
- v) Remove the cell culture medium, rinse once with 0.01 M PBS, pH 7.2, then three times briefly with a cold mixture of acetone 30% and ethanol 70% (v/v) (stored at –20°C).
- vi) Let the fixative act for 15 minutes. A volume of 0.5 ml is adequate for 2 cm<sup>2</sup> of cell monolayer.
- vii) Allow the cell monolayers to air-dry for at least 30 minutes and process immediately or freeze at –20°C.
- viii) Prepare a solution of purified VHSV antibody or serum in 0.01 M PBST, pH 7.2, at the appropriate dilution (which has been established previously or is given by the reagent supplier).
- ix) Rehydrate the dried cell monolayers by using four rinsing steps with the PBST solution and remove this buffer completely after the last rinse.
- x) Treat the cell monolayers with the antibody solution for 1 hour at 37°C in a humid chamber and do not allow evaporation to occur, e.g. by adding a piece of wet cotton in the humid chamber. The volume of solution to be used is 0.25 ml/2 cm<sup>2</sup> well<sup>-1</sup>.
- xi) Rinse four times with PBST as above.

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- xii) Treat the cell monolayers for 1 hour at 37°C with a solution of fluorescein isothiocyanate (FITC)- or tetramethylrhodamine-5-(and-6-) isothiocyanate (TRITC)-conjugated antibody to the immunoglobulin used as the primary antibody and prepared according to the instructions of the supplier. These conjugated antibodies are most often rabbit or goat antibodies.
- xiii) Rinse four times with PBST.
- xiv) Examine the treated cell monolayers on plastic plates immediately, or mount the cover-slips using, for example glycerol saline, pH 8.5 prior to microscopic observation.
- xv) Examine under incident UV light using a microscope with ×10 eye pieces and ×20–40 objective lens having numerical aperture >0.65 and >1.3 respectively. Positive and negative controls must yield the expected results prior to any other observation.

Other IFAT or immunocytochemical (alkaline phosphatase or peroxidase) techniques of demonstrated performance may be used instead.

Always include positive control such as wells or coverslip with cells infected with a known VHSV isolate.

**4.10. Other serological methods****4.10.1. Neutralisation test**

- i) Collect the culture medium of the cell monolayers exhibiting CPE and centrifuge it at 2000 *g* for 15 minutes at 4°C, or filter through a 0.45 µm (or 450 nm) pore membrane to remove cell debris.
- ii) Dilute virus-containing medium from 10<sup>-2</sup> to 10<sup>-4</sup>.
- iii) Mix aliquots (for example 200 µl) of each dilution with equal volumes of a VHSV antibody solution and, likewise, treat aliquots of each virus dilution with cell culture medium. The neutralising antibody [NAb] solution must have a 50% plaque reduction titre of at least 2000.
- iv) In parallel, another neutralisation test must be performed against a homologous virus strain (positive neutralisation test).
- v) If required, a similar neutralisation test may be performed using antibodies to IPNV.
- vi) Incubate all the mixtures at 15°C for 1 hour.
- vii) Transfer aliquots of each of the above mixtures on to 24–48 hour-old monolayers, overlaid with cell culture medium containing 10% FCS (inoculate two wells per dilution), and incubate at 15°C; 24- or 12-well cell culture plates are suitable for this purpose, using a 50 µl inoculum.
- viii) Check the cell cultures for the onset of CPE and read the result as soon as it occurs in non-neutralised controls (cell monolayers being protected in positive neutralisation controls). Results are recorded either after a simple microscopic examination (phase contrast preferable) or after discarding the cell culture medium and staining cell monolayers with a solution of 1% crystal violet in 20% ethanol.
- ix) The tested virus is identified as VHSV when CPE is prevented or noticeably delayed in the cell cultures that received the virus suspension treated with the VHSV-specific antibody, whereas CPE is evident in all other cell cultures.
- x) In the absence of any neutralisation by NAb to VHSV, it is mandatory to conduct an RT-PCR, an ELISA or IFAT, using the suspect sample. Some cases of antigenic drift of surface antigen have been observed, resulting in occasional failure of the neutralisation test using NAb to VHSV.

Other neutralisation tests of demonstrated performance may be used instead.

**5. Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations**

Virus isolation, real-time RT-PCR and conventional PCR are the recommended tests for surveillance to demonstrate freedom of disease in apparently healthy population.

## 6. Corroborative diagnostic criteria

This section only addresses the diagnostic test results for detection of infection in the absence (Section 6.1.) or in the presence of clinical signs (Section 6.2.) but does not evaluate whether the infectious agent is the cause of the clinical event.

The case definitions for a suspect and confirmed case have been developed to support decision making related to trade and confirmation of disease status at the country, zone or compartment level. Case definitions for disease confirmation in endemically affected areas may be less stringent. It is recommended that all samples that yield suspect positive test results in an otherwise pathogen-free country or zone or compartment should be referred immediately to the OIE Reference Laboratory for confirmation, whether or not clinical signs are associated with the case. If a laboratory does not have the capacity to undertake the necessary diagnostic tests it should seek advice from the appropriate OIE Reference Laboratory.

### 6.1. Apparently healthy animals or animals of unknown health status<sup>11</sup>

#### 6.1.1. Definition of suspect case in apparently healthy animals

The presence of infection with VHSV shall be suspected if at least one of the following criteria is met:

- i) VHSV-typical CPE in cell cultures before confirmation;
- ii) A positive result from a real-time PCR assay;
- iii) A positive result from a conventional PCR assay;
- iv) Detection of antibodies (by Ab-ELISA or serum neutralisation in adults only).

#### 6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with VHSV is considered to be confirmed if, in addition to the criteria in Section 6.1.1., one or more of the following criteria are met:

- i) VHSV isolation in cell culture followed by virus identification by conventional RT-PCR, and by sequencing of the amplicon;
- ii) VHSV isolation in cell culture, followed by virus identification by real-time RT-PCR, Ag-ELISA, or IFAT and detection of VHSV in tissue preparations by conventional RT-PCR and sequencing of the amplicon;
- iii) VHSV isolation in cell culture, followed by virus identification by real-time RT-PCR, Ag-ELISA, or IFAT and detection of VHSV in tissue preparations by real-time RT-PCR;
- iv) Detection of VHSV in tissue preparations by real-time RT-PCR, and by conventional RT-PCR and sequencing of the amplicon.

Reference Laboratories should be contacted for specimen referral when testing laboratories cannot undertake any of the recommended test methods and testing is being undertaken that will result in notification to the OIE.

## 6.2. Clinically affected animals

No clinical signs are pathognomonic for infection with VHSV however, they may narrow the range of possible diagnoses.

### 6.2.1. Definition of suspect case in clinically affected animals

The presence of infection shall be suspected if at least one of the following criteria are met:

- i) Gross pathology or clinical signs associated with infection with VHSV as described in this chapter, with or without elevated mortality;

<sup>11</sup> For example transboundary commodities.

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- ii) Histopathological changes consistent with infection with VHSV as described in this chapter;
- iii) A positive result from real-time PCR, conventional PCR, or IFAT;
- iv) A positive result from a conventional PCR;
- v) Cytopathic effect in cell culture.

**6.2.2. Definition of confirmed case in clinically affected animals**

The presence of infection with VHSV shall be confirmed if positive results has been obtained on at least one animal from two tests used in the following combination:

- i) VHSV isolation in cell culture, followed by virus identification by conventional RT-PCR, and sequencing of the amplicon;
- ii) VHSV isolation in cell culture, followed by virus identification by real-time RT-PCR, Ag-ELISA, or IFAT and detection of VHSV in tissue preparations by conventional RT-PCR and sequencing of the amplicon;
- iii) VHSV isolation in cell culture, followed by virus identification by real-time RT-PCR, Ag-ELISA, or IFAT and detection of VHSV in tissue preparations by real-time RT-PCR;
- iv) VHSV isolation in cell culture, followed by virus identification by real-time RT-PCR, Ag-ELISA, or IFAT and a positive result from immunohistopathology);
- v) Detection of VHSV in tissue preparations by real-time RT-PCR and by conventional RT-PCR, followed by sequencing of the amplicon.

Reference Laboratories should be contacted for specimen referral when testing laboratories cannot undertake any of the recommended test methods and testing is being undertaken that will result in notification to the OIE.

**6.3. Diagnostic sensitivity and specificity for diagnostic tests**

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (n)	DSp (n)	Reference test	Citation
Cell culture	Surveillance	Experimentally infected fish	Kidney, heart and spleen	Rainbow trout	86 (84)	–	Real-time RT-PCR	Jonstrup <i>et al.</i> , 201
Real-time RT-PCR	Surveillance	Experimentally infected fish	Kidney	Atlantic salmon	93 (30)	100 (70)	Cell culture	Garver <i>et al.</i> , 2011
Real-time RT-PCR	Surveillance	Experimentally infected fish	Kidney, heart and spleen	Rainbow trout	90 (84)	100 (43)	Cell culture	Jonstrup <i>et al.</i> , 201

DSe: = diagnostic sensitivity, DSp = diagnostic specificity.

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\*  
\* \*

**NB:** There are OIE Reference Laboratories for Infection with viral haemorrhagic septicaemia 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/scientific-expertise/reference-laboratories/list-of-laboratories/> ).

Please contact the OIE Reference Laboratories for any further information on infection with viral haemorrhagic septicaemia virus

**NB:** FIRST ADOPTED IN 1995 AS VIRAL HAEMORRHAGIC SEPTICAEMIS;  
MOST RECENT UPDATES ADOPTED IN 2012.



**ASSESSMENT OF INFECTION WITH DECAPOD IRIDESCENT VIRUS-1 (DIV1)  
FOR LISTING IN CHAPTER 1.3. OF THE  
AQUATIC ANIMAL HEALTH CODE**

**EU comment:**

**We support the listing of Infection with decapod iridescent virus-1 (DIV1)**

**Overall Assessment**

The OIE Aquatic Animal Health Standards Commission (the Commission) assessed infection with Decapod iridescent virus-1 (DIV1) against the criteria for listing aquatic animal diseases in Article 1.2.2. of the *Aquatic Code* and agreed that infection with (DIV1) meets the OIE criteria for listing, notably 1.: International spread of the disease is likely; 2.: At least one country may demonstrate country or zone freedom from the disease; 3.: A precise case definition is available and a reliable means of detection and diagnosis exists, and 4b.: The disease has been shown to affect the health of cultured aquatic animals at the level of a country or a zone resulting in significant consequences e.g. production losses, morbidity or mortality at a zone or country level (see Table 1 below).

**Table 1.** Summary of assessment of infection with (DIV1)

	Listing criteria						Conclusion
	1	2	3	4a	4b	4c	
Infection with DIV1	+	+	+	NA	+	-	The disease meets the criteria for listing

NA = not applicable.

**Background**

A novel member of family *Iridoviridae*, named as Decapod iridescent virus 1 (DIV1) (ICTV, 2019), with a double-stranded DNA genome about 166K bp (Li *et al.*, 2017; Qiu *et al.*, 2017b), has been identified as the cause of mass mortalities in shrimp, prawn and crayfish productions (Xu *et al.*, 2016; Qiu *et al.*, 2017a; Qiu *et al.*, 2019a). Shrimp Infection with DIV1 has so far been detected in red claw crayfish (*Cherax quadricarinatus*) (Xu *et al.*, 2016), white-leg shrimp (*Penaeus vannamei*) (Qiu *et al.*, 2017), giant freshwater prawn (*Macrobrachium rosenbergii*) (Qiu *et al.*, 2019a), red swamp crayfish (*Procambarus clarkia*) (Qiu *et al.*, 2019a), oriental river prawn (*Macrobrachium nipponense*) (Qiu *et al.*, 2019a) and ridgetail white prawn (*Exopalaemon carinicauda*). Two species of crab, Chinese mitten crab (*Eriocheir sinensis*) and striped shore crab (*Pachygrapsus crassipes*) have been shown to become infected with DIV1 in experimental challenge through unnatural pathways (Pan *et al.*, 2017). The Commission has recognised the potential significance of infection with DIV1 to many countries given the worldwide importance of crustacean farming and trade. At the moment, infection with DIV1 is considered an “emerging disease” and, as such, should be reported in accordance with Article 1.1.4. of the *Aquatic Code*.

Historically, *P. vannamei* have been traded internationally as broodstock and postlarvae for production in new geographic regions, and shrimp. *P. vannamei* products are traded internationally, thus the potential of international spread is likely.

**Criteria for listing an aquatic animal disease (Article 1.2.2.)**

**Criterion No. 1. International spread of the pathogenic agent (via aquatic animals, aquatic animal products, vectors or fomites) is likely.**

**Assessment**

The virus has been detected by PCR or nested PCR method in white-leg shrimp (*P. vannamei*), giant freshwater prawn (*M. rosenbergii*), red swamp crayfish (*P. clarkia*), oriental river prawn (*M. nipponense*) and ridgetail white prawn (*E. carinicauda*) in farms in China (People’s Rep. of) (Xu *et al.*, 2016; Qiu *et al.*, 2017a; Qiu *et al.*, 2018b; Qiu *et al.*, 2019b). Historically, *P. vannamei* and other susceptible crustacean species have been traded internationally as

Annex 14 (contd)

broodstock and postlarvae for production in new geographic regions, and *P. vannamei* products are traded internationally. Histopathology, visualization under TEM and *in-situ* hybridisation provide evidence that the virus can be found in haematopoietic tissue, gills, hepatopancreas, periopods and muscle (Qiu *et al.*, 2017a). Quantitative PCR detection in experimentally infected shrimp showed that haemolymph and haemopoietic tissues had the highest DIV1 load and muscle tissues had the lowest load (Qiu *et al.*, 2018a; Qiu *et al.*, 2019a).

Conclusion

The criterion is met.

**AND**

**Criterion No. 2. At least one country may demonstrate country or zone freedom from the disease in susceptible aquatic animals.**

Assessment

Currently, infection with DIV1 has only been detected in China (People's Rep. of) but the distribution of the virus may be wider than what has been reported if mortality events have not been investigated. However, because of the broad distribution of *P. vannamei*, *M. rosenbergii*, and other susceptible species to infection with DIV1, as well as extensive trade in these species, and likely expression of clinical disease and mortality, it is expected that the disease would have been reported elsewhere if the virus had spread widely.

In addition, the disease has been listed as a notifiable disease by the Network of Aquaculture Centres in Asia-Pacific (NACA) in its 'Quarterly Aquatic Animal Disease report' (Asia and Pacific Region) since January 2019. It is, therefore, likely that the disease at least one country may demonstrate country or zone freedom from the disease in susceptible aquatic animals.

Conclusion

The criterion is met.

**AND**

**Criterion No. 3. A precise case definition is available, and a reliable means of detection and diagnosis exists.**

Assessment

Infected *P. vannamei* exhibit empty stomach and guts in all diseased shrimp, slight loss of colour on the surface and in the area of the hepatopancreas, and soft shell. In some individuals slight reddening of the body is observed. Moribund shrimp lose their swimming ability and sink to the bottom of the pond (Qiu *et al.*, 2017a). Diseased *M. rosenbergii* exhibit a white triangle inside the carapace at the base of rostrum which is the location of hematopoietic tissue (Qiu *et al.*, 2019a).

To date, a nested PCR method (Qiu *et al.*, 2017a), a TaqMan probe based real-time PCR (TaqMan qPCR) method (Qiu *et al.*, 2018a), and *in situ* hybridization method (Qiu *et al.*, 2017a) and an *in situ* DIG-labeling-loop-mediated DNA amplification (ISDL) method (Chen *et al.*, 2019) have been published and are available for DIV1 detection. The PCR primers and TaqMan probe have been shown to be specific for DIV1 (no cross-reaction with other shrimp pathogens), with a low detection limit (4 copies per reaction) and high sensitivity and specificity (95.3% and 99.2%, respectively). Validation of the nested PCR method and TaqMan probe based real-time PCR method has occurred.

It can be concluded that a) reliable means of detection and diagnosis is available, and b) a precise case definition based on clinical signs and the use of the available diagnostic tests can be developed.

Conclusion:

Criterion is met.

**AND**

**Criterion No. 4. a. *Natural transmission to humans has been proven, and human infection is associated with severe consequences.***

Assessment:

No available data to assess.

Conclusion

Criterion not applicable.

**OR**

**Criterion No. 4.b. *The disease has been shown to affect the health of cultured aquatic animals at the level of a country or a zone resulting in significant consequences e.g. production losses, morbidity or mortality at a zone or country level.***

Assessment

High mortality (>80%) have been observed in affected *P. vannamei* and *M. rosenbergii* populations in farms in China (People's Rep. of) (Qiu *et al.*, 2017a; Qiu *et al.*, 2019a). Experimental infection trials mimicking the natural infection pathway (*per os* and reverse garvage) in *P. vannamei* has shown 100% cumulative mortality within 2 weeks (Qiu *et al.*, 2017a). Injection challenges in *P. vannamei*, *C. quadricarinatus*, and *P. clarkii* also exhibited 100% cumulative mortalities (Xu *et al.*, 2016; Qiu *et al.*, 2017a). Since 2014, some disease events with massive losses of *P. vannamei* and *M. rosenbergii* in coastal provinces of China (People's Rep. of) have been associated with infection with DIV1 (Qiu *et al.*, 2017a). Targeted surveillance in China in 2017 and 2018 revealed that DIV1 has been detected in 11 of 16 provinces (Qiu *et al.*, 2018b; Qiu *et al.*, 2019b). Losses are significant at a country level.

Conclusion

Criterion is met.

**OR**

**Criterion No. 4.c. *The disease has been shown to, or scientific evidence indicates that it would affect the health of wild aquatic animals resulting in significant consequences e.g. morbidity or mortality at a population level, reduced productivity or ecological impacts.***

Assessment

Infection with DIV1 has been shown to have a significant effect on the health of cultured shrimp, crayfish, or lobsters resulting in significant consequences including morbidity and mortality. It is possible that the disease would affect wild aquatic animals; however, there are no available data to demonstrate impact (e.g. morbidity or mortality) of the disease on wild aquatic animals at a population level.

Annex 14 (contd)Conclusion

Criterion is not met.

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Organisation  
Mondiale  
de la Santé  
Animale

World  
Organisation  
for Animal  
Health

Organización  
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de Sanidad  
Animal

Annex 15

Original: English  
September 2019

**REPORT OF THE OIE AD HOC GROUP ON SUSCEPTIBILITY  
OF FISH SPECIES TO INFECTION WITH OIE LISTED DISEASES**

**November 2018-September 2019**

**EU Comment:**

**The EU thanks the OIE for the report of the ad hoc group on susceptibility of fish species to infection with OIE listed diseases.**

**We have included a comment in the body of the text.**

This report covers the work of the OIE *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases (the *ad hoc* Group) between November 2018 and September 2019.

The list of participants and the Terms of Reference are presented in [Annex II](#) and [Annex III](#), respectively.

During this period the *ad hoc* Group had worked electronically and had applied the criteria to host species to determine susceptibility to infection with viral haemorrhagic septicaemia virus (VHSV). This was done by the three-stage approach, outlined in Article 1.5.3. of the *Aquatic Code*, to assess susceptibility of a species to infection with VHSV, as described below:

- 1) criteria to determine whether the route of transmission is consistent with natural pathways for the infection (as described in Article 1.5.4.);
- 2) criteria to determine whether the pathogenic agent has been adequately identified (as described in Article 1.5.5.);
- 3) criteria to determine whether the evidence indicates that presence of the pathogenic agent constitutes an infection (as described in Article 1.5.6.)

**Stage 1: criteria to determine whether the route of transmission is consistent with natural pathways for the infection (as described in Article 1.5.4.)**

Route of infection Key

*N: Natural infection.*

*E: Experimental (non-invasive).*

*EI: Experimental (invasive).*

References that reported invasive experimental procedures as the route of transmission were not used as evidence for infection (i.e. Article 1.5.4.).

**Stage 2: criteria to determine whether the pathogenic agent has been adequately identified as described in Article 1.5.5.**

Accurate pathogenic agent identification might not have been carried out in older publications because molecular typing techniques were not available at the time. In these circumstances a weight of evidence approach, using combined data from relevant studies, were considered and used to assess susceptibility.

Annex 15 (contd)

**Stage 3: criteria to determine whether the evidence indicates that presence of the pathogenic agent constitutes an infection as described in Article 1.5.6.**

Criteria A to D in Article 1.5.6. were used to determine if there was sufficient evidence for infection with the pathogenic agent in the suspect host species. Evidence to support criterion A alone was sufficient to determine infection. In the absence of evidence to meet criterion A, satisfying at least two of criteria B, C or D were required to determine infection.

- A. The pathogenic agent is multiplying in the host, or developing stages of the pathogenic agent are present in or on the host;
- B. Viable pathogenic agent is isolated from the proposed susceptible species, or infectivity is demonstrated by way of transmission to naïve individuals;
- C. Clinical or pathological changes are associated with the infection;
- D. The specific location of the pathogen corresponds with the expected target tissues.

*Table 1. Criteria for susceptibility to infection with VHSV*

**EU Comment:**

**We would like to receive clarification in relation to the difference between B: ‘isolation of virus from internal organs by cell culture’ and D: ‘recover virus from internal organs’**

A: Replication	B: Viability / Infectivity	C: Pathology / Clinical signs	D: Location
Sequential virus titration showing increase in viral titres or high virus titres in internal organs (>10 <sup>5</sup> TCID <sub>50</sub> /g)  <b>OR</b> TEM  <b>OR</b> Immunohistochemistry  <b>OR</b> Product of virus replication detected	Isolation of virus from internal organs by cell culture  <b>OR</b> Passage to a susceptible host	The occurrence of the following signs should lead to extended clinical examination for VHS: rapid onset of mortality, lethargy, darkening of the skin, exophthalmia, anaemia (pale gills), haemorrhages at the base of the fins, gills, eyes and skin, petechial haemorrhages in muscle, abnormal swimming such as flashing and spiralling, and a distended abdomen due to oedema in the peritoneal cavity (from the <i>Aquatic Manual</i> )*  <u>Microscopic methods</u>  The kidney and liver are prime targets and examination of histological sections from diseased fish reveals degeneration and necrosis of haematopoietic tissues of the kidney (and the spleen) with focal degeneration and necrosis of the liver. Sections of the skeletal muscle may show many foci of red blood cells, while the muscle fibres remain undamaged.	Recover virus from internal organs  <b>OR</b> RT-PCR from internal organs

\*not all the clinical signs will be found in all species

**Pathogen identification for VHSV:**

Pathogen isolation on BF-2, EPC, FHM, or CHSE cell lines with confirmation using immunological or molecular test. Immunological test could include virus neutralization, IFAT, or ELISA. Molecular tools include RT-PCR, DNA probes, sequencing. RT-PCR could also be done directly on infected tissues.

Evidence of infection Key Stage 3

Y: Demonstrates criterion is met.

N: Criterion is not met or was not assessed.

ND: Not determined

**Outcome key used by the *ad hoc* Group when assessing the susceptibility of the species:**

<b>1.</b>	<i>Species that were classified as susceptible (as described in Article 1.5.7.) were proposed for inclusion in Article 10.10.2. of Chapter 10.10. Infection with viral haemorrhagic septicaemia virus (VHSV) of the Aquatic Code and Section 2.2.1. of Chapter 2.3.10. Viral haemorrhagic septicaemia (VHSV) of the Aquatic Manual.</i>
<b>2.</b>	<i>Species that were classified as species for which there is partial evidence for susceptibility (as described in Article 1.5.8.) were proposed for inclusion in Section 2.2.2. Species with incomplete evidence for susceptibility of Chapter 2.3.10. Viral haemorrhagic septicaemia (VHSV) of the Aquatic Manual.</i>
<b>3.</b>	<i>Species that were found not to meet the criteria were not proposed for inclusion in either the Aquatic Code or Aquatic Manual. The exception were species where there had been reported pathogen-specific positive PCR results. These species were included in a separate paragraph in Section 2.2.2. Species with incomplete evidence for susceptibility of Chapter 2.3.10. Viral haemorrhagic septicaemia (VHSV) of the Aquatic Manual.</i>
<b>4.</b>	<i>There is evidence of non-susceptibility and the species is not proposed for inclusion in either the Aquatic Code or Aquatic Manual.</i>

The *ad hoc* Group recommended that invertebrate species and turtle species assessed and listed in Table 2 be included in Section 2.2.6. Vectors of Chapter 2.3.10. Viral haemorrhagic septicaemia (VHSV) in the *Aquatic Manual*. These species were considered to be vectors for transmission of VHSV rather than true susceptible species because it was difficult to determine viral replication within the invertebrate and turtle species.

Where there is conflicting evidence in the scientific literature for the same host species, or assessments differed (e.g. assessments ranging between '1' and '3'), the *ad hoc* Group provided some explanatory text in the relevant Annex as to their rationale for the final outcome.

The *ad hoc* Group considered that if only a single publication provided evidence for a score of 1, some form of corroborating evidence was required in addition, specifically:

- 1) Internal corroboration in the published study. Multiple lines of evidence within the same publication. This could result from i) a research cruise that amasses positive fish from multiple dates and locations or ii) an experimental study testing several isolates or routes of exposure (e.g. immersion and cohab). In these instances, assuming the research is sound, the species was scored a 1 from a single peer-reviewed publication.
- 2) External corroboration: evidence from other publications or sources. Examples might include data found in a government website, a separate publication that scores a 2 or better, or evidence of expert judgement (e.g., source for a permissive cell line, or records from a reference lab).

The detailed assessments for VHSV assessed by the *ad hoc* Group are provided in Annex I.

**UNOFFICIAL VERSION**

**ASSESSMENT OF HOST SUSCEPTIBILITY TO INFECTION WITH VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUS (VHSV)**

The assessments for host susceptibility to infection with VHSV are provided in Table 2.

**Table 2.** Outcome of assessments for host susceptibility to infection with VHSV

Common name	Genus	Species	Genotype	Stage 1: Transmission	Stage 2: Pathogen identification	Stage 3: Evidence for infection				Outcome	References
						A	B	C	D		
<b>Score 1</b>											
American gizzard shad	<i>Dorosoma</i>	<i>cepedianum</i>	IVb	N	Virus isolation, RT-PCR, sequencing	ND	Y	Y	Y	1	Faisal 2012; USGS/NACSE database
Atlantic cod	<i>Gadus</i>	<i>morhua</i>	Ib, III	N	Viral isolation, cell culture and ELISA	ND	Y	Y	Y	1	Smail, 2000; Skall <i>et al.</i> , 2005
Atlantic herring	<i>Clupea</i>	<i>harengus</i>	Ib, III	N	Cell culture, ELISA, RT-PCR	ND	Y	N	Y	1	Dixon <i>et al.</i> , 1997; Mortensen <i>et al.</i> , 1999; King <i>et al.</i> , 2001a
Atlantic salmon	<i>Salmo</i>	<i>salar</i>	Ia, Ib, II, III, IVa	N, E	Cell culture, ELISA and RT-PCR, IHC	Y	Y	Y	Y	1	King <i>et al.</i> , 2001b, Lovy <i>et al.</i> , 2013
Atlantic stargazer	<i>Uranoscopus</i>	<i>scaber</i>	Ie	N	Cell culture, ELISA and PCR	ND	Y	N	Y	1	Ogut & Altuntas, 2014
Ballan wrasse	<i>Labrus</i>	<i>bergylta</i>	III	N	Virus isolation, ELISA, RT-PCR and sequencing	ND	Y	y	Y	1	Hall <i>et al.</i> , 2012; Munro <i>et al.</i> , 2015

Annex 15 (contd)Annex I (contd)

Common name	Genus	Species	Genotype	Stage 1: Transmission	Stage 2: Pathogen identification	Stage 3: Evidence for infection				Outcome	References
						A	B	C	D		
Bastard halibut	<i>Paralichthys</i>	<i>olivaceus</i>	IVa	N	Viral isolation, PCR, cell culture	ND	Y	ND	Y	1	Isshiki <i>et al.</i> , 2001; Takano <i>et al.</i> , 2000 and 2001
Black crappie	<i>Pomoxis</i>	<i>nigromaculatus</i>	IVb	N	Virus isolation, PCR, sequencing	ND	Y	ND	Y	1	Faisal, 2012; USGS/NACSE database
Blue Whiting	<i>Micromesistius</i>	<i>poutassou</i>	Ib, III	N	Cell culture, ELISA and PCR	N	Y	N	Y	1	Mortensen <i>et al.</i> , 1999; Brudeseth <i>et al.</i> , 2002
Bluegill	<i>Lepomis</i>	<i>macrochirus</i>	IV, IVb	N	Virus isolation RT-PCR, IHC	Y	Y	Y	Y	1	Al-Hussinee <i>et al.</i> , 2011; Department of Wisconsin Natural Resources, 2007
Bluntnose minnow	<i>Pimephales</i>	<i>notatus</i>	IVb	N	VI, RT-PCR	ND	Y	N	Y	1	Fratini, 2011; Department of Wisconsin Natural Resources, 2007
Brown bullhead	<i>Ictalurus</i>	<i>nebulosus</i>	IVb	N	Virus isolation, RT-PCR, sequencing	ND	Y	N	Y	1	Faisal <i>et al.</i> , 2012; USGS/NACSE database
Brown trout	<i>Salmo</i>	<i>trutta</i>	Ia, Ib	N	Virus isolation	ND	Y	Y	N	1	Ogut & Altunas, 2011; Jørgensen, 1980
Chinook salmon	<i>Oncorhynchus</i>	<i>tshawytscha</i>	IVa, IVb	N	Cell culture, sequencing	ND	Y	ND	Y	1	Faisal <i>et al.</i> , 2012; Garver <i>et al.</i> , 2013
Coho salmon	<i>Oncorhynchus</i>	<i>kisutch</i>	IVa	N	Cell culture, neutralization and immunoblot assay	ND	Y	N	Y	1	Winton <i>et al.</i> , 1989; Meyers & Winton, 1995

Annex 15 (contd)

Annex I (contd)

Common name	Genus	Species	Genotype	Stage 1: Transmission	Stage 2: Pathogen identification	Stage 3: Evidence for infection				Outcome	References
						A	B	C	D		
Common dab	<i>Limanda</i>	<i>limanda</i>	Ib	N	Cell culture, ELISA	ND	Y	N	Y	1	Skall <i>et al.</i> , 2005
Common whitefish	<i>Coregonus</i>	<i>lavaretus</i>	Ia	N/E	Virus isolation, ELISA, cell culture and neutralization	ND	Y	Y	Y	1	Meier <i>et al.</i> , 1986; Skall <i>et al.</i> , 2004
Corkwing wrasse	<i>Symphodus</i>	<i>melops</i>	III	N	Virus isolation, ELISA, RT-PCR and sequencing	ND	Y	y	Y	1	Hall <i>et al.</i> , 2012; Munro <i>et al.</i> , 2015
Cuckoo wrasse	<i>Labrus</i>	<i>mixtus</i>	III	N	Virus isolation, ELISA, RT-PCR and sequencing	ND	Y	y	Y	1	Hall <i>et al.</i> , 2012; Munro <i>et al.</i> , 2015
Emerald shiner	<i>Notropis</i>	<i>atherinoides</i>	IVb	N	Cell culture and PCR	ND	Y	Y	Y	1	Boonthai <i>et al.</i> , 2018
Eulachon	<i>Thaleichthys</i>	<i>pacificus</i>	IVa	N	Cell culture and RT-PCR	ND	Y	N	N	1	Hedrick <i>et al.</i> , 2003
European anchovy	<i>Engraulis</i>	<i>encrasicolus</i>	Ie	N	Cell culture, ELISA and PCR	ND	Y	N	Y	1	Ogut & Altuntas, 2014
European Flounder	<i>Platichthys</i>	<i>flesus</i>	Ib	N	Cell culture, ELISA	ND	Y	N	Y	1	Skall <i>et al.</i> , 2005

Annex 15 (contd)Annex I (contd)

Common name	Genus	Species	Genotype	Stage 1: Transmission	Stage 2: Pathogen identification	Stage 3: Evidence for infection				Outcome	References
						A	B	C	D		
European plaice	<i>Pleuronectes</i>	<i>platessa</i>	III	N	Cell culture, ELISA, sequencing	ND	Y	N	Y	1	Skall <i>et al.</i> , 2005; Wallace <i>et al.</i> , 2015
European sprat	<i>Sprattus</i>	<i>sprattus</i>	Ib	N	Cell culture, ELISA and PCR	N	Y	N	Y	1	Mortensen <i>et al.</i> , 1999; Skall <i>et al.</i> , 2005
Fathead Minnow	<i>Pimephales</i>	<i>promelas</i>	IVb	E	Virus isolation, RT-PCR, IHC	Y	Y	Y	Y	1	Al-Hussinee <i>et al.</i> , 2010
Freshwater drum	<i>Aplodinotus</i>	<i>grunniens</i>	IVb	N	Virus Isolation, Rt-PCR, IHC	Y	Y	Y	Y	1	Lumsden <i>et al.</i> , 2007; Al-Hussinee & Lumsden, 2011
Goldsinny wrasse	<i>Ctenolabrus</i>	<i>rupestris</i>	III	N/E	Virus isolation, ELISA, RT-PCR and sequencing	Y	Y	Y	Y	1	Munro et al 2015; Matejusova <i>et al.</i> , 2016
Grayling	<i>Thymallus</i>	<i>thymallus</i>	I	N/E	Cell culture, neutralization, IFAT	ND	Y	Y	N D	1	Meier & Wahli, 1988
Lake cisco	<i>Coregonus</i>	<i>artedi</i>	IVb	N/E	Cell culture, PCR/sequence	Y	Y	Y	Y	1	Weeks <i>et al.</i> , 2011; USGS/NACSE database
Lake trout	<i>Salvelinus</i>	<i>namaycush</i>	Ia, IVa, IVb	N/E	Virus isolation, sequencing	ND	Y	Y	Y	1	Dorson <i>et al.</i> , 1991; USGS/NACSE database
Lake whitefish	<i>Coregonus</i>	<i>clupeaformis</i>	IVb	N	Virus isolation, RT-PCR, sequencing	ND	Y	Y	Y	1	Faisal 2012; USGS/NACSE database
Largemouth bass	<i>Micropterus</i>	<i>salmoides</i>	IVb	N/E	Virus isolation, RT-PCR, sequencing	ND	Y	ND	Y	1	Faisal, 2012; Throckmorton <i>et al.</i> , 2017
Lumpfish	<i>Cyclopterus</i>	<i>lumpus</i>	IVd	N/E	Virus isolation, RT-PCR and sequencing	y	Y	Y	Y	1	Guðmundsdóttir <i>et al.</i> , 2018



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Annex I (contd)

Common name	Genus	Species	Genotype	Stage 1: Transmission	Stage 2: Pathogen identification	Stage 3: Evidence for infection				Outcome	References
						A	B	C	D		
Marble trout	<i>Salmo</i>	<i>marmoratus</i>	Ia	E	Cell culture and RT-PCR	ND	Y	Y	Y	1	Pascoli <i>et al.</i> , 2015
Mediterranean horse mackerel	<i>Trachurus</i>	<i>mediterraneus</i>	Ie	N	Cell culture, ELISA and PCR	ND	Y	N	Y	1	Ogut & Altuntas, 2014
Mummichog	<i>Fundulus</i>	<i>heteroclitus</i>	IVc	N	Virus identification, RT-PCR, sequencing, serum neutralization	ND	Y	Y	Y	1	Gagne <i>et al.</i> , 2007
Muskellunge	<i>Esox</i>	<i>masquinongy</i>	IVb	N/E	Virus isolation, RT-PCR, IHC, cell culture	Y	Y	Y	Y	1	Al-Hussinee & Lumsden, 2011; Kim & Faisal, 2012
Northern pike	<i>Esox</i>	<i>lucius</i>	IVb	N	Virus isolation, RT-PCR, sequencing	ND	Y	Y	Y	1	Faisal, 2012
Norway pout	<i>Trisopterus</i>	<i>esmarkii</i>	III, Ib	N	Cell culture, ELISA and PCR	N	Y	N	Y	1	Mortensen <i>et al.</i> , 1999; King <i>et al.</i> , 2001a
Pacific Chub mackerel	<i>Scomber</i>	<i>japonicus</i>	IVa	N	Cell culture RT-PCR	ND	Y	N	Y	1	Hedrick <i>et al.</i> , 2003
Pacific cod	<i>Gadus</i>	<i>macrocephalus</i>	IVa	N	Neutralization, immunoblot assay, DNA probe	ND	Y	N	Y	1	Meyers <i>et al.</i> , 1992; Meyers & Winton, 1995
Pacific herring	<i>Clupea</i>	<i>pallasii pallasii</i>	IVa	N	Cell culture and neutralisation	N	Y	Y	Y	1	Meyers <i>et al.</i> , 1993; Meyers <i>et al.</i> , 1994
Pacific sand lance	<i>Ammodytes</i>	<i>hexapterus</i>	IVa	N/E	Cell culture	Y	Y	Y	Y	1	Kocan <i>et al.</i> , 2001

Annex 15 (contd)Annex I (contd)

Common name	Genus	Species	Genotype	Stage 1: Transmission	Stage 2: Pathogen identification	Stage 3: Evidence for infection				Outcome	References
						A	B	C	D		
Pilchard	<i>Sardina</i>	<i>pilchardus</i>		N	Cell culture, ELISA and PCR	ND	Y	N	Y	1	Ogut & Altuntas, 2014
Pontic shad	<i>Alosa</i>	<i>immaculata</i>	le	N	Cell culture, ELISA and PCR	ND	Y	N	Y	1	Ogut & Altuntas, 2014
Pumpkinseed	<i>Lepomis</i>	<i>gibbosus</i>	IVb	N	Cell culture, RT- PCR	ND	Y	N	Y	1	Cornwell <i>et al.</i> , 2015
Rainbow trout	<i>Oncorhynchus</i>	<i>mykiss</i>	la-e, III, IVb	E	Virus isolation, RT-PCR, IHC	Y	Y	Y	Y	1	Dale <i>et al.</i> , 2009
Rainbow trout X coho salmon hybrids	<i>Oncorhynchus</i>	<i>mykiss X kisutch</i>	la	E	Cell culture	Y	Y	Y	Y	1	Ord <i>et al.</i> , 1976
Red mullet	<i>Mullus</i>	<i>barbatus</i>	le	N	Cell culture, ELISA and PCR	ND	Y	N	Y	1	Ogut & Altuntas, 2014
River lamprey	<i>Lampetra</i>	<i>fluviatilis</i>	II	N	VI, RT-PCR, sequencing	ND	Y	N	Y	1	Gadd <i>et al.</i> , 2010
Rock bass	<i>Ambloplites</i>	<i>rupestris</i>	IVb	N	Cell culture, RT- PCR	ND	Y	N	Y	1	Cornwell <i>et al.</i> , 2015
Rock cook wrasse	<i>Centrolabrus</i>	<i>exoletus</i>	III	N	Virus isolation, ELISA, RT-PCR and sequencing	ND	Y	y	Y	1	Hall <i>et al.</i> , 2012; Munro <i>et al.</i> , 2015
Round goby	<i>Neogobius</i>	<i>melanostomus</i>	IVb	N	Cell culture, RT- PCR	ND	Y	Y	Y	1	Groocock <i>et al.</i> , 2007
Sand goby	<i>Pomatoschistus</i>	<i>minutus</i>	lb	N	Cell culture ELISA	ND	Y	N	Y	1	Skall <i>et al.</i> , 2005a
Senegalese sole	<i>Solea</i>	<i>senegalensis</i>	III	N	Cell culture, ELISA and PCR	ND	Y	Y	Y	1	Lopez-Vazquez <i>et al.</i> , 2011

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Common name	Genus	Species	Genotype	Stage 1: Transmission	Stage 2: Pathogen identification	Stage 3: Evidence for infection				Outcome	References
						A	B	C	D		
Shiner perch	<i>Cymatogaster</i>	<i>aggregata</i>	IVa	N	Neutralization, IFAT	ND	Y	Y	Y	1	Meyers & Winton, 1995
Smallmouth bass	<i>Micropterus</i>	<i>dolomieu</i>	IVb	N	Virus isolation RT-PCR, IHC	Y	Y	Y	Y	1	Al-Hussinee <i>et al.</i> , 2011
South American pilchard	<i>Sardinops</i>	<i>sagax</i>	IVa	N/E	Cell culture PCR	ND	Y	Y	Y	1	Traxler <i>et al.</i> , 1999; Hedrick <i>et al.</i> , 2003
Spottail shiner	<i>Notropis</i>	<i>hudsonius</i>	IVb	N/IP	Virus isolation, PCR, sequencing	ND	Y	N	Y	1	Faisal, 2012
Striped bass	<i>Morone</i>	<i>saxatilis</i>	IVb, IVc	N	VI, RT-PCR, sequencing, serum neutralization	ND	Y	N	Y	1	Gagne <i>et al.</i> , 2007
Thornback ray	<i>Raja</i>	<i>clavata</i>	Ie	N	Cell culture, ELISA and PCR	ND	Y	N	Y	1	Ogut & Altuntas, 2014
Three-bearded rockling	<i>Gaidropsarus</i>	<i>vulgaris</i>	Ie	N	Cell culture, ELISA and PCR	ND	Y	N	Y	1	Ogut & Altuntas, 2014
Three-spine stickleback	<i>Gasterosteus</i>	<i>aculeatus</i>	IVc	N	VI, RT-PCR, sequencing, serum neutralization	ND	Y	Y	Y	1	Gagne <i>et al.</i> , 2007
Turbot	<i>Psetta</i>	<i>maxima</i>	Ib, III	N	Cell culture, ELISA and PCR	Y	Y	Y	Y	1	King <i>et al.</i> , 2001b; Snow & Smail, 1999

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Common name	Genus	Species	Genotype	Stage 1: Transmission	Stage 2: Pathogen identification	Stage 3: Evidence for infection				Outcome	References
						A	B	C	D		
Walleye	<i>Sander</i>	<i>vitreum</i>	IVb	N	Virus isolation, PCR, sequencing	ND	Y	Y	Y	1	Faisal, 2012
White Bass	<i>Morone</i>	<i>chrysops</i>	IVb	N	Virus isolation, sequencing	ND	Y	ND	Y	1	Bain <i>et al.</i> , 2010; USGS/NACSE database
White Perch	<i>Morone</i>	<i>americana</i>	IVb	N	qRT-PCR and cell culture	N	Y	N	Y	1	Bain <i>et al.</i> , 2010; USGS/NACSE database
Whiting	<i>Merlangius</i>	<i>merlangus</i>	le	N	Cell culture, ELISA and PCR	ND	Y	N	Y	1	Ogut & Altuntas, 2014
Yellow perch	<i>Perca</i>	<i>flavescens</i>	IVb	N	Virus isolation, qRT-PCR	ND	Y	Y	Y	1	Olson <i>et al.</i> , 2013
Zebra fish	<i>Danio</i>	<i>rerio</i>	IVa	E	VI, RT-PCR	ND	Y	Y	Y	1	Novoa <i>et al.</i> , 2006
<b>Score 2</b>											
Alaska pollock	<i>Theragra</i>	<i>chalcogramma</i>	IVa	N	Cell culture and PCR	ND	Y	Y	Y	2	Meyers <i>et al.</i> , 1999
Alewife	<i>Alosa</i>	<i>pseudoharengus</i>	IVb	N	RT-PCR	ND	N	N	Y	2	Cornwell <i>et al.</i> , 2015
Arctic charr	<i>Salvelinus</i>	<i>alpinus</i>	la	N	Virus isolation, IFAT	N	Y	N	Y	2	Knuesel <i>et al.</i> , 2003
Armoured cusk	<i>Hoplobrotula</i>	<i>armata</i>	IV	N	PCR	ND	N	N	Y	2	Lee <i>et al.</i> , 2007
Atlantic Halibut	<i>Hippoglossus</i>	<i>hippoglossus</i>	III	E	Cell culture and ELISA	ND	Y	Y	Y	2	Bowden <i>et al.</i> , 2003
Banded Killifish	<i>Fundulus</i>	<i>diaphanus</i>	IVb	N	qRT-PCR and cell culture	N	N	N	Y	2	Bain <i>et al.</i> , 2010
Black scorpionfish	<i>Scorpaena</i>	<i>porcus</i>	le	N	Cell culture, ELISA and PCR	ND	Y	N	Y	2	Ogut & Altuntas, 2014
Blackfin flounder	<i>Glyptocephalus</i>	<i>stelleri</i>	IVa	N	PCR	ND	N	N	Y	2	Lee <i>et al.</i> , 2007

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Common name	Genus	Species	Genotype	Stage 1: Transmission	Stage 2: Pathogen identification	Stage 3: Evidence for infection				Outcome	References
						A	B	C			
Brook trout	<i>Salvelinus</i>	<i>fontinalis</i>	le	E	Virus isolation, ELISA	ND	Y	N	N	2	Ogut & Altunas, 2011
Burbot	<i>Lota</i>	<i>lota</i>	IVb	N	Cell culture, sequencing	ND	Y	ND	Y	2	Department of Wisconsin Natural Resources, 2007
Channel catfish	<i>Ictalurus</i>	<i>punctatus</i>	IVb	N	Cell culture, sequencing	ND	Y	N	Y	2	USGS/NACSE database
Cloudy catshark	<i>Scyliorhinus</i>	<i>torazame</i>	IV	N	PCR	ND	N	N	Y	2	Lee <i>et al.</i> , 2007
Cubed snailfish	<i>Liparis</i>	<i>tessellatus</i>	IV	N	PCR	ND	N	N	Y	2	Lee <i>et al.</i> , 2007
European eel	<i>Anguilla</i>	<i>anguilla</i>	III	N	Viral isolation, neutralization test	ND	Y	ND	N D	2	Jorgensen <i>et al.</i> , 1994
European sea bass	<i>Dicentrarchus</i>	<i>labrax</i>	le	E	Cell cultivature and ELISA	ND	N	N	Y	2	Ogut & Altuntas 2014
Fallfish	<i>Semotilus</i>	<i>corporalis</i>	IVb	N	RT-PCR	ND	N	N	Y	2	Cornwell <i>et al.</i> , 2015
Flathead grey mullet	<i>Mugil</i>	<i>cephalus</i>	IVa	N	PCR	ND	N	N	Y	2	Lee <i>et al.</i> , 2007
Fourbeard rockling	<i>Enchelyopus</i>	<i>cimbrius</i>	Ib	N	Cell culture, ELISA	ND	Y	N	Y	2	Mortensen <i>et al.</i> , 1999
Garfish	<i>Belone</i>	<i>belone</i>	le	N	Cell culture, ELISA and PCR	ND	Y	N	Y	2	Ogut & Altuntas, 2014
Golden shiner	<i>Notemigonus</i>	<i>crysoleucas</i>	IVb	N	RT-PCR	ND	N	N	Y	2	Cornwell <i>et al.</i> , 2015
Gray gurnard	<i>Eutrigla</i>	<i>gurnardus</i>	III	N	Cell culture, ELISA, sequencing	ND	Y	N	Y	2	Wallace <i>et al.</i> , 2015
Greater amberjack	<i>Seriola</i>	<i>dumerili</i>	IVa	N	PCR, cell culture, IFAT	ND	Y	Y	Y	2	OIE, 2013

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Common name	Genus	Species	Genotype	Stage 1: Transmission	Stage 2: Pathogen identification	Stage 3: Evidence for infection				Outcome	References
						A	B	C			
Greenland halibut	<i>Reinhardtius</i>	<i>hippoglossoides</i>	III	N	Cell culture, IFAT, RT-PCR	ND	Y	N	Y	2	Dopazo <i>et al.</i> , 2002
Haddock	<i>Melanogrammus</i>	<i>aeglefinus</i>	III	N	Cell culture IFAT and ELISA	ND	Y	N	N	2	Smail, 2000
Izu scorpionfish	<i>Scorpaena</i>	<i>izensis</i>	IV	N	PCR	ND	N	N	Y	2	Lee <i>et al.</i> , 2007
Japanese fluvial sculpin	<i>Cottus</i>	<i>pollux</i>	IVb	E	RT-PCR and cell culture	ND	Y	Y	Y	2	Ito & Olesen, 2013
Japanese rice fish	<i>Oryzias</i>	<i>latipes</i>	IVb	E	RT-PCR and cell culture	ND	Y	Y	Y	2	Ito & Olesen, 2013
Largehead hairtail	<i>Trichiurus</i>	<i>lepturus</i>	IVa	N	PCR	ND	N	N	Y	2	Lee <i>et al.</i> , 2007
Lesser Argentine	<i>Argentina</i>	<i>sphyraena</i>	Ib	N	Cell culture, ELISA and PCR	N	Y	N	Y	2	Mortensen <i>et al.</i> , 1999
Marine medaka	<i>Oryzias</i>	<i>dancena</i>	IVa	E	Ref. strain FYoesu05	ND	Y	Y	N D	2	Wi-Sik Kim <i>et al.</i> , 2013 (Marine medaka)
North Pacific hake	<i>Merluccius</i>	<i>productus</i>	IVa	N	Cell culture and neutralisation	N	Y	Y	Y	2	Meyers <i>et al.</i> , 1999
Poor cod	<i>Trisopterus</i>	<i>minutus</i>	III	N	Virus isolation, cell culture and ELISA	ND	Y	ND	Y	2	King <i>et al.</i> , 2001a
Rainbow trout X arctic charr hybrids	<i>Oncorhynchus X Salvelinus</i>	<i>mykiss X alpinus</i>	Ia	E	Ref strains (07- 71, 34-86, 23-75)	ND	Y	Y	Y	2	Dorson <i>et al.</i> , 1991
Rainbow trout X lake trout hybrids	<i>Oncorhynchus X Salvelinus</i>	<i>mykiss X namaycush</i>	Ia	E	Ref strains (07- 71, 34-86, 23-75)	ND	ND	Y	N D	2	Dorson <i>et al.</i> , 1991
Rainbow trout X brown trout hybrids	<i>Oncorhynchus X Salmo</i>	<i>mykiss X trutta</i>	Ia	E	Ref strains (07- 71, 34-86, 23-75)	ND	N	Y	N	2	Dorson <i>et al.</i> , 1991

Annex 15 (contd)

Annex I (contd)

Common name	Genus	Species	Genotype	Stage 1: Transmission	Stage 2: Pathogen identification	Stage 3: Evidence for infection				Outcome	References
						A	B	C			
Sandeel	<i>Ammodytes</i>	<i>personatus</i>	lb	N	Cell culture ELISA	ND	Y	N	Y	2	Skall <i>et al.</i> , 2005
Shorthead redhorse	<i>Moxostoma</i>	<i>macrolepidotum</i>	IVb	E	Virus isolation, RT-PCR, sequencing	ND	Y	Y	Y	2	Bowser, 2009
Silver pomfret	<i>Pampus</i>	<i>argenteus</i>	IV	N	PCR	ND	N	N	Y	2	Lee <i>et al.</i> , 2007
Silver redhorse	<i>Moxostoma</i>	<i>anisurum</i>	IVb	N	Virus isolation, RT-PCR, sequencing	ND	Y	Y	N	2	Faisal, 2012
Silvery pout	<i>Gadiculus</i>	<i>argenteus</i>	lb	N	RT-PCR, sequencing	ND	N	N	Y	2	Sandlund <i>et al.</i> , 2014
Striped mullet	<i>Mugil</i>	<i>cephalus</i>	IVa	N	Viral isolation, PCR	ND	Y	ND	Y	2	Kim & Park, 2004
Surf smelt	<i>Hypomesus</i>	<i>pretiosus</i>	ND	N/E	Cell culture and RT-PCR	ND	Y	Y	Y	2	Hedrick <i>et al.</i> , 2003
Tiger muskellunge X Northern pike hybrids	<i>Esox</i>	<i>masquinongy X lucius</i>	IVb	N	Cell culture, PCR	ND	Y	N	Y	2	Getchell <i>et al.</i> , 2013
Trout Perch	<i>Percopsis</i>	<i>omiscomaycus</i>	IVb	N	Virus isolation, sequencing	ND	Y	ND	Y	2	USGS/NACSE database
White Crappie	<i>Pomoxis</i>	<i>annularis</i>	IVb	N	Virus isolation RT-PCR, IHC	Y	Y	Y	Y	2	Al-Hussinee <i>et al.</i> , 2011
White sucker	<i>Catostomus</i>	<i>commersonii</i>	IVb	N	RT-PCR, sequencing	ND	N	N	Y	2	Cornwell <i>et al.</i> , 2011
Yellow croaker	<i>Larimichthys</i>	<i>polyactis</i>	IV	N	PCR	ND	N	N	Y	2	Lee <i>et al.</i> , 2007
Yoshinobori (Japanese goby)	<i>Rhinogobius</i>	Sp. (undescribed species)	IVb	N	Virus isolation, RT-PCR	ND	Y	Y	Y	2	Ito & Olesen, 2013

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Common name	Genus	Species	Genotype	Stage 1: Transmission	Stage 2: Pathogen identification	Stage 3: Evidence for infection				Outcome	References
						A	B	C			
<b>Score 3</b>											
Black cod = Sablefish	<i>Anoplopoma</i>	<i>fimbria</i>		N	PCR	ND	ND	ND	Y	3	Hedrick <i>et al.</i> , 2003
<b>Score 4</b>											
No species were found to be non-susceptible to infection with VHSV											
<b>Vectors</b>											
Amphipod	<i>Hyalella</i>	<i>spp.</i>	IVb	N	rRT-PCR	ND	N	N	N	3	Throckmorton <i>et al.</i> , 2017
Amphipod	<i>Diporeia</i>	<i>ssp.</i>	IVb	N	Virus isolation, RT-PCR, sequencing	ND	Y	N	N	3	Faisal & Winters, 2011
Common snapping turtle	<i>Chelydra</i>	<i>serpentine</i>	IVb	IP/E	RT-PCR	ND	N	N	Y	2	Goodwin & Merry, 2011
Leech	<i>Myzobdella</i>	<i>lugubris</i>	IVb	N	Virus isolation, RT-PCR, sequencing	ND	Y	N	N	3	Faisal & Schultz, 2009
Northern map turtle	<i>Graptemys</i>	<i>geographica</i>	IVb	IP/E	Cell culture, RT-PCR	ND	Y	N	Y	2	Goodwin & Merry, 2011
Water flea	<i>Moina</i>	<i>macrocopa</i>	Ia	N	Cell culture and RT-PCR	N	Y	N	N	3	Ito & Olesen, 2017
<b>Species that were assessed but not scored due to insufficient or absence of scientific evidence</b>											
Black rockfish	<i>Sebastes</i>	<i>inermis</i>									
Blackhead seabream	<i>Acanthopagrus</i>	<i>schlegeli</i>									
Chum salmon	<i>Oncorhynchus</i>	<i>keta</i>									
English sole	<i>Parophrys</i>	<i>vetulus</i>									



Common name	Genus	Species	Genotype	Stage 1: Transmission	Stage 2: Pathogen identification	Stage 3: Evidence for infection			Outcome	References
						A	B	C		
Golden trout	<i>Oncorhynchus</i>	<i>aguabonita</i>								
Goldfish	<i>Carassius</i>	<i>auratus</i>								
Hong Kong grouper	<i>Epinephelus</i>	<i>akaara</i>								Not scored
Japanese amberjack	<i>Seriola</i>	<i>quinqueradiata</i>								Not scored
Korean rockfish	<i>Sebastes</i>	<i>schlegeli</i>								Not scored
Marbled flounder	<i>Pleuronectes</i>	<i>yokohamae</i>								Not scored
Pacific lamprey	<i>Entosphenus</i>	<i>tridentatus</i>								Not scored
Red sea bream	<i>Pagrus</i>	<i>major</i>								Not scored
Sea lamprey	<i>Petromyzon</i>	<i>marinus</i>								Not scored
Sockeye salmon	<i>Oncorhynchus</i>	<i>nerka</i>								Not scored
Splake: Hybride ( <i>Salvelinus</i> <i>namaycush</i> x <i>Salvelinus fontinalis</i> )	<i>Salvelinus</i>	<i>namaycush</i> X <i>fontinalis</i>								Not scored
Tube-snout	<i>Aulorhynchus</i>	<i>flavidus</i>								Not scored
Yellowback seabream	<i>Dentex</i>	<i>tumifrons</i>								Not scored

The common names of fish species are in line with FAOTERM (<http://www.fao.org/faoterm/collection/faoterm/en/>) and scientific fish names with the Fishbase (<https://www.fishbase.se/search.php>). Where the common fish name was not found in FAOTERM the species naming was done in line with the Fishbase.

Annex 15 (contd)

Annex I (contd)

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**REPORT OF THE OIE AD HOC GROUP ON SUSCEPTIBILITY  
OF FISH SPECIES TO INFECTION WITH OIE LISTED DISEASES**

**November 2018–September 2019**

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**UNOFFICIAL VERSION**

**REPORT OF OF THE OIE AD HOC GROUP ON SUSCEPTIBILITY OF  
FISH SPECIES TO INFECTION WITH OIE LISTED DISEASES  
November 2018–September 2019**

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**Terms of reference**

**Background**

A new Chapter 1.5. ‘Criteria for listing species as susceptible to infection with a specific pathogen’ was introduced in the 2014 edition of the *Aquatic Code*. The purpose of this chapter is to provide criteria for determining which host species are listed as susceptible in Article X.X.2. of each disease-specific chapter in the *Aquatic Code*. The criteria are to be applied progressively to each disease-specific chapter in the *Aquatic Code*.

These assessments will be undertaken by *ad hoc* Groups and the assessments will be provided to Member Countries for comment prior to any change in the list of susceptible species in Article X.X.2. of the disease-specific chapters in the *Aquatic Code*.

For species where there is some evidence of susceptibility but insufficient evidence to demonstrate susceptibility through the approach described in Article 1.5.3., information will be included in the relevant disease-specific chapter in the *Aquatic Manual*.

**Purpose**

The *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases will undertake assessments for the ten OIE listed fish diseases.

**Terms of Reference**

1. Consider evidence required to satisfy the criteria in Chapter 1.5.
2. Review relevant literature documenting susceptibility of species for OIE listed fish diseases.
3. Propose susceptible species for OIE listed diseases for fish based on Article 1.5.7.
4. Propose susceptible species for OIE listed diseases for fish based on Article 1.5.8.

**Expected outputs of the *ad hoc* Group**

1. Develop a list of susceptible species for inclusion in the relevant Article X.X.2. of fish disease-specific chapters in the *Aquatic Code*.
2. Develop a list of species with incomplete evidence for susceptibility for inclusion in Section 2.2.2. of the *Aquatic Manual*.
3. Draft a report for consideration by the Aquatic Animals Commission at their September 2019 meeting.



## ASSESSMENT OF HOST SUSCEPTIBILITY TO INFECTION WITH SPRING VIRAEMIA OF CARP VIRUS – ZEBRAFISH (*Danio rerio*)

### Background

The zebrafish (*Danio rerio*) was found not to meet the criteria to be listed as susceptible to infection with spring viraemia of carp virus when it was assessed in 2018. This was done by the *ad hoc* Group on Susceptibility of fish species to infection with OIE-listed diseases and the Aquatic Animals Commission in accordance with the criteria in Chapter 1.5. Criteria for listing species as susceptible to infection with a specific pathogen. It was considered that the conditions of experimental challenge did not meet the requirements of Article 1.5.7. concerning evidence of a route of transmission that is consistent with natural pathways for the infection. The study considered for this assessment had used temperatures outside of the natural range of zebrafish and the authors had acknowledged that this had likely resulted in immunosuppression. The conclusion was to include zebrafish in Section 2.2.2. of the Aquatic Manual and further evidence could be considered on this issue if it becomes available.

Before its September 2019 meeting the Commission had asked the *ad hoc* Group to re-assess the species, considering that new studies were available. The Commission found that, based on new available scientific evidence, zebrafish now met the criteria for listing as susceptible species as described in Chapter 1.5. It proposed the species to be added to Article 10.9.2. Scope.

### Assessment

Criteria for susceptibility to infection with spring viraemia of carp virus (SVCV) are detailed in Table 1 (as per Article 1.5.6. of the *Aquatic Code*). This table includes Replication (A), Viability/Infectivity (B), Pathology/Clinical Signs (C) and Location (D). Hosts were considered to be infected with SVCV if they fulfilled either criterion A, or at least two of criteria B, C and D (as per point 3 of Article 1.5.7. of the *Aquatic Code*).

### Criteria for susceptibility to infection with SVCV

**Stage 1:** Natural infection (e.g. disease outbreak) or experimental transmission by cohabitation, immersion, feeding (injection not appropriate).

**Stage 2:** Virus isolation followed by serological test using validated antisera (serum neutralisation test) or RT-PCR + sequencing.

#### OIE definition of confirmed case:

The presence of SVCV should be **suspected** if at least one of the following criteria are met:

- Presence of rapid and significant mortalities in susceptible fish species;
- Presence of typical clinical signs of the disease in susceptible fish species;
- Presence of typical histopathology;
- Virus isolation with typical CPE.

The presence of SVCV should be considered as **confirmed** if the following criteria are met:

Virus isolation with typical CPE and positive for serological tests using validated antisera;

OR

Virus isolation with typical CPE and positive for RT-PCR using extracted RNA from isolated virus as template and sequencing.

## Annex 16 (contd)

**Table 1.** Criteria for susceptibility to infection with SVCV (Stage 3)

<b>A: Replication</b>	<b>B: Viability/ Infectivity</b>	<b>C: Pathology/Clinical signs</b>	<b>D: Location</b>
<p>Sequential virus titration showing increase in viral titres or high virus titres in organs (&gt;10<sup>5</sup> TCID<sub>50</sub>/g);</p> <p>OR</p> <p>Demonstration of increasing copy number over time by qPCR with confirmatory PCR/sequencing;</p> <p>OR</p> <p>TEM showing virions in host cells;</p> <p>OR</p> <p>Products (e.g. antigens) of virus replication detected.</p>	<p>Isolation by cell culture.</p> <p>OR</p> <p>Passage to a susceptible host with infection confirmed by PCR/sequencing and demonstrating at least two of the following: i. clinical signs, with or without associated mortality, ii. Histopathology, iii. Re-isolation of virus in cell culture.</p>	<p>Typical clinical signs include exophthalmia, pale gills, haemorrhages on the skin, base of the fins and the vent, abdominal swelling, ascites and a protruding vent (anus), often with trailing mucoid faecal casts.</p> <p>Necrosis and degeneration in major organs.</p>	<p>High titre in liver, heart and kidney.</p> <p>Lower titre in spleen, gills and brain.</p> <p>As a systemic infection virus will be located in all tissues.</p>

For this assessment only virus isolates identified as SVCV according to Stone *et al.* 2003 (genotype 1) are regarded as SVCV.

**ASSESSMENT FOR HOST SUSCEPTIBILITY**

The assessment for host susceptibility of zebrafish (*Danio rerio*) to infection with SVCV is provided in Table 2.

**Table 2.** Outcome of assessment for host susceptibility to infection with SVCV

Common name	Genus	Species	STAGE 1: Transmission	STAGE 2: Pathogen identification	STAGE 3: Evidence of infection				Outcome	References
					A	B	C	D		
Zebrafish	<i>Danio</i>	<i>rerio</i>	E	Ref strain 56/70, RT-PCR	ND	Y	Y	Y	1*	Sanders <i>et al.</i> , 2003
Zebrafish	<i>Danio</i>	<i>rerio</i>	E	Ref strain ATCCVR-1390; RT-PCR, VI	ND	ND	Y	Y	1	Lopez-Munoz <i>et al.</i> , 2010
Zebrafish	<i>Danio</i>	<i>rerio</i>	E	Ref strain 56/70, RT-PCR	Y	Y	Y	Y	1	Martinez-Lopez <i>et al.</i> , 2014
Zebrafish	<i>Danio</i>	<i>rerio</i>	E	Ref strain 56/70, RT-PCR	Y	ND	ND	ND	1	Medina-Gali <i>et al.</i> , 2018a
Zebrafish	<i>Danio</i>	<i>rerio</i>	E	Ref strain 56/70, RT-PCR	Y	ND	Y	ND	1	Medina-Gali <i>et al.</i> , 2018b
Zebrafish	<i>Danio</i>	<i>rerio</i>	E	Ref strain 56/70, RT-PCR	Y	ND	Y	Y	1	Bello-Perez <i>et al.</i> , 2019

\*For this species the conditions of experimental challenge did not meet the requirements of Article 1.5.7. concerning evidence of a route of transmission that is consistent with natural pathways for the infection. The study considered for this assessment had used temperatures outside of the natural range of zebrafish and the authors had acknowledged that this had likely resulted in immunosuppression.

Annex 16 (contd)

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**EU Comment:**

**Thank you for providing the revised workplan for 2020.**

**We welcome the establishment of an *ad hoc* group to assess the susceptible species for listed mollusc diseases and we note the order in which it is proposed to work on this list. Given that we are keen to examine the outcome of these deliberations well in advance of the application of Regulation 2016(EU) 429 with a view to aligning the EU and OIE lists where this is possible, we would request that the following diseases might be assessed as a priority:**

**Infection with *B.exitiosa***

**Infection with *B.ostreae***

**Infection with *M.refringens***

**Infection with *P.marinus***

**We note also, the workplan for the assessment of susceptible species for fish and crustacean diseases and for the same reason as outlined above, we would request that the following diseases might be added to the workplan as a priority:**

**Infection with whitespot syndrome virus**

**Epizootic haematopoietic necrosis**

**Koi herpes virus disease**

**Should you be willing to facilitate this request, we would be grateful if the new lists of susceptible species could be made available by September 2020, to allow sufficient time for us to examine them and to make changes to the current legislation, should that be necessary.**

<b>AQUATIC CODE</b>		
<b>Chapter/Subject</b>	<b>Activity</b>	<b>Status September 2020</b>
<b>Section 1</b>	Reviewing Chapter 1.3. Diseases listed by the OIE to decide if work should be initiated to either assess new diseases for listing or to de-list diseases	Commission to review at all meetings
<b>Glossary, Sections 1 and 8-11</b>	Explore improvements to the standards of the <i>Aquatic Code</i> for demonstration of freedom from OIE listed diseases. These standards are provided through several interacting parts of the <i>Aquatic Code</i> , for example: Articles X.X.4. (free country) and X.X.5. (free zone or compartment) of each disease-specific chapter (except Infection with ISAV, for which numbering differs); Chapter 1.4. on <i>Aquatic animal health surveillance</i> ;	September 2018 – Discussion paper on approaches for determining periods required to demonstrate disease freedom first circulated for Member comments. September 2019 – The discussion paper to be revised by following consideration of Member comments and provided for further comment. February 2020 – Commission to consider revised articles of disease-specific chapters and revised structure for Chapter 1.4.

	and relevant definitions in the glossary (e.g. basic biosecurity conditions and early detection system)	
<b>Section 4. Disease prevention and Control</b>	Finalising new chapter on Biosecurity for Aquaculture Establishments (Chapter 4.X.)	September 2019 – The draft chapter circulated for Member Comments for the 3 <sup>rd</sup> time. February 2020 – expected to be proposed for adoption in May 2020.
	Draft new chapters on emergency disease preparedness (Chapter 4.X.) and on disease outbreak management (Chapter 4.X.)	February 2020 – Secretariat and Commission to consider scoping documents on the structure and content of these chapters.
	Revision of Chapters 4.2., 4.7. and 4.8. for alignment with Chapter 4.X. Biosecurity for Aquaculture Establishments.	September 2020 – Necessary revisions to be considered following adoption of Chapter 4.X. on Biosecurity for Aquaculture Establishments.
	Draft new chapter on application of zoning	Yet to be prioritised
<b>Section 5. Trade measures</b>	Draft new chapter on ornamental aquatic animals	September 2020 – Commission to consider scoping document on the structure and content of this chapter
	Draft new standards on trade in genetic material	February 2021 – Commission to consider scoping document on the structure and content of this chapter
<b>Section 6. Antimicrobial use in Aquatic Animals</b>	Consider the next steps of the work on antimicrobial use in aquatic animals in light of the new OIE work plan	February 2020 – For Commission discussion
<b>Section 8. Diseases of Amphibians</b>	Update list of susceptible species in Article X.X.2 of disease-specific chapters	Yet to be prioritised
<b>Section 9. Diseases of Crustaceans</b>	Update list of susceptible species in Article 9.8.2. of Chapter 9.8. Infection with white spot syndrome virus	Yet to be prioritised

## Annex 17 (contd)

<b>Section 9. Diseases of Crustaceans</b>	Update list of susceptible species in Article 9.2.2. of Chapter 9.2. Infection with <i>Aphanomyces astaci</i> (Crayfish plague)	Yet to be prioritised
<b>Section 10. Diseases of Fish</b>	Update list of susceptible species in Article 10.10.2. of Chapter 10.10. Infection with viral haemorrhagic septicaemia virus	September 2020 – amended Article circulated for Member comments
	Update list of susceptible species in Article 10.8.2. of Chapter 10.8. Infection with red sea bream iridovirus (and infectious Spleen and kidney Necrosis Virus (ISKNV))	Yet to be prioritised
	Update list of susceptible species in Article 10.2.2. of Chapter 10.2. Infection with <i>Aphanomyces invadans</i> (Epizootic ulcerative syndrome)	Yet to be prioritised
<b>Section 11. Diseases of Molluscs</b>	Update list of susceptible species in Article X.X.2 of disease-specific chapters	An <i>ad hoc</i> Group has been established and will have its first two meetings in December 2019 and January 2020. They will assess susceptible species for the different diseases in the following order: <ul style="list-style-type: none"> <li>– Infection with <i>Bonamia exitiosa</i></li> <li>– Infection with <i>Bonamia ostreae</i></li> <li>– Infection with abalone herpesvirus</li> <li>– Infection with <i>Xenohaliotis californiensis</i></li> <li>– Infection with <i>Marteilia refringens</i></li> <li>– Infection with <i>Perkinsus marinus</i></li> <li>– Infection with <i>Perkinsus olseni</i></li> </ul> February 2020 – Commission to consider <i>ad hoc</i> Group report and propose amendments to relevant <i>Aquatic Code</i> and <i>Aquatic Manual</i> chapters
<b>Section 8-11: improving standards</b>	Review structure and application for different trade purposes of articles in disease-specific chapters	September 2020 – Proposed structure to be presented for Member comments in the Commission report
	Safe commodities: review the structure of Article X.X.3. of all disease specific chapters	Review structure at the February 2020 meeting and send for Member comments
<b>AQUATIC MANUAL</b>		
<b>Part 1 – General provisions</b>	Review and update introductory text to the disease-specific chapters	February 2020
	The use of environmental DNA methods for aquatic animal disease surveillance	February 2020 – Commission to consider the need for standards to validate the performance of environmental DNA methods
<b>Part 2 – Update and reformat disease chapters using the new template.</b>	Chapter 2.3.9. Spring viraemia of carp	September 2020 – circulated for Member comments for the second time and expected to be proposed for adoption in May 2020

<b>Part 2 – Update and reformat disease chapters using the new template (contd).</b>	New draft Chapter 2.1.X. – Infection with <i>Batrachochytrium salamandrivorans</i>	September 2020 – Circulated for Member comments twice and expected to be proposed for adoption in May 2020
	Chapter 2.3.2. Infection with <i>Aphanomyces invadans</i> (epizootic ulcerative syndrome)	February 2020 – revised draft to be reviewed by the Commission
	Chapter 2.3.3. Infection with <i>Gyrodactylus salaris</i>	February 2020 – revised draft to be reviewed by the Commission
	Chapter 2.3.4. Infection with infectious haematopoietic necrosis virus	September 2019 – revised draft circulated for the first time for Member comments
	Chapter 2.3.6. Infection with salmonid alphavirus	February 2020 – revised draft to be reviewed by the Commission
	Chapter 2.3.7. Infection with koi herpesvirus	February 2020 – revised draft to be reviewed by the Commission
	Chapter 2.3.10. Viral haemorrhagic septicaemia	September 2019 – revised draft circulated for the first time for Member comments
<b>Ad hoc Groups</b>		
	<i>Ad hoc</i> Group on Susceptibility of fish species to infection with OIE listed diseases	Remaining work: assessments of fish species susceptible to infection with red sea bream iridovirus (and infectious spleen and kidney necrosis virus) and infection with <i>Aphanomyces invadans</i> (Epizootic ulcerative syndrome) September 2020 – Commission to consider <i>Ad hoc</i> Group report and propose revised texts for comment
	Electronic <i>ad hoc</i> Group on tilapia lake virus	Was established in December 2017 and is expected to finalise its work in 2020 September 2019 – Commission consider an update from the <i>Ad hoc</i> Group on progress to assess the performance of available assays
	<i>Ad hoc</i> Group on Susceptibility of mollusc species to infection with OIE listed diseases	Will have its first two meetings in December 2019 and January 2020



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