Original: English
September 2021

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

Virtual meeting, 22–29 September 2021

The OIE Aquatic Animal Health Standards Commission (the Aquatic Animals Commission) held its meeting electronically from 22 to 29 September 2021. The list of participants is attached as **Annex 1**.

The Aquatic Animals Commission wished to thank the following 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 in Part B of the Commission's February 2021 report: Australia, Bangladesh, Canada, Chile, China (People's Republic of), Chinese Taipei, Japan, Korea (Republic of), New Zealand, Thailand, United Kingdom (the UK), United States of America (the USA), the African Union Interafrican Bureau for Animal Resources (AU-IBAR) on behalf of African Member Countries of the OIE, the Permanent Veterinary Committee of the Southern Cone (CVP) on behalf of Argentina, Bolivia, Brazil, Chile, Paraguay and Uruguay, and the Member States of the European Union (EU). The Commission also wished to acknowledge the valuable advice and contributions from numerous experts of the OIE scientific network.

The Commission reviewed all comments that were submitted on time and were supported by a rationale. The Commission made amendments to draft texts, where relevant, in the usual manner by '<u>double underline</u>' and '~~strikethrough~~'. In relevant Annexes, amendments proposed at this meeting are highlighted with a coloured background in order to distinguish them from those made previously. Due to the large number of comments, the Commission was not able to provide a detailed explanation for the reasons for accepting or not each of the comments considered, and focused its explanations on significant issues. Where amendments were of an editorial nature, no explanatory text has been provided. The Commission wished to note that not all texts proposed by Members to improve clarity were accepted; in these cases, it considered the text clear as currently written.

The Aquatic Animals Commission informed Members that *ad hoc* Group reports would no longer be annexed to its report. Instead a hyperlink will be provided for relevant *ad hoc* Group reports that will take the reader to the dedicated webpages for all *ad hoc* Group reports on the OIE Website. The Commission encourages Members to consider relevant information in previous Commission and *ad hoc* Group reports when preparing comments, especially on longstanding issues.

The table of contents includes all of the agenda items addressed by the Aquatic Animals Commission at this meeting and includes links to relevant items within this report.

Texts in **Annexes 2 to 24** are presented for Member comments.

Comments on relevant texts in this report must reach OIE Headquarters by the **<u>9 January 2022</u>** to be considered at the February 2022 meeting of the Aquatic Animals Commission.

All comments should be sent to the OIE Standards Department at: AAC.Secretariat@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 presented in the relevant Annex, and include any amendments to the proposed text, 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 may be lost in the process of collating Members' submissions into 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 Aquatic Animals Commission strongly encourages Members to participate in the development of the OIE's international standards by submitting comments on this report.

Agenda

1. MEETING WITH THE DEPUTY DIRECTOR GENERAL

Dr Matthew Stone, OIE Deputy Director General International Standards and Science, welcomed the Aquatic Animals Commission and congratulated members on their election. Dr Stone together with Dr Gillian Mylrea, Head of the Standards Department, conducted an induction session at the start of the meeting. This was the final session of the Specialist Commission induction programme. In previous months induction sessions had been conducted for new Commission members, Presidents and all Commission members and secretariats, to meet each other and share information relevant to this new term.

During this induction session, Dr Stone presented, for the consideration of members, a discussion on managing the workload, roles and responsibilities, process innovation, and the performance management system.

Dr Stone recalled that the February 2021 Commission reports had been produced in two parts, A (texts for adoption) and B (texts for comments and information) to ensure early publication of texts that were to be proposed for adoption ahead of the virtual General Session. He noted that the OIE will continue with this approach in 2022. Dr Stone also recalled that Pre-General Session webinars hosted by Commission members to explain the standards being proposed for adoption were well received and will be repeated in the future. Dr Stone also encouraged Commission members to conduct webinars in their respective regions for Delegates and relevant Focal Points after the September meeting to explain decisions made.

Dr Stone also informed the Aquatic Animals Commission that the OIE will undertake a one-year pilot study of an online commenting system for the collation and review of Member comments. Dr Stone explained that this pilot study will be applied to the work of the Aquatic Animals Commission using a small number of Members to assess whether the system should be applied to all Specialist Commissions.

Dr Mylrea facilitated a short session on agreed ways of working in which members discussed expectations and how they would like to work as a group in the coming 3 years. The President of the Aquatic Animals Commission, Dr Ingo Ernst, shared his expectations for the new term, and acknowledged the excellent support provided to them by the OIE Secretariat.

2. MEETING WITH THE DIRECTOR GENERAL

Dr Monique Eloit, the OIE Director General, met the Aquatic Animals Commission on 28 September 2021 and congratulated the new and re-elected members of the Commission. Dr Eloit provided an update on progress in the implementation of the 7th OIE Strategic Plan and highlighted one example of new work that will be undertaken to assess the OIE science system including OIE Reference Centres and expertise in OIE *ad hoc* Groups, Working Groups, and how the OIE can ensure the best use of these networks of experts. Dr Eloit also acknowledged the large workload of the Commission and highlighted that prioritisation of its work programme is critical during this coming period.

The members of the Commission congratulated Dr Eloit for her election for a second term as OIE Director General and expressed the commitment of the Commission to support the achievement of OIE objectives. Dr Ernst highlighted the importance of the new OIE Aquatic Animal Health Strategy that was launched at the 88th General Session in May 2021. In particular, he emphasised the need to continue to strengthen OIE resources and expertise to support Members to improve aquatic animal health and welfare to keep pace with the rapid growth of aquatic animal production and the increased risk of diseases.

3. COOPERATION WITH OTHER SPECIALIST COMMISSIONS

The Aquatic Animals Commission and the Terrestrial Animal Health Standards Commission (the Code Commission) continued to work together to coordinate their respective work on the revision of the glossary definitions for ‘Competent Authority’, ‘Veterinary Authority’ and ‘Aquatic Animal Health Services’ in the *Aquatic Code* with the glossary definitions for ‘Competent Authority’, ‘Veterinary Authority’ and ‘Veterinary

Services' in the *Terrestrial Code*, noting the importance of ensuring alignment of these definitions, except where differences are required (see Item 5.1.2.2. for more details).

As part of the discussion of the next steps for the revision of Section 4 of the *Aquatic Code*, the OIE Secretariat provided the Aquatic Animals Commission with a summary report of relevant work completed or planned by the Code Commission. The Aquatic Animals Commission appreciated this information and acknowledged that some of the work of the Code Commission may be helpful for its work given that many of the topics addressed in Section 4 are relevant for both the aquatic and terrestrial domains.

The Aquatic Animals Commission also discussed the need for a review of some chapters in Section 5 and the importance of coordinating this work with parallel work being considered by the Code Commission.

The Aquatic Animals Commission will continue to work with the Code Commission on relevant items in their respective work programmes to share information and ensure alignment, as appropriate.

4. WORK PLAN OF THE AQUATIC ANIMALS COMMISSION

The Aquatic Animals Commission considered ongoing work plan items at this meeting and the anticipated milestones for their completion. The Commission agreed to convene an additional meeting to review and prioritise new work. Any new work that is prioritised will be added to the workplan and provided for Member comments in the Commission's February 2022 report. Prioritisation of any new work will take into account multiple factors including the expected improvement to standards, benefit to Members, Member comments, activities of the OIE Aquatic Animal Health Strategy, capacity constraints, Headquarters' comments, and progress on the previous Commission's work plan.

The work plan of the Aquatic Animal Commission is presented as **Annex 2** for Member comments.

5. THE OIE *AQUATIC ANIMAL HEALTH CODE*

5.1. Texts for Member comment

5.1.1. User's Guide

The Aquatic Animals Commission amended the User's Guide to include Chapter 4.1. Biosecurity for aquaculture establishments, that was adopted at the 88th General Session, to ensure that it was consistent with the 2021 version of the *Aquatic Code*. The Commission also proposed some additional amendments to improve readability.

The revised User's Guide is presented as **Annex 3** for Member comments.

5.1.2. Glossary definitions:

5.1.2.1. 'Basic biosecurity conditions', 'Early detection system', 'Passive surveillance' and 'Biosecurity plan'

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

Background

In its September 2020 report, the Aquatic Animals Commission proposed amendments to the Glossary definitions for 'Basic biosecurity conditions' and 'Early detection system'. It also proposed a new Glossary definition for 'Passive surveillance' to ensure alignment with proposed amendments to Chapter 1.4. Aquatic Animal Health Surveillance.

Previous Commission reports where this item was discussed:

February 2021 report (Part B: Item 1.1., page 3).

September 2021

The Commission did not agree with a comment to add a new definition for 'Active surveillance' to the Glossary as active surveillance had been removed from the amended Chapter 1.4. Aquatic Animal Health Surveillance.

Basic biosecurity conditions

The Commission did not agree to remove the reference to 'a particular disease' from the definition as the application of the standards is on a disease basis. The Commission did, however, agree to replace 'particular' with 'specific' in the same paragraph for improved clarity.

The Commission did not agree with a comment to add 'aquaculture establishments' as this definition applies at a higher level than an establishment.

Biosecurity plan

During the Commission's review of the model Articles X.X.4.–X.X.8., it agreed that a reference to Chapter 4.1. Biosecurity for aquaculture establishments, should be included in the definition of 'Biosecurity plan' given that Chapter 4.1. provides specific recommendations for development of a biosecurity plan.

Early detection system

In the first sentence, the Commission agreed with comments to delete 'an efficient' and to replace 'for ensuring' with 'which ensure' for improved clarity.

The Commission agreed to delete 'diagnostic' before 'investigation' in the last sentence, as an investigation may extend beyond diagnostic activities.

Passive surveillance

The Commission agreed with comments to amend this definition to include more details on observational aquatic animal health surveillance information generated by an early detection system.

The revised Glossary definitions for 'Basic biosecurity conditions', 'Biosecurity plan', 'Early detection system' and 'Passive surveillance' are presented as **Annex 4** for Member comments.

5.1.2.2. 'Competent Authority', 'Veterinary Authority' and 'Aquatic Animals Health Services'

Comments on the Commission's September 2020 report were received from Armenia, Canada, Chinese Taipei, Cuba, New Caledonia, Switzerland, Thailand, the UK and the EU.

Background

In September 2018, the Terrestrial Animal Health Standards Commission (the Code Commission) agreed to revise the Glossary definitions for 'Competent Authority', 'Veterinary Authority' and 'Veterinary Services' in the *Terrestrial Code* following Member requests and feedback from the *ad hoc* Group on Veterinary Services (2018 report). The revised definitions were circulated for comments in the Code Commission's September 2018 report. The *ad hoc* Group on Veterinary

Services considered the comments submitted in June 2019 and proposed revised definitions. The Code Commission and the Aquatic Animals Commission agreed to discuss the proposed amendments to ensure alignment between the *Aquatic Code* and *Terrestrial Code*, where relevant. The revised Glossary definitions for Competent Authority', 'Veterinary Authority' and 'Veterinary Services' in the *Terrestrial Code* and Competent Authority', 'Veterinary Authority' and 'Aquatic Animal Health Services' in the *Aquatic Code* were circulated for comments in the September 2020 reports of the Code Commission and the Aquatic Animals Commission, respectively.

In preparation for the September 2021 meeting, the Presidents of the two Commissions met to review all comments received and to consider if additional amendments were needed whilst also considering the importance of aligning these definitions, where relevant. They acknowledged that the comments received indicated some confusion amongst Members as to the intended meaning and use of these terms and that their September 2020 Commission reports did not provide sufficient information about the rationale for the proposed amendments. The Presidents agreed that the proposed definitions did not need significant changes and they proposed to provide a more detailed explanation of the rationale for the proposed amendments in the September 2021 Commission reports, as well as some more detailed information on the use of these terms in each Code.

At the September 2021 meeting, each President informed its respective Commission about these discussions and sought input and agreement from Commission members.

Previous Commission reports where this item was discussed:

September 2020 (Item 4.5.3., page 9).

September 2021 meeting

The Aquatic Animals Commission considered the comments received on its September 2020 report as well as the feedback from the President regarding discussion with the Code Commission President, and the outcome of the Code Commission's discussions at its September 2021 meeting. The Code Commission had agreed that the proposed definitions did not need further edits and that its September 2021 report should include a more detailed explanation on the purpose and current use of these definitions, as well as a clearer explanation of the proposed changes.

The text presented below reflects the opinion of both Commissions and is presented in the September 2021 reports of the Aquatic Animals Commission and Code Commission to ensure a shared understanding in the context of both Codes.

General consideration on Glossary definitions

The objective of the glossaries in the *Aquatic* and *Terrestrial Codes* is to provide definitions of key terms that require precise interpretation for the purpose of their use in the Codes. These definitions might deviate from those provided by common dictionary definitions. It is desirable to pursue harmonisation where possible to assist interpretation by users of both Codes. Glossary terms should be used consistently throughout all chapters.

The Glossary definitions are expected to be concise and should not contain unnecessary descriptive detail or further elaborations beyond what is necessary to define the term. Further descriptive detail or explanation that may be necessary for the implementation of a standard are provided within the contents of the relevant chapters.

Purpose of the definitions of 'Competent Authority', 'Veterinary Authority' and 'Veterinary Services'/'Aquatic Animal Health Services'

The purpose of these terms in the Codes is to differentiate responsibilities for implementation of the OIE standards. It is important to note that the definitions apply only for the purposes of each of the Codes and are not intended to dictate the administrative structure, or the naming of governmental authorities, within a Member Country. To achieve this purpose, the definitions must be applicable to the diversity of administrative arrangements among Members and must be sufficiently precise to provide clarity on the responsibilities for the implementation of the standards by relevant governmental authorities or public or private services.

Current application of these definitions

The *Aquatic Code* currently uses the terms 'Competent Authority' and 'Aquatic Animal Health Services' but uses 'Veterinary Authority' only in certain Glossary definitions and in Section 5, Trade measures, importation/exportation procedures and health certification. This approach was previously adopted (i.e. 'Competent Authority' in place of 'Veterinary Authority') because governmental responsibilities for aquatic animal health and welfare are not necessarily the responsibility of a veterinary governmental authority/agency. The Aquatic Animals Commission is aware that there are currently some inconsistent and incorrect uses of the terms within the *Aquatic Code*. Proposals to address these issues will be made and proposed for comments once the revised definitions have been adopted.

The *Terrestrial Code* uses the three terms extensively ('Competent Authority', 'Veterinary Authority' and 'Veterinary Services') across its different sections. The Code Commission considers that these terms are generally applied correctly in the *Terrestrial Code*, as explained above, and in line with the relevant horizontal recommendations in Section 3. Veterinary Services, notably Chapter 3.4. on Veterinary legislation. However, the use of the terms will be reviewed once the revised definitions have been adopted.

Proposed changes to the definitions of 'Competent Authority', 'Veterinary Authority' and 'Veterinary Services' / 'Aquatic Animal Health Services'

A decision was made to revise these definitions because many users found they lacked clarity, which led to contradicting interpretations among Members, with significant discrepancies in the understanding of the terms. It is important to note that the changes proposed to the definitions are not intended to change their meaning or application, only to bring clarity.

Some cross-references between the Codes within these definitions have been removed because they are irrelevant (e.g. references to the *Aquatic Code* within definitions in the *Terrestrial Code*).

a) Competent Authority

The proposed wording recognises that, in many countries, more than one governmental authority is responsible for implementing standards of the *Terrestrial* or *Aquatic Codes*. The term *Competent Authority* is intended to apply to any governmental authority with some responsibility for the implementation of some OIE standards.

Key changes to the definitions include:

- *'responsibility … for implementation' was* deemed simpler, clearer language than the current reference to 'competence for ensuring implementation';
- '*in the whole or part of the territory'* reflects that under some administrative arrangements government authorities may have responsibility for certain standards over the whole territory of a country, or just over a part of it, e.g. provincial or state authorities;
- '*certain standards'* reflects that governmental authorities may have responsibility for a clearly defined area of standards. Responsibility for implementation of other standards of the *Codes* would be part of the mandate of different Competent Authorities within the same country.

These revisions are consistent with Article 3.4.5. Competent Authorities, of the *Terrestrial Code*. There is no equivalent chapter on Veterinary Legislation within the *Aquatic Code*.

b) Veterinary Authority

The level of detail in the existing definition was deemed unnecessary, and the definition was simplified to make it clearer. This term distinguishes the role of a single Competent Authority that has responsibility for communicating with the OIE and an overarching responsibility for implementation of OIE standards. Examples of the differentiated role for a Veterinary Authority include disease notification requirements or demonstrating compliance with international standards for international trade or for disease free status.

The Aquatic Animals Commission agreed that it was necessary to include reference to coordinating the implementation of standards 'by Competent Authorities' in the Glossary definition of 'Veterinary Authority' for the purpose of the *Aquatic Code*. These words add clarity given that 'Competent Authority' is the primary term used within the *Aquatic Code* (refer to the section 'current application of the definitions' above) and also reflects the fact that the Veterinary Authority itself may not always be the Competent Authority with responsibility for the implementation of specific standards of the *Aquatic Code*. The Code Commission did not consider this to be necessary in the definition for Veterinary Authority in the *Terrestrial Code*.

Key changes to the definitions include:

- '*comprising veterinarians, other professionals and paraprofessionals'* was removed as these words do not define the term and do not distinguish it from other governmental authorities;
- '*primary responsibility'* was included to distinguish the Veterinary Authority from other Competent Authorities;
- '*having the responsibility and competence for ensuring or supervising the implementation'* was changed to '*having the primary responsibility … for coordinating the implementation'* as this is more concise and direct language and reflects the fact that some standards may not be under the direct responsibility or competence of the Veterinary Authority;
- '*implementation of the standards of'* was included to replace *'animal health and* welfare *measures, international veterinary certification and other standards of*' as the latter includes unnecessary detail.

c) Veterinary Services/Aquatic Animal Health Services

This term covers a broad range of actors that are involved in the implementation of OIE standards and are not necessarily part of governmental authorities or regulatory agencies. This may be the case for standards that involve a complex chain of responsibilities to be appropriately implemented. The definition has been reduced substantially to the key defining elements.

This term does not refer to a defined governmental structure but to a combination of individuals and organisations, public and private, which cannot be individually listed in the definition.

Key changes to the definitions include:

- The word '*individuals'* was added to ensure that private veterinarians, aquatic animal health professionals, veterinary paraprofessionals and others, would be covered under the definition when appropriate.

- The terms '*Private sector organisations, aquatic animal health professionals, veterinarians,* veterinary *paraprofessionals or aquatic animal health professionals'* were removed as these were considered unnecessary, and could exclude other relevant actors.

- '*that implement animal health and welfare measures and other standards and recommendations'* was changed to '*that perform activities to implement standards'*, to better differentiate from the more specific role of responsible government authorities, which are covered by the terms Competent Authority and Veterinary Authority.

- '*implement standards of the Aquatic Code/Terrestrial Code'* was included to replace '*animal health and welfare measures and other standards and recommendations in the OIE Terrestrial Code* and *the OIE Aquatic Code',* as the latter includes unnecessary detail.

- The current reference to the Veterinary Authority within the definition of Veterinary Services was not considered necessary, as the definition of Veterinary Authority is sufficiently clear, and was removed.

- '*Private sector organisations, veterinarians, veterinary paraprofessionals or aquatic animal health* professionals *are normally accredited or approved by the Veterinary Authority to deliver the delegated functions'* was deleted to keep the definition simple and to the point, and as these elements are described in the relevant chapters of Section 3 of the Codes.

The revised Glossary definitions for 'Competent Authority', 'Veterinary Authority' and 'Aquatic Animal Health Services', are presented as **Annex 5** for Member comments.

5.1.3. Chapter 1.3. Diseases listed by the OIE – Listing of infection with Tilapia Lake Virus

Background

Tilapia lake virus continues to be reported in new countries and poses a significant threat to many countries given the worldwide importance of tilapia farming and international trade in this species. In September 2017, the Aquatic Animals Commission reviewed the assessment of infection with tilapia lake virus (TiLV) against the criteria in Article 1.2.2. of Chapter 1.2. Criteria for listing aquatic animal diseases. The Commission agreed that the disease could not be proposed for listing at that time, as it did not meet criterion 3, 'a precise case definition is available and a reliable means of detection and diagnosis exists'. The Commission convened an *ad hoc* Group to evaluate available diagnostic methods for TiLV.

Previous Commission reports where this item was discussed:

September 2016 (Item 5., page 7), February 2017 (Item 4.4., page 7), September 2017 (Item 2.3., page 8).

September 2021

The *ad hoc* Group on infection with tilapia lake virus conducted its work electronically between November 2017 and September 2021 and submitted its final report for the consideration of the Commission (also see Item 7.1.).

The Commission considered the *ad hoc* Group's report and commended its members for its comprehensive work. The Commission encouraged Members to refer to the September 2021 *ad hoc* Group report available on the OIE Website, for more details on the recommendations of the *ad hoc* Group.

In light of available information on diagnostic methods for TiLV, including the recommendations of the *ad hoc* Group, the Commission reviewed its assessment of infection with TiLV against the criteria in Article 1.2.2. The Commission agreed that criterion 3 of Article 1.2.2. was now met. The Commission also agreed that infection with TiLV meets criteria 1, 2, 4b and 4c of Article 1.2.2. and concluded that the disease should be proposed for listing in Article 1.3.1. of Chapter 1.3. Diseases listed by the OIE.

The Commission advised Members that should new scientific evidence become available that could impact the outcome of this assessment for listing, the Commission would review its assessment, and encouraged Members to provide any such information for its consideration.

The revised Article 1.3.1. of Chapter 1.3. Diseases listed by the OIE is presented as **Annex 6** for Member comments.

The revised Assessment for listing infection with tilapia lake virus is presented as **Annex 6** for Members' information.

5.1.4. Approaches to demonstrate disease freedom

Background

A discussion paper, developed by the Aquatic Animals Commission, on approaches for determining periods required to demonstrate disease freedom, was first circulated for comments in the Commission's September 2018 report. The Commission considered comments received and circulated a revised discussion paper in its September 2019 report. Model Articles to replace the existing Articles X.X.4.–X.X.6. within the disease-specific chapters of the *Aquatic Code* were provided for Member comments in the Commission's February 2020 report.

At its September 2020 meeting, the Commission considered all comments received and agreed that a revised Chapter 1.4. Aquatic animal health surveillance, was necessary to complement the model articles and ensure all comments were addressed appropriately. The revised Chapter 1.4. and the model Articles to replace X.X.4.–X.X.6., were provided to Members for comment in its February 2021 report.

Previous Commission reports where this item was discussed:

September 2018 report (Item 2.10., page 11); September 2019 report (Item 6.6., page 9); February 2020 report (Item 7.2.2., page 15); September 2020 (Item 6.2., page 16), February 2021 (Part B: Item 1.2., page 4).

5.1.4.1. Chapter 1.4. Aquatic Animal Health Surveillance

Comments were received from Australia, Bangladesh, Canada, China (People's Rep. of), Chinese Taipei, the UK, the USA, AU-IBAR and the EU.

Previous Commission reports where this item was discussed:

February 2021 report (Part B: Item 1.2.1., page 4).

September 2021

The Commission reminded Members that the revision of Chapter 1.4. is intended to align with the approaches proposed in the discussion paper previously provided to Members for comment. The revised Chapter 1.4. is focused on providing guidance for self-declaration of freedom from disease, rather than providing general guidance on aquatic animal health surveillance.

The Commission did not agree with comments to revert to the original title, as the revised title reflects the new approach for this chapter. The revised chapter provides recommendations for methods of surveillance to detect aquatic animal diseases, with a focus on self-declaration of freedom from disease.

The Commission did not agree with a comment to include some Glossary definitions in Chapter 1.4. and noted that definitions used in the *Aquatic Code* are provided in the Glossary.

Article 1.4.1. Purpose

The Commission agreed with a comment to add '...and maintain...' to reflect that this chapter addresses the continued demonstration of freedom from disease.

Article 1.4.2. Introduction and scope

In point 3, the Commission agreed with a comment that additional clarification was required to specify the four pathways for claiming freedom, and added a reference to Article 1.4.3., as well as some additional details in other articles.

Article 1.4.3. Pathways for demonstrating freedom from disease

In point 2, the Commission did not agree with a comment to remove the reference to a country's early detection system as the Commission considered that this clarifies that the role of the early detection system is to generate passive surveillance information.

In point 2, the Commission agreed with a comment that passive surveillance may provide more qualitative information than just data and replaced 'passive surveillance data' with 'passive surveillance information' as well as throughout the rest of the chapter. The Commission agreed with a comment that general knowledge generated through the awareness, readiness, and competence of the aquatic animal health infrastructure are key elements of passive surveillance, but decided that is better described in Article 1.4.7.

In the title of point 3, the Commission agreed with a comment to amend the title to 'Targeted surveillance', noting that while all pathways utilise 'surveillance' in some way, the third pathway is primarily the use of targeted surveillance to achieve freedom from disease. The Commission also added 'targeted' in the rest of this chapter to identify this pathway, where appropriate.

In the first sentence of point 3, the Commission reminded Members that a country may choose targeted surveillance rather than the first two pathways for many reasons and not only if Pathway 1 or 2 cannot be met.

The Commission agreed to add a sentence to point 3 that passive surveillance could be used within the targeted surveillance pathway. A similar sentence was also added to point 2 to provide consistency with this change.

The Commission did not agree with a comment that the key difference between passive surveillance and historical freedom is the generation of empirical data and explained that passive surveillance can be subject to quantitative analysis if required such as through scenario tree analysis.

In Table 1.1, the Commission agreed with comments to replace 'Active Surveillance' with specific types of primary surveillance evidence and added 'Surveys, historical data, import records, environmental information' to align with Article 1.4.11. that describes the type of evidence required. The Commission did not agree with a comment to add compartment as an applicable level of application for Pathway 1, as it considered that targeted surveillance should be undertaken to establish free status for a compartment.

Article 1.4.4. Publication by the OIE of a self-declaration of freedom from disease by a Member Country

The Commission reminded Members that the Standard Operating Procedure on the publication of the self-declaration of animal health status of Members as well as the list of self-declarations are available on the OIE website: https://www.oie.int/en/what-we-offer/self-declared-disease-status/.

In the first paragraph, the Commission agreed to change 'may' to 'should' to indicate that all claimed statuses should be declared to the OIE given that it is the OIE that processes these self-declarations. The Commission also agreed to add 'for a country, zone or compartment' after 'claimed status' to reflect the three different types of self-declarations.

In point 1, the Commission agreed to add 'or return to freedom' to reflect a self-declaration using Pathway 4.

In point 2, the Commission agreed to replace 'general requirements' with a specific reference to 'basic biosecurity conditions and the requirements' to highlight the specific requirements for self-declaration.

In the fourth paragraph, the Commission agreed to add a sentence to inform Members of the linkage between OIE-WAHIS and the published list of self-declarations and to clarify that if a notification of a disease outbreak for a country, zone or compartment is received, for a self-declared status, the OIE website would be updated accordingly.

Article 1.4.5. Biosecurity and surveillance system requirements

In the first paragraph, the Commission agreed to add 'biosecurity and' before 'surveillance system' in line with the title of this article and the to reflect that the articles that follow are not solely concerned with surveillance system requirements. The Commission also agreed to add 'in the given compartment, zone or country' at the end of the paragraph to clarify the scope of the subitems.

The Commission did not agree to remove point 3, as it provided an explicit reference to early detection systems.

In point 5, the Commission agreed to add 'and expertise' after 'capacity' as both capacity and expertise are needed for disease investigations.

The Commission did not agree to delete point 5, as it considered that a reference to capacity and expertise for disease investigations emphasised the importance of these elements.

In point 6, the Commission agreed to add '(from a laboratory with a quality management system that meets requirements of Chapter 1.1.1. of the *Aquatic Manual*)' after 'appropriate diagnostic capability' to highlight the importance of a quality management in veterinary testing laboratories.

Article 1.4.6. Basic biosecurity conditions

The Commission agreed to delete point 1 on notification, as a compulsory requirement for reporting of a disease to the Competent Authority is addressed in point 5 of Article 1.4.7. Early detection system.

In the new point 1 (previously point 2), the Commission did not agree with a comment to add 'how they support the Competent Authority's' before 'early detection system', noting that both the public and private sectors contribute to a functioning early detection system.

In the new point 2 (previously point 3), the Commission did not agree to delete 'within or' as the aim is to minimise the spread from infected establishments, including to other establishments in the infected zone and to establishments in the protection zone.

In the last paragraph, the Commission agreed with a comment to add 'a specific' after 'self-declaration of freedom' to emphasise that the self-declaration is for a specific disease. The Commission also added 'continuously' at the end of the paragraph to emphasise that the basic biosecurity conditions must be continuously met to make a self-declaration of freedom. The Commission agreed to simplify some other wording to improve clarity, including deletion of the second reference to 'basic biosecurity conditions'.

Article 1.4.7. Early detection system

In the first paragraph, the Commission agreed to make the following amendments to improve clarity:

- 'data' was replaced by 'information' to reflect that passive surveillance may provide qualitative information or empirical data. Similar amendments were made in Articles 1.4.8., 1.4.12., 1.4.16. and 1.4.17.;

- 'underpins any' was replaced by 'is important to collect' to reflect the role of the early detection system in collecting passive surveillance information.

In the second paragraph, the Commission agreed to replace 'five characteristics' with 'requirements' as this is more appropriate wording.

In point 1, the Commission agreed to add the word 'observers' and to provide examples. The Commission highlighted the importance that awareness of the characteristic signs of listed and emerging diseases does not only apply to farmers.

In point 4, the Commission agreed to add '(from a laboratory with a quality management system that meets requirements of Chapter 1.1.1. of the *Aquatic Manual*)' to highlight the importance for the Aquatic Animal Health Services to have access to laboratories that meet the OIE standards for diagnostic testing. The Commission did not agree with a comment questioning the reference to emerging diseases in this point, and noted that the reference to emerging disease is important because investigations of morbidity and mortality may rule out listed diseases but there is also an obligation to investigate and report emerging diseases. The Commission also added 'the capacity and expertise to investigate' before 'emerging diseases' to reflect the change made in Article 1.4.5.

In point 5, in response to a comment that this point (a legal/compulsory requirement to report) is duplicated in point 1 of Article 1.4.6., the Commission noted that it had proposed to delete point 1 of Article 1.4.6. (also see above) and to retain point 5 in Article 1.4.7. as this is the more appropriate article to reference this requirement.

In point 5, the Commission agreed with a comment to include other relevant persons to report suspicions of disease occurrence such as 'others with an occupational role with aquatic animals', in addition to veterinarians and aquatic animal professionals as they should also have a legal obligation to report.

In point 5, the Commission added 'listed and emerging diseases' after 'report suspicion of' to reflect that Article 1.4.2. includes both listed and emerging diseases.

In the third paragraph, the Commission agreed with comments that the obligation of the Competent Authority is to demonstrate that efforts have been made to make more than farmers aware of the signs of listed diseases and emerging diseases, and that this obligation also applies to reporting suspicions of disease. The Commission was of the opinion that these obligations would be

applicable to more observers than farmers and fishers, and decided to add ‘aquatic animal health professionals, veterinarians and others’ after ‘farmers’ in the second sentence to emphasise the importance of reporting by these groups and replace ‘farmers with ‘relevant observers (e.g. farmers and fishers)’ in the third sentence and to include 'with an occupational role with aquatic animals’ after ‘others’ at the end of the same sentence. A relevant observer could include the owner of, or any person attending, aquatic animals; any person accompanying aquaculture animals during transport or any other person with an occupational relationship to aquatic animals.

In the third paragraph, the Commission did not agree with a comment to delete the second sentence as it considered that farmers are the key observers and that aquatic animal health professionals may be occasional observers.

In the last paragraph, the Commission clarified that the early detection system generates general knowledge (better described as information, rather than data which implies something empirical) generated through the awareness, readiness, and competence of the aquatic animal health infrastructure. Strong systems (combined with a pathogenic agent that manifests clinically) should, given enough time, detect disease if it was present. The Commission edited the paragraph to clarify that a quantitative assessment of the early detection system is generally not required and that a qualitative assessment would be sufficient for the self-declaration of freedom.

Article 1.4.8. Requirements for passive surveillance

In point 1 a), the Commission did not agree with a comment to replace ‘infection’ with ‘disease’ as it would appear tautological. The Commission did, however, agree to add ‘at least seasonally’ at the end of the sentence to clarify that conditions to not need to be continuously conducive to clinical expression of the disease.

In point 1 b), the Commission agreed to replace ‘reporting’ with ‘investigation and where appropriate, reporting to the Competent Authority’. This reflects that farmers, in many cases, would be the first to detect and report signs of disease to a private veterinarian or aquatic animal health professional who has the obligation to report to the Competent Authority, if they suspected a listed disease.

In point 1 c), the Commission agreed to delete ‘in all relevant production systems’ to clarify that passive surveillance requires sufficient observation of the animals such that the clinical signs would be observed if they were to occur – whether that be in production systems or other contexts (e.g. transport).

In point 1 d) i), the Commission did not agree with comments to delete ‘sufficient’ in front of ‘observation’ as observations need to be sufficient to achieve the outcomes of observation and reporting. The Commission agreed to delete the word ‘clinical’ in front of ‘signs of disease’ as it is redundant. The Commission deleted ‘clinical’ in front of ‘signs of disease’ throughout the chapter, for consistency.

In point 1 d) ii), the Commission agreed to amend the text to reduce wordiness and clarify that wild aquatic animals must be epidemiologically linked to farmed populations such that disease occurring in wild populations would also occur in farmed populations and be observed and reported.

In point 2, the Commission:

– agreed to add ‘veterinarians and others’ to the examples of observers in the first sentence as veterinarians in the private sector have a role to play in passive surveillance and should therefore be included together with aquatic animal health professionals;

- agreed to add ‘recognizing signs that are suspicious of a listed disease’ after the example in brackets, in the first sentence, to ensure consistency with the Glossary definition of ‘early detection system’;

- agreed to replace ‘are unlikely to be’ with ‘may not be’ to reflect the possible relevance of passive surveillance for wild populations;

- did not agree to delete ‘under most’ before ‘circumstances’ in the second sentence as it considered that this wording is accurate;

- agreed that the correct reference in the second sentence was 1 d) i) rather than 4 a);

- agreed in the last sentence to replace ‘provides appropriate sensitivity for’ with ‘will result in’ as the defined term “sensitivity” is not appropriate in this context.

In point 4 a), the Commission agreed to amend the text by specifying that the environmental conditions must be permissive for the development of clinical signs at least seasonally.

In point 4 b), the Commission did not agree with a comment to add ‘representative’ in front of ‘presence’ as this was not relevant for passive surveillance.

In point 5, the Commission agreed to restructure the sentence to avoid duplications and to improve clarity.

In point 6, the Commission agreed to amend the text to emphasise the importance of observations and initial investigations by farmers and private veterinarians or aquatic animal health professionals through passive surveillance to be rapidly reported to the Competent Authority for subsequent investigation.

Article 1.4.9. Required periods for basic biosecurity conditions

In point 1 a), the Commission agreed with a comment to amend the text to clarify that the application of this clause is to a specific pathogenic agent that is the subject of a declaration of freedom.

The Commission agreed with a comment to rephrase point 2 to clarify that the minimum periods that basic biosecurity conditions should be in place prior to a self-declaration of freedom from disease are defaults. These default periods are included in each disease-specific chapter, however, following consideration of the factors specified in this article longer periods may be deemed necessary for inclusion in the relevant chapter. The Commission confirmed that the factors in this article are used to set the requirements in each disease-specific chapter and are not provided for application by Competent Authorities.

In point 2 b), the Commission made amendments to clarify that for Pathway 2, the default minimum period of basic biosecurity conditions required prior to a self-declaration, for all listed diseases, is ten years.

In the same point, the Commission did not agree with a comment to allow for a shorter period to achieve 95% likelihood of freedom if the annual likelihood of detection can be demonstrated to substantially exceed 30%, noting that these are default minimum periods. The Commission noted that if a shorter period was to be included as an option, standards for quantitative assessment of passive surveillance sensitivity would be required and the standards would need to be updated to include guidance on the requirements for the quantitative assessment.

The Commission clarified that Pathway 2 would not be applicable for emerging diseases as it would not be possible to have basic biosecurity conditions in place for a disease if it had not been a known disease for at least 10 years.

In point 2 b) v), the Commission did not agree with a request to add 'in susceptible species' after 'clinical expression' as it considered this to be redundant because clinical expression would only occur in susceptible species.

In point 2 c), the Commission did not agree with a comment to allow for a default minimum period of basic biosecurity conditions of less than one year prior to commencement of targeted surveillance. The Commission had chosen a default one-year minimum period as it expected that this period will be sufficient under most circumstances for a disease to reach a prevalence sufficiently high to be detected by a survey designed in accordance with the recommendations of Chapter 1.4.

Article 1.4.10. Required periods for targeted surveillance

In the second paragraph, the Commission agreed to add a cross reference to 'Article 3.1. Selection of populations and individual specimens, of relevant disease-specific chapters of the *Aquatic Manual*', to clarify that this paragraph is about the sampling frame and not the populations that will be sampled. The Commission informed Members that the intention here was not to suggest every species or population be sampled, but rather that all populations of susceptible species be considered when designing surveys, and then the prioritised populations, based on likelihood of infection, would be sampled.

In the second paragraph, the Commission did not agree with a comment that surveys with relatively continuous sampling could accrue results within a six-month period without a three-month break between surveys. The Commission considered that there was a need to ensure there is a distinction between time-limited targeted surveys for the purpose of declaring freedom and routine sampling that is unlikely to be optimised for detection of the target pathogen. Targeted surveys should be designed to generate a 95% confidence of detection; however, sample size calculations and analysis of the results are considerably more complex when sampling a small number of animals on a frequent or continuous basis in comparison to at a limited number of sampling events at specified time points. This same rationale was applied to a comment in Article 1.4.12.

In the penultimate paragraph, the Commission agreed to amend the text to emphasise that if a different period for targeted surveillance is stipulated in the disease-specific chapter, the minimum required period would be more than one year.

In response to a comment, the Commission agreed to amend the last paragraph, to clarify that one survey in a compartment is required to ensure that basic biosecurity conditions are effective. The Commission also added text to clarify that the minimum period of targeted surveillance required prior to returning to free status will be specified in the relevant disease specific chapter of the *Aquatic Code*.

Article 1.4.11. Pathway 1 – Absence of susceptible species

In point 1, the Commission did not agree to delete 'sound' at the beginning of the sentence or to insert 'in the country/zone/compartment' in front 'of susceptible species' as the intent is that the pathway would not be applicable for diseases with an uncertain host range; for example, where the host range is broad and is expected to increase with exposure of hosts in new geographic areas. The pathway would not be available for some diseases with uncertainty regarding their host range and would not be included as an option in the relevant disease-specific chapter. This is explained in the penultimate paragraph of this article.

In point 2, the Commission agreed with a comment to delete 'based on active surveillance' and added 'demonstrated by the following forms of evidence' to indicate the forms of evidence that could be used to establish the absence of susceptible species. The Commission did not agree with a comment to add a definition for active surveillance as the above change meant the term would not be in use.

The Commission did not agree with a comment to add text in the last paragraph on intentional or non-accidental introduction of susceptible species that are not documented to be part of the country's natural fauna and subsequent use of Pathway 1 for declaration of freedom. If susceptible species have been introduced, through intentional or accidental means prior to a self-declaration of freedom being made, targeted surveillance, through Pathway 3, would be required to have confidence in the disease free status.

Article 1.4.12. Pathway 2 – Historically free

The Commission amended the title to 'historical freedom' to harmonise with the use of terminology in the rest of the chapter.

The Commission did not agree with a comment to add information regarding annual sensitivity of the surveillance system and potential for changing pathways as it determined that the text was clear as written.

Requirements for passive surveillance

In the second paragraph, the Commission agreed with a comment to delete '(and ideally a quantitative assessment of sensitivity would be included)', noting that a qualitative assessment is considered to be sufficient in most circumstances. The Commission also note that a requirement for quantitative assessment of passive surveillance sensitivity would put a claim of historical freedom out of reach for most Members.

Need for targeted surveillance

The Commission agreed with a comment to rephrase the text to improve clarity, and noted that the intent of this sentence was to indicate that this pathway should only be used if passive surveillance information is the primary form of evidence that the disease is absent.

The Commission did not agree with a request that Pathway 2, could be applied for compartments.

The Commission did also not agree with a comment to allow for a shorter period to claim historic freedom and advised that Pathway 3 should be used in such cases.

Article 1.4.13. Pathway 3 – Surveillance

The Commission amended the title to 'Targeted surveillance' to harmonise with the use of terminology in the rest of the chapter.

The Commission agreed with a comment that information provided in point 1 is duplicated in the subsequent paragraph. It agreed to delete the paragraph entitled 'Requirements for basic biosecurity conditions' and insert additional information at the beginning of point 1 for clarity.

Requirements for targeted surveillance

In the first paragraph, the Commission agreed to delete the phrase 'the rate' as it was not needed.

In the third paragraph, the Commission did not agree with a comment, also received on Article 1.4.10., that surveys with relatively continuous sampling could accrue results within a six-month period without a three-month break between surveys. See Article 1.4.10. for the Commission's explanation.

In the third paragraph, the Commission agreed to replace 'an overall' with 'at least', as 95% confidence is the minimum standard and is consistent with text in Article 1.4.14. The Commission also agreed to add 'set to a maximum' after 'should be' and delete 'or lower' after '2%' as 2% is consistent with Article 1.4.14.

The Commission agreed to delete the last paragraph as this was covered in Article 1.4.15.

Other sources of data

The Commission agreed to replace 'structured surveillance' with 'targeted surveillance', as it was a more appropriate term.

Article 1.4.14. Pathway 4 – Returning to freedom

In the third paragraph, the Commission amended the text for improved clarity and to align with proposed amendments in Article 1.4.10.

In the fourth paragraph, the Commission agreed to add 'and update' after 'review' to ensure that if there was a breach of basic biosecurity conditions then these would also need to be updated.

1. Infected zone and protection zone

In the second paragraph, the Commission agreed with a comment to add text that consideration should also be given to the type of aquaculture production system (open or closed). Vectors was added in sentences 2 and 4 to ensure that movements of vectors are considered in addition to wild susceptible species.

2. Requirements for targeted surveillance in a country or zone

In point 2 b), the Commission agreed with a comment to replace 'geographical' with 'hydrographical' to reflect the fact that sites can be geographically close but not epidemiologically linked by water.

The Commission agreed with a request to add a sentence at the end of the last paragraph to clarify that if disease is detected in wild populations of susceptible species, and eradication is not possible, the country or zone remains infected. The Commission did not agree to add recommendations on further actions (e.g. establishing compartments) as this was not thought to be appropriate. Any further actions would be decided by the Competent Authority.

3. Requirements for targeted surveillance in a compartment

The Commission agreed with a comment to add 'or at the maximum length of time allowed by production cycle of species' after '6 months' to clarify that the production cycle of some species is shorter than six months.

In response to a comment about the need to ensure a flexible approach to determining survey design within compartments, the Commission reminded Members that this was included in the discussion papers and that Members had indicated that at least one survey should be required. The Commission also reminded Members that the requirements for surveillance to maintain freedom would also have to be met.

Article 1.4.15. Maintenance of disease free status

The Commission agreed that the requirements to maintain freedom should be based on the level of confidence that a country, zone or compartment remains free, rather than the initial pathway utilised to achieve freedom. The Commission also considered that the information included in model Article X.X.8. for each listed disease was applicable to all diseases and better suited for inclusion in Article 1.4.15. The Commission noted that additional information regarding maintenance of freedom may be necessary in Article X.X.8. of disease-specific chapters based on the epidemiology of a disease.

The Commission revised Article 1.4.15. consistent with the above approach based on the guidance included in both model Article X.X.8. and Article 1.4.15. (also see Item 5.1.4.2.).

The Commission agreed with a comment that Competent Authorities should ensure prompt investigation of any health events or other information that may raise suspicion of the occurrence of a listed disease from which a country, zone or compartment that has been declared free. Text to this effect was added to the revised article.

Article 1.4.16. Design of surveys to demonstrate freedom from disease

In the first sentence of the first paragraph, the Commission agreed to add 'and maintain freedom' after 'Article 1.4.14.' as this article applies to the maintenance of a claim of freedom, as well as regaining freedom.

In the first paragraph, the Commission did not agree with a comment to remove 'clinical' in 'clinical expression of disease' as it considered that 'clinical' was appropriate in the context of 'conditions conducive to clinical expression of disease'.

1. Population

In the second paragraph, the Commission agreed with a comment to add 'within the selected population' after 'all individuals' to avoid misunderstanding that all susceptible species may require sampling. Similarly to Article 1.4.10., the intention is not to suggest that every species or population be sampled, but rather that all populations be considered when designing surveys (i.e. within the sampling frame), and populations would then be prioritised for sampling based on the likelihood of infection.

In the fourth paragraph, the Commission agreed with a comment to add 'Similarly, wild aquatic animal populations are not evenly distributed within a zone', as it considered that this should be considered in the design of surveys as it could lead to clusters of infection.

In the fourth paragraph, the Commission agreed with a comment to delete 'ponds' as ponds may not be a good example of a first stage sampling group for a two-stage sampling survey.

In the last sentence of the fourth paragraph, the Commission agreed to replace 'selected' with 'first stage sampling' to make the text more explicit about two-stage sampling.

3. Statistical Methodology

In the second point b), the Commission agreed with a comment to add 'that can remain sub-clinical' because there is a difference between transient infection and subclinical infection. Transient disease is not detected effectively even at very low design prevalence (e.g. for infection with ISAV HPR0), whereas subclinical disease (e.g. for infection with KHV) is present at very low levels but can be detected at a low (1% or less) design prevalence.

4. Risk based sampling

In point a), the Commission agreed with a comment to amend the text to reflect the five transmission pathways that are outlined in Article 1.4.7. of Chapter 4.1. Biosecurity for aquaculture establishments.

In point b), the Commission agree with a comment to delete 'quarantine facilities' as this did not align with the definition for quarantine in the Glossary.

5. Test characteristics

In the second paragraph, the Commission did not agree to delete 'not' before 'be pooled' as it is not acceptable to pool samples for a declaration of freedom without evidence to support such an approach. The Commission also noted that the text includes a reference to the disease-specific chapters of the *Aquatic Manual* that provide guidance on pooling.

7. Multi-stage structured survey design

In the second sentence of paragraph 1, the Commission agreed with a comment to add 'or discrete populations of wild susceptible species' after '(or villages)' and 'or defined stocks within a wild population' after (or village) to provide examples on how sampling levels can be applied to wild populations.

8. Discounting

In the first paragraph, the Commission agreed with a comment to add 'of disease in a population' after 'clinical expression' to ensure consistency of terminology within the chapter.

Article 1.4.18. Diagnostic confirmation of a listed disease or an emerging disease

In the fifth paragraph, the Commission agreed to include a cross reference to Chapter 1.1.1. of the *Aquatic Manual*.

In the final paragraph, the Commission did not agree to add an additional sentence regarding the implementation of disease control measures when the presence of a listed or emerging disease is suspected or confirmed as this is outside the scope of Article 1.4.18. and covered elsewhere in the chapter.

The revised Chapter 1.4. Aquatic Animal Health Surveillance, is presented as **Annex 7** for Member comments.

5.1.4.2. Model Articles X.X.4. to X.X.8. for disease-specific chapters to address declaration of freedom from [Pathogen X]

Comments were received from Canada, Japan, the UK and the EU.

Background

At its February 2020 meeting, the Aquatic Animals Commission considered comments received on the discussion paper 'Approaches for determining periods required to demonstrate disease freedom', and presented model Articles X.X.4., to X.X.8. for the disease-specific chapters of the *Aquatic Code* for comments.

At its February 2021 meeting the Commission considered comments on the model Articles X.X.4. to X.X.8. These articles were amended in conjunction with amendments to the draft revised Chapter 1.4. Aquatic Animal Health Surveillance. The Commission highlighted that relevant cross-references to articles of Chapter 1.4. have been included in the model articles.

Previous Commission reports where this item was discussed:

February 2020 report (Item 7.2.2., page 15); February 2021 report (Part B: Item 1.2.2., page 5).

September 2021

The Commission noted that time periods in these model articles will be determined by the Commission for each disease-specific chapter based on the criteria that are included the revised Chapter 1.4. For this reason, periods are shown as [X] to indicate that the period is yet to be determined for each specific disease. Assessment of the periods for each disease-specific chapter of the *Aquatic Code* will occur following adoption of the revised Chapter 1.4. and the model articles X.X.4 to X.X.8.

Article X.X.5. Country free from infection with [PATHOGEN X]

In point 2 a), the Commission did not agree with a comment to replace 'conditions that are conducive to the clinical expression of infection with [PATHOGEN X], as described in the corresponding chapter of the *Aquatic Manual*' with 'passive surveillance was undertaken in accordance with the conditions outlined in Chapter 1.4., Articles 1.4.7. and 1.4.8.'. The Commission explained that this is not appropriate given that point 2 b) requires that basic biosecurity conditions have been continuously met, including that an early detection system be in place. The Commission also did not agree with the same comment on point 2 a) of Article X.X.6.

In point 1), the Commission agreed to amend the default minimum period from two years to six months to align with the revised Article 1.4.9.2. a) of Chapter 1.4. The same amendment was made to Article X.X.6. point 2 b).

In the first line of point 4 d), the Commission agreed with a comment to add 'in wild and farmed susceptible species' to make it explicit that targeted surveillance would be required in both wild and farmed susceptible species to facilitate a self-declaration of freedom when farms and wild populations are epidemiologically linked. Under these circumstances, at least two years of surveillance would be required.

In the sixth paragraph of point 4, the Commission reminded Members that these are model articles and once adopted, the relevant amendments, including time periods (presented in square brackets) will be applied to each of the disease-specific chapters. The default minimum periods will be applied unless consideration of the relevant factors in Chapter 1.4. indicates that a longer period would be required.

Article X.X.6. Zone free from infection with [Pathogen X]

In point 4 d), the Commission did not agree with a comment to add 'using a suitable sample size, and under conditions including water temperature, which are conducive to the clinical expression of the disease' as it considered that there are numerous considerations for targeted surveillance throughout Chapter 1.4. and 'as described in Chapter 1.4.' ensures that all considerations for targeted surveillance are considered.

Article X.X.7. Compartment free from infection with [Pathogen X]

In point 2 b) the Commission agreed that basic biosecurity conditions do not include a biosecurity plan and so it added 'compartment' to the first line. The Commission agreed to amend the definition of biosecurity plan to refer to Chapter 4.1. (also see Item 5.1.2.1.).

In point 2 c), the Commission did not agree to add 'However, a different period (less than one year) may be stipulated if warranted by the epidemiology of the disease and the criteria described in Article 1.4.10. of Chapter 1.4.' at the end of the point. The Commission noted that Article 1.4.10.

describes the criteria for setting the period for targeted surveillance for each disease-specific chapter and that changes have been proposed to Article 1.4.10. for clarity (see Item 5.1.4.1.).

Article X.X.8. Maintenance of free status

The Commission agreed to amend model Article X.X.8. and Article 1.4.15. as described above (see Item 5.1.4.1.). Much of the information that had been included in this model article was moved to Article 1.4.15. as it was better suited there. A cross reference to Article 1.4.15. was provided and the commission noted that, based on the epidemiology of a specific disease, additional information regarding maintenance of freedom may sometimes be necessary in model Article X.X.8. of disease-specific chapters.

The revised model Articles X.X.4. to X.X.8. for disease-specific chapters to address declaration of freedom from [Pathogen X] are presented as **Annex 8** for Member comments.

5.1.5. Safe Commodities – Articles X.X.3 for disease-specific chapters

Background

At its September 2020 meeting, the Aquatic Animals Commission reviewed Article X.X.3. of all disease-specific chapters to address comments that the recommended time and temperature treatments in these articles represented different levels of thermal treatment and that some were not commercially feasible as they would diminish product quality. The Commission noted that it was difficult to propose a uniform model Article X.X.3. because of differences in time/temperature treatments as well as products in Article X.X.3. between disease-specific chapters. Therefore, the Commission developed an example article, Article 9.8.3. Infection with white spot syndrome virus, to demonstrate the suggested approach for Member comments.

At its February 2021 meeting, the Commission considered comments and amended the example article and then applied these amendments to all of the disease-specific chapters in Section 9 of the *Aquatic Code*, Diseases of crustaceans. The time/temperature treatments provided in Articles 9.X.3. for all of the crustacean disease-specific chapters were adjusted in line with the information provided in the 'Safe commodity assessments for OIE listed aquatic animal diseases' published in 2016.

The Commission also proposed a specific time/temperature heat treatment for meal in Articles 9.X.3. As a result of this amendment, the Commission indicated that the use of the definition of 'meal' throughout the *Aquatic Code* would be discussed at the Commission's September 2021 meeting to determine if the addition of a core time/temperature for meal in Article X.X.3. will require the Glossary definition be amended.

September 2021

The Commission reviewed the use of 'meal' throughout the *Aquatic Code* and agreed that the addition of a specific time/temperature heat treatment for meal included in Articles 9.X.3. did not impact the definition of meal in the Glossary. As a result, no amendments have been proposed for the definition of meal in the glossary.

The Commission noted that in some of the articles of the disease-specific chapters (e.g. Articles 9.X.7., 9.X.8., 9.X.9. and 9.X.10.) there is a cross-reference to 'point 1 of Article X.X.3.'. Given the proposed amendments to point 1 of Article 9.X.3. and 10.X.3., the Commission agreed that the cross reference to 'point 1' should be deleted from the relevant articles once the proposed amendments to Articles 9.X.3. and 10.X.3. are adopted.

The Commission agreed to implement these revisions one section at a time and to circulate for comment the revised Articles 9.X.3.(crustacean diseases) and Articles 10.X.3. (fish diseases); revised Articles 8.X.3. (amphibian diseases) and Articles 11.X.3. (mollusc diseases) would be circulated for comment in its February 2022 report.

5.1.5.1. Revised Articles 9.X.3. for crustacean disease-specific chapters

Comments were received from the USA and the EU.

Previous Commission reports where this item was discussed:

September 2020 (Item 4.7., page 10), February 2021 (Part B: Item 1.4., page 8).

September 2021 meeting

Article 9.X.3. of all crustacean disease-specific chapters

The Commission agreed with a comment that when a time period is presented as a fraction of a minute that this may be difficult to interpret when translated into different languages. The Commission agreed to ensure that any fractions of minutes used in the *Aquatic Code* will be amended to appear as seconds. In addition, when the time/temperature heat treatment requires only one minute for inactivation this has been amended to read as sixty seconds. For time periods greater than one minute, the time will remain in minutes.

The Commission re-ordered the aquatic animal products listed in Articles X.X.3. so that all thermal treatments for inactivation of a specific pathogenic agent appear in the list before other products and treatment types; for example, meal was moved above oil.

Article 9.1.3.1. Acute hepatopancreatic necrosis disease (AHPND)

In point 1 a), the Commission did not agree with a comment to remove 'sufficient to attain a core temperature of at least 100°C for at least one minute (or a time/temperature equivalent that has been demonstrated to inactivate VpAHPND)', and noted that this information is based on the core temperatures required for the inactivation of AHPND as presented in the commodity assessments available at https://www.oie.int/en/what-we-do/standards/standards-setting-process/ad-hoc-groups/.

The revised Articles 9.X.3. for crustacean disease-specific chapters are presented as **Annex 9** for Member comments.

5.1.5.2. Revised Articles 10.X.3. for fish disease-specific chapters

September 2021 meeting

The time/temperature treatments provided in Article 10.X.3. of all fish disease-specific chapters were amended in line with the information provided in the 2016 Safe commodity assessments for OIE listed aquatic animal diseases. These articles were also amended in line with the proposed changes in the crustacean disease-specific Articles 9.X.3. (see Item 4.1.5.1.).

Article 10.3.3. – Infection with *Gyrodactylus salaris*

The Commission agreed to not apply the proposed time/temperature heat treatment for *G. salaris* given that *G. salaris* would not survive in a pasteurised or retorted product as the parasite would not survive in any heated product.

The revised Articles 10.X.3. for fish disease-specific chapters are presented as **Annex 10** for Member comments.

5.1.6. Draft Chapter 9.X. Infection with decapod iridescent virus 1

Background

Following the adoption of infection with decapod iridescent virus 1 (DIV1) in Article 1.3.1. of Chapter 1.3. Diseases listed by the OIE, at the 88th General Session in May 2021, the Commission developed a draft Infection with DIV1 chapter for the *Aquatic Code*.

September 2021

The Commission developed a draft of Chapter 9.X. Infection with decapod iridescent virus 1, based on the article structure of other disease-specific chapters in Section 9. This new chapter includes proposed horizontal amendments to article structure, such as the model Articles X.X.4. to X.X.8. (see also Item 5.1.4.2.) and Articles 9.X.3. (see also Item 5.1.5.1.). The Commission noted that the proposed article structure for 9.X.3., and 9.X.4. to 9.X.8., is dependent on adoption of the model articles by Members.

The Commission noted that the susceptible species in Article 9.X.2. would be placed under study pending assessment against Chapter 1.5. Criteria for listing species as susceptible to infection with a specific pathogen. The aquatic animal products listed in Articles 9.X.3. and 9.X.14. would be placed under study pending assessment against Chapter 5.4. Criteria to assess the safety of aquatic animal commodities.

The Commission agreed that the default periods for basic biosecurity conditions and targeted surveillance presented in the revised Chapter 1.4. Aquatic Animal Disease Surveillance, would be appropriate for infection with DIV1. The Commission noted that following the adoption of the revised Chapter 1.4. an assessment of these periods would be required for all listed diseases, including infection with DIV1.

The new draft Chapter 9.X. Infection with decapod iridescent virus 1, is presented as **Annex 11** for Member comments.

5.1.7. Article 10.1.2. of Chapter 10.1. Infection with epizootic haematopoietic necrosis virus

The Aquatic Animals Commission agreed to list fish species susceptible to infection with epizootic haematopoietic necrosis virus in a table format, in line with the convention to list susceptible species in table format if there are more than ten species susceptible. (see also Item 6.1.3.).

The revised Article 10.1.2. of Chapter 10.1. Infection with epizootic haematopoietic necrosis virus, is presented as **Annex 12** for Member comments.

5.1.8. Article 10.10.2. of Chapter 10.10. Infection with koi herpesvirus

The Aquatic Animals Commission noted that common carp X crucian carp hybrids (*Cyprinus carpio x Carassius carassius)* had been omitted from Article 10.7.2. despite these hybrids having been found susceptible through the assessment of the ad hoc group of susceptibility of fish species. The Commission agreed to correct this omission (see also Item 6.1.5.).

The amended Article 10.7.2. of Chapter 10.7. Infection with koi herpesvirus, is presented as **Annex 13** for Member comments.

5.1.9. Susceptible species- Section 11. Diseases of Molluscs

5.1.9.1. Articles 11.1.1. and 11.1.2. of Chapter 11.1. Infection with abalone herpesvirus

The Commission considered the *ad hoc* Group report on Susceptibility of mollusc species to infection with OIE listed diseases and commended its members for their comprehensive work.

The *ad hoc* Group had applied the criteria for listing species as susceptible to infection with a abalone herpesvirus in accordance with Chapter 1.5. Criteria for listing species as susceptible to infection with a specific pathogen.

The Commission amended Article 11.1.1. to ensure consistency with other mollusc disease-specific chapters.

The Commission agreed to amend the list of susceptible species in Article 11.1.2. in line with recommendations of the *ad hoc* Group. It noted that the four species currently included in Article 11.1.2. meet the criteria for listing as susceptible to infection with abalone herpesvirus, i.e., small abalone (*Haliotis diversicolor*), greenlip abalone (*Haliotis laevigata*), blacklip abalone (*Haliotis rubra*) and hybrids of greenlip x blacklip abalone (*Haliotis laevigata x Haliotis rubra*) and were proposed for retention in Article 11.1.2. No new species were found to meet the criteria for listing as susceptible to infection with abalone herpesvirus.

Relevant sections of Chapter 2.4.1. Infection with abalone herpesvirus, in the *Aquatic Manual* were also amended in line with the recommendations of the *ad hoc* Group (see Item 6.2.).

The Commission encouraged Members to refer to the *ad hoc* Group's June 2021 report available on the OIE Website (https://www.oie.int/en/what-we-do/standards/standards-setting-process/ad-hoc-groups/), for details of the assessments conducted by the *ad hoc* Group.

The revised Articles 11.1.1. and 11.1.2. of Chapter 11.2. Infection with abalone herpesvirus, are presented as **Annex 14** for Member comments.

5.1.9.2. Articles 11.2.1. and 11.2.2. of Chapter 11.2. Infection with Bonamia exitiosa

Comments were received from Canada, China (People's Rep. of) and the EU.

Background

At its February 2021 meeting, the Aquatic Animals Commission reviewed the December 2020 report of the *ad hoc* Group on Susceptibility of mollusc species to infection with OIE listed diseases. The *ad hoc* Group had applied the criteria for listing species as susceptible to infection with *Bonamia exitiosa* in accordance with Chapter 1.5. of the *Aquatic Code*.

The Commission had agreed to amend Article 11.2.1. to ensure consistency with other mollusc disease-specific chapters.

The Commission had agreed to amend the list of susceptible species in Article 11.2.2., in line with the recommendations of the *ad hoc* Group.

Previous Commission reports where this item was discussed:

February 2021 report (Part B: Item 1.5., page 10).

September 2021

The Commission acknowledged a comment requesting additional information be provided in the assessment table of the *ad hoc* Group reports regarding the route of infection for Stage 1 and the method for pathogen identification for Stage 2. The Commission agreed that this information should be considered for inclusion in future *ad hoc* Group reports to support Members' understanding of the assessments and to ensure consistency between the assessments completed for the different species groups.

The Articles 11.2.1. and 11.2.2. of Chapter 11.2. Infection with *Bonamia exitiosa*, are presented as **Annex 15** for Member comments.

5.2. Texts for Member information

5.2.1. Emerging diseases

5.2.1.1. Infection with carp edema virus (CEV)

Comments were received from Japan.

Background

At its February 2021 meeting, the Aquatic Animals Commission reviewed new scientific information on infection with carp edema virus (CEV) and reported that infection with CEV had spread from the Asia-Pacific region to many European countries and had caused mortalities in common carp and koi carp. The Commission noted that mortalities caused by infection with CEV in New Caledonia have demonstrated the virulence of CEV to koi carp, and the spread of infection and mortalities caused by CEV in koi carp farms in China (People's Rep. of) have significant impacts. The Commission also noted that the decrease in mortality rates in some countries was likely to be the result of successful mitigation measures.

Previous Commission reports where this item was discussed:

February 2020 report (Item 7.3.3., page 17); September 2020 (Item 6.3., page 17); February 2021 (Part B: Item 2.2., page 11).

September 2021

The Commission reviewed the latest scientific evidence and noted that infection with CEV continues to be reported to impact production and cause mortality events in wild and farmed populations but the severity of the impacts is unclear.

The Commission did not agree with a comment that infection with CEV did not meet the definition of an emerging disease, and reiterated that detections of infection with CEV should be reported to the OIE as an emerging disease, in accordance with Article 1.1.4. of the *Aquatic Code*.

The Commission encouraged Members to investigate mortality and morbidity events in carp, emphasising that a better understanding of the virus is essential for efforts to control its possible spread and impacts on carp populations.

The Commission will continue to monitor the global situation for infection with CEV and seek further information from scientists working on the disease.

5.2.1.2. Infection with Entercytozoon hepatopenaei

The Aquatic Animals Commission reviewed the scientific evidence to determine whether infection with *Entercytozoon hepatopenaei* (EHP) meets the OIE definition of an emerging disease. The Commission noted that there have been reports of significant economic and production impacts related to the disease, particularly in Asia, that have been ongoing for some time, that there are available diagnostic methods and evidence that EHP can be spread through trade.

The Commission agreed that infection with EHP meets the definition of an emerging disease, but recognised that there is some uncertainty in regard to the spread and impacts of the disease outside of Asia.

The Commission agreed that any detections of infection with *EHP* should be reported to the OIE as an emerging disease, in accordance with Article 1.1.4. of the *Aquatic Code.*

The Commission encouraged Members to investigate morbidity and mortality events in shrimp and invited Members to provide further information on the disease, particularly from Members in Africa and South America.

5.2.2. New draft Chapters 4.X. Emergency disease preparedness and 4.Y. Disease outbreak management

Comments were received from Chile, AU-IBAR and CVP.

Background

The Aquatic Animals Commission further developed the article structure of the new draft Chapters 4.X. Emergency disease preparedness, and 4.Y. Disease outbreak management. Given the importance of this work to support Members in these critical areas, the Commission agreed to circulate the article structure of the new draft chapters to Members for comments before starting work to draft the detailed text.

Previous Commission reports where this item was discussed:

February 2020 (Item 7.3.2., page 16), September 2020 (Item 6.1., page 16), February 2021 (Part B: Item 1.2.2., page 5).

September 2021 meeting

The Commission thanked Members for their constructive comments on the proposed article structure for the new two draft chapters and informed Members that these comments would be taken into consideration as the chapters are developed.

The Commission requested an *ad hoc* Group be convened to develop the text for the two new chapters and requested that *ad hoc* Group start work on the new draft Chapter 4.X. Emergency disease preparedness.

5.2.3. Chapter 10.10. Infection with viral haemorrhagic septicaemia virus

Background

At its September 2020 meeting, the Aquatic Animals Commission confirmed its decision that genotypes should not be included in Article 10.10.2. of Chapter 10.10. Infection with viral haemorrhagic septicaemia (VHSV), as the Commission had not assessed whether VHSV genotypes can be differentiated for the purpose of distinguishing risk management measures for traded commodities.

At the Commission's February 2021 meeting, in response to several requests by Members to initiate an assessment of VHSV genotypes with respect to strain differentiation as had been conducted for ISAV, the Commission agreed that further guidance on the principal of applying risk assessments to justify mitigation measures directed at specific genotypes would be useful. It noted that this issue could be addressed through the possible restructuring of articles of disease-specific chapters and that approaches in the *Terrestrial Code* should be considered. At that time, the Commission indicated that the next Commission would need to prioritise this activity against other items in its workplan.

Previous Commission meeting reports where this item was discussed:

February 2021 (Part A: Item 3.16., page 11).

September 2021 meeting

The Commission discussed the feasibility and benefits of differentiating VHSV genotypes for the purposes of trade.

The Commission noted that there is significant complexity associated with the different VHSV genotypes and gaps in knowledge. The Commission agreed that it would be difficult to develop standards to reflect strain differentiation of VHSV genotypes, in a way that could be adopted and easily managed by Members. The Commission also expected that VHSV strain differentiation would not result in a significant outcome for members in the terms of improved utility of the standards.

Following these considerations, the Commission agreed not to pursue strain differentiation for VHSV. However, the Commission reiterated that a Member may, based on a risk assessment and a claim of freedom from a specified VHSV genotype, take appropriate measures to protect its declared free status.

The Commission also discussed the considerations regarding a comment made in its September 2020 report requesting the Commission to reconsider the listing of Atlantic salmon (*Salmo salar*) as susceptible to all genotypes of VHSV in Section 2.2.1. of Chapter 2.3.10. of the *Aquatic Manual*, and specifically susceptibility to genotypes I, II and III. The Commission agreed that Atlantic salmon should remain listed in Article 10.10.2., as susceptibility to specific genotypes is not included in the *Aquatic Code*. However, the Commission agreed to refer the question of susceptibility of Atlantic salmon to genotypes I, II and III to the *ad hoc* Group for Susceptibility of fish species to infection with OIE listed diseases.

6. OIE *MANUAL OF DIAGNOSTIC TESTS FOR AQUATIC ANIMALS*

6.1. Texts for Members' comment

Members were reminded that the Aquatic Animals Commission has commenced the process of progressively reformatting the disease-specific chapters of the Aquatic Manual into a new template. As the reformatted and updated chapters have substantial changes, at its meeting in September 2019, the Commission agreed that only clean versions of the chapters would be provided in the report. Subsequent changes made to these initial revisions following Member comments would be indicated in the usual style (i.e., strikethrough for deletions and double underline for additions).

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 upon request from the OIE Standards Department (AAC.Secretariat@oie.int).

6.1.1. Guidance document on the use of environmental DNA methods for aquatic animal disease surveillance

Comments were received from Australia, Bangladesh, Canada, China (People's Rep. of), the UK, the USA, AU-IBAR and the EU.

Background

The monitoring of aquatic systems using environmental DNA (eDNA) is a rapidly advancing research field that will provide opportunities for rapid, cost-effective, non-destructive methods to screen for pathogens, especially in wild aquatic populations where sampling may be difficult or removal of animals undesirable. The Aquatic Animals Commission is aware that eDNA methods exist for detecting pathogenic agents of several listed diseases, including infection with *Xenohaliotis californiensis*, infection with *Batrachochytrium dendrobatidis*, infection with *Aphanomyces astaci* and infection with *Gyrodactylus salaris*.

The Commission agreed that as these methods are available and currently in use, it would be advisable for guidance to be provided on appropriate application and potential limitations. The Commission noted that as accurate estimates of diagnostic performance are not available for designing surveillance programmes using eDNA assays, data obtained from eDNA methods may not be suitable to support declaration of freedom from listed diseases. The Commission also noted that confirmation of infection by listed diseases could not be made using eDNA methods; however, positive results could be appropriate criteria for a suspect case.

At its February 2021 meeting, the Commission developed a discussion document outlining the benefits and limitations of eDNA detection within a diagnostic or disease surveillance context. This document is intended to guide the appropriate purposes of use and assay performance reporting required for an eDNA assay to be considered for inclusion in the *Aquatic Manual*.

Previous Commission reports where this item was discussed:

February 2020 (Item 8.4.2, page 22), September 2020 (Item 6.4, page 17), February 2021 (Part B: Item 3.1, page 12).

September 2021 meeting

The Commission thanked Members for the thorough engagement and feedback received on the guidelines provided for the use of environmental DNA methods for the detection of OIE listed aquatic animal diseases that were circulated in its February 2021 Part B report. The Commission noted that the comments were generally positive and in support of the approaches presented in the discussion paper.

The Commission did not agree to broaden the scope of the definition to include host-derived materials, such as faeces and mucous which are usually shed into the environment. The Commission noted that these host-derived sample types present different considerations (e.g. sampling, processing, extraction)as opposed to environmenal samples (e.g. water or soil).

In point 9, the Commission did not agree to amend the Glossary definition of ‘case’ as it considered it important to retain the current nomenclature as it is consistent with that of Section 6 of each disease-specific chapter of the *Aquatic Manual*. The Commission reminded Members that a positive result obtained from an eDNA method recommended in the *Aquatic Manual* is not considered to provide appropriate evidence to confirm a case in apparently healthy animals, and would only be suitable as a criterion for a suspect case.

The Commission will consider the second round of comments at its February 2022 meeting, after which the guidelines will be published on the OIE website.

The revised guidance document on the use of environmental DNA methods for aquatic animal disease surveillance, is presented as **Annex 16** for Member comments.

6.1.2. Chapter 2.3.0. General information (diseases of fish)

The Aquatic Animals Commission noted the need to add a sentence to Section 2.5. Use of molecular techniques for surveillance testing, confirmatory testing and diagnosis, of the general information chapter on the possibility of false-negative results (positive samples giving a negative result) occurring in PCR reactions due to the presence of a new variant that is not recognised by the PCR primer/probe set).

The revised Chapter 2.3.0. General information (diseases of fish), is presented as **Annex 17** for Member comments.

6.1.3. Chapter 2.3.2. Infection with epizootic haematopoietic necrosis virus

The Aquatic Animals Commission reviewed Chapter 2.3.2. Infection with epizootic haematopoietic necrosis virus, 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;
- updated information on the aetiological agent, its survival and stability outside the host and the sections on likelihood of infection by species, host life stage, population or sub-populations and aquatic animal reservoirs of infection;
- in the section on disease pattern updated information on modes of transmission and life cycle and on geographical distribution;
- updated information on specimen selection, sample collection, transportation and handling;
- updated the section on diagnostic methods including completing Table 4.1 OIE recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals, and revising the molecular diagnostic tests;
- revised definitions of suspect and confirmed case in apparently healthy and clinically affected animals.

The revised Chapter 2.3.2. Infection with epizootic haematopoietic necrosis virus, is presented as **Annex 18** for Member comments.

6.1.4. Chapter 2.3.4. Infection with HPR-deleted or HPR0 infectious salmon anaemia virus

Comments were received from Armenia, Canada, China (People's Rep. of), Cuba, Switzerland, Thailand, the UK, the EU and the USA.

Background

The Aquatic Animals Commission, at its September 2020 meeting, reviewed Chapter 2.3.5. Infection with HPR-deleted or HPR0 infectious salmon anaemia virus, which had been updated by the OIE Reference Laboratory experts and reformatted using the new disease chapter template.

Previous Commission reports where this item was discussed:

September 2020 (Item 5.4, Page 15).

September 2021

The Commission did not agree with a comment to jointly describe HPR-deleted ISAV and HPR0 ISAV rather than separately in the chapter. The Commission confirmed that the clinical expression of disease, epidemiology and control measures differ and justify leaving their descriptions separate.

In Section 1. Scope, the Commission did not agree with a proposal to replace 'disease outbreaks' with 'HPR-deleted ISAV genotypes' in a sentence on a suggested link between non-pathogenic HPR0 ISAV and pathogenic HPR-deleted ISAV as the change would alter the intended meaning of the sentence. The Commission noted that there is sufficient evidence in the cited references to

support the statement to indicate the relationship between the presence of HPR deletion and the occurrence of clinical disease.

In Section 2.1.1. Aetiological agent, the Commission agreed to move a sentence on the properties of ISAV to the beginning of the section, but did not agree to delete the word 'physicochemical' as the sentence correctly indicates that the physicochemical characteristics of ISAV are consistent with those of the members of the Family *Orthomixoviridae*. The Commission also agreed to include a more detailed description of the virus and new references to this section. Finally, the Commission did not agree to replace 'transient' with 'sometimes below the threshold of detection' in a sentence on the seasonal nature of HRP0 ISAV: transient is the correct term and describes the natural behaviour of the pathogen while below the threshold of detection would mean that it cannot be detected.

In Section 2.1.3. Survival and stability outside the host, the Commission expanded the information on the infectivity of ISAV in seawater under different physical conditions and updated the references.

In Section 2.2.2. Species with incomplete evidence for susceptibility, the Commission agreed to insert 'RT' before PCR.

A number of Members had commented on Section 2.2.3. Non-susceptible species. The Commission reiterated that this section had been deleted from the template for disease-specific chapters in the *Aquatic Manual* (also see the Commission's February 2021 meeting). The comments were therefore not considered.

In Section 2.2.4. Likelihood of infection by species, host life stage, population or sub-populations, the Commission deleted 'only' before 'a few cases have been reported in the freshwater stage' as disease outbreaks are increasing in freshwater.

In Section 2.2.5. Distribution of the pathogen in the host, the Commission agreed to add a reference to support the statement that clinical disease and macroscopic organ lesions appear foremost in severely anaemic Atlantic salmon in HPR-deleted ISAV. In the HPR0 ISAV paragraph, the Commission did not agree to mention 'non-HPR0 specific' as it is not the commonly used expression.

In Section 2.2.6. Aquatic animal reservoirs of infection, the Commission agreed to insert 'HPR-deleted' before 'ISAV' in the last sentence to improve clarity.

In Section 2.3.1. Mortality, morbidity and prevalence, the Commission agreed to add a new sentence at the start of the paragraph: 'The disease pattern with HPR-deleted ISAV depends on many factors including the strain of the virus', but did not support the inclusion of a second proposed sentence 'Not all strains result in noticeable disease on the farm' as the statement is not correct. The Commission agreed to insert a new reference to support the statement that morbidity and mortality may vary greatly between net pens and between farms during outbreaks of infection with HPR-deleted ISAV. The Commission deleted the last sentence in the paragraph as it was not relevant to the section.

In Section 2.3.4. Modes of transmission and life cycle, the Commission clarified that the first sentence on the main route of infection refers to horizontal transmission, and added a sentence and reference on vertical transmission. The Commission agreed that ISAV may be shed in skin and thus included it in the list, and deleted 'but shedding from localised gill infection may be most important' as there is no strong evidence to support this statement.

In Section 2.3.6. Geographical distribution, the Commission agreed to remove the second sentence on disease occurrence as this information can be found in the OIE-WAHIS interface.

In Section 2.4.1. Vaccination, the Commission did not agree to delete the information on the history of vaccination in certain countries believing that Members could find this text to be of interest. The Commission made some editorial changes in the section.

In Section 2.4.7. General husbandry, the Commission provided a reference to support the statement that good biosecurity and husbandry practices reduce the risk of outbreaks of infection with HPR deleted ISAV.

In Section 3.1. Selection of populations and individual specimens, the Commission detailed which fish should be sampled for virus detection methods and added cross references to Sections 2.3.2. Clinical signs, including behavioural changes, and 2.3.3. Gross pathology, for clarity. For consistency with the other updated fish disease chapters, the Commission added information on sampling for surveillance and for disease outbreak situations.

In Section 3.2.2. Detection of HPR0 ISAV, the Commission kept the first part of the sentence 'Gill tissue is recommended' and deleted the rest as the detection of HPR0 in organs other than gills is controversial.

In Section 3.4. Non-lethal sampling, the Commission clarified that blood is preferred for non-lethal sampling for HPR-deleted ISAV.

In Section 3.5.1. Samples for pathogen isolation, the Commission agreed to delete the reference to bioassay as it is not recommended in Table 4.1.

In Section, 3.5.2. Preservation of samples for molecular detection, the Commission corrected the PCR to 'real-time RT-PCR' and agreed to delete mention of commercial products name.

In Section 3.6. Pooling of samples, the Commission added the standard text clarifying that pooling of samples from more than one individual animal for a given purpose should only be recommended where robust supporting data on diagnostic sensitivity and diagnostic specificity have been evaluated and found to be suitable. Regarding small life stages, the Commission agreed to delete 'up to 0.5 g' from the pooling recommendation because 0.5 g of tissue is the minimum amount needed to perform virus isolation, but not necessarily the other test methods referenced in this chapter.

In Table 4.1, the Commission corrected some of the terminology used in the methods column to conform to the template. The Commission also modified footnote 2 (also see Item 8.2.).

In Section 4.3. Cell culture for isolation, the Commission added more details on cell culture procedures to provide more fulfilling information in this section.

Sections 4.4.1. Real-time PCR and 4.4.2. Conventional PCR, the Commission agree to replace 'PCR' with 'RT-PCR'. In Section 4.4.2., the Commission did not agree to replace the words 'if necessary' with 'if the laboratory has no access to real-time methodology' in a sentence stating that primers for segment 7 and 8 may also be used for conventional RT-PCR as it considered the change not necessary. The Commission did agree to delete the last three paragraphs from the section as they are already in Section 4.4.1.

In Section 4.7. Immunohistochemistry (IHC), the Commission agreed to create a new subsection 4.7.1. IHC on paraffin sections from formalin-fixed tissue, and to clarify that Section 4.7.2. is for the indirect fluorescent antibody test on tissue imprints and blood smears. The Commission agreed to delete the word 'polyclonal' from the start of the first paragraph of Section 4.7.1. as the text refers to both monoclonal and polyclonal antibodies. And finally in Section 4.7.1. ii) *Staining procedure for IHC*, the Commission removed reference to the monospecific rabbit antibody against ISAV nucleoprotein as other suitable antibodies could be used, and placed reference to goat anti-rabbit IgG with a reference to species specific IgG.

In Section 6. Corroborative diagnostic criteria, the Commission did not agree with a general comment not to separate the case definitions into 'apparently healthy' and 'clinically affected' animals. The Commission reminded Members that they had opportunities to comment on the disease chapter template when it was appended to the report of the February 2018 meeting before it was implemented from February 2019. The template has been consistently applied to all the fish disease chapters, and the case definitions are consistent with the purposes of the diagnostic tests given in Table 4.1 (also see Item 4.1. of the Commission's February 2021 report). The Commission did not agree with a comment to mention the fact that having an established link with a confirmed case can also create a suspect case, as this information is already mentioned in the below the first paragraph of Section 6.1.

In Section 6.1. Apparently healthy animals or animals of unknown health status, the Commission did not agree to add text stating that healthy populations are sampled 'for early detection of disease' as this purpose is not addressed in Section 5.

In Section 6.1.1. Definition of suspect case in apparently healthy animals, the Commission agree to delete criteria i) ISAV typical CPE in cell cultures (HPR deleted only) as this is not recommended in Table 4.1. For consistency with the terminology used throughout the fish disease chapters, the conventional RT-PCR was replaced with RT-PCR.

In Section 6.1.2. Definition of confirmed case in apparently healthy animals, under '*Definition of confirmed case of infection with HPR-deleted ISAV* and Section 6.2.2. Definition of confirmed case in clinically affected animals, the Commission clarified that the PCR methods are of segment 6 of the gene, and replaced 'HE gene' with 'amplicon'. In Section 6.1.2., Commission reduced the number of criteria from six criteria to three to simplify the definitions. Under *'Definition of confirmed case of infection with HPR0 ISAV*, the Commission revised criterion i) by adding real-time RT-PCR for detection of ISAV in tissue preparations and clarified that sequencing of the amplicon is to verify HPR0-deletion.

In Section 6.2.1. Definition of suspect case in clinically affected animals, the Commission did not agree to amend the first sentence before the list of criteria as the text is from the template and is standard throughout the chapters.

In Section 6.2.2. Definition of confirmed case in clinically affected animals, the Commission did not agree to add 'in addition to the criteria in Section 6.2.1.' to the first sentence before the list of criteria as they considered it clear as written. The Commission agreed to delete the existing criteria i) as HPR0 does not grow in cell culture and there is no need to sequence, and replaced it with a new criterion i) on virus isolation in cell culture and virus identification. The Commission reduced the number of criteria from six criteria to three to simplify the definitions.

The revised Chapter 2.3.4. Infection with HPR-deleted or HPR0 infectious salmon anaemia virus, is presented in **Annex 19** for Member comment.

6.1.5. Chapter 2.3.6. Infection with koi herpesvirus

Comments were received from Armenia, Canada, China (People's Rep. of), Cuba, Japan, Switzerland, the UK the EU and the USA.

Background

At its September 2020 meeting, the Aquatic Animals Commission reviewed Chapter 2.3.7. Infection with koi herpesvirus (KHV), which had been updated by the OIE Reference Laboratory experts and reformatted using the new disease chapter template.

The Commission agreed that the disease name 'infection with koi herpesvirus' should be retained and used in the *Aquatic Code* and *Aquatic Manual* for reasons of continuity and familiarity. CyHV-

3, the virus name recognised by the ICTV is, however, referred to in Section 1 of the chapter. This is a similar approach used for other listed diseases where the official pathogen name may be relatively unfamiliar.

Previous Commission reports where this item was discussed:

September 2020 (Item 5.5, page 15).

September 2021

The Commission reiterated its decision to refer to the pathogenic agent throughout the chapter as KHV rather than CyHV-3 for continuity (also see the Commission's September 2020 report).

In reply to a request to recommend the most suitable detection methods for KHV, the Commission informed Members that such guidance is available in the chapter in Table 4.1 OIE recommended diagnostic methods and their level of validation for surveillance of healthy animals and investigation of clinically affected animals, and on Section 6 Corroborative diagnostic criteria.

The Commission did not agree to delete 'all genotypes of' [the pathogenic agent] from Section 1 Scope, because the pathogenic agent does include all genotypes of the virus and this is substantiated by the references provided.

In Section 2.1.1. Aetiological agent, the Commission agreed to expand the description of the aetiological agent and to include new references.

In response to a comment to delete the last sentence of Section 2.1.3. Survival and stability outside the host, on the infective period of the virus in sterilised environmental water samples, the Commission agreed to maintain the sentence and to add a reference to support the statement.

In Section 2.2.1. Susceptible host species, the Commission agreed to add 'and subspecies' [of common carp] after 'all varieties', to delete '/goldfish' before 'hybrids' and to add '*Cyprinus carpio* × *Carassius Carassius*' all of which is in line with the recommendations made by the *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases (also see Item 5.1.8.).

In Section 2.2.2. Species with incomplete evidence for susceptibility, the Commission agreed to delete the rainbow trout (*Oncorhynchus mykiss*) in accordance with the recommendations of the *ad hoc* Group on Susceptibility of fish species.

A number of Members had commented on Section 2.2.3. Non-susceptible species. The Commission reiterated that this section had been deleted from the template for disease-specific chapters in the *Aquatic Manual* (also see the Commission's February 2021 report). The comments were therefore not considered.

In Section 2.2.3. Likelihood of infection by species, host life stage, population or sub-population, the Commission inserted a new sentence to provide guidance on the three life stages used in Table 4.1.

In Section 2.2.7. Vectors, the Commission reduced the existing text to the most relevant information. Also, consistent with the definition of vector in the *Aquatic Code*, a new sentence was included to state that no species of vector have been demonstrated to transmit KHV to susceptible species.

In Section 2.3.4. Modes of transmission and life cycle, the Commission rejected a suggestion to add intestine along with gills as the major portal of virus entry into carp, as the publication

provided to support this comment and the references already cited do not indicate intestine as port of entry.

In Section 2.4.1. Vaccination, the Commission agreed to update the information by adding text that reflects developments in research on vaccine candidates against KHV.

In Section 2.4.5. Inactivation methods, the Commission included other methods of inactivation of the virus and a reference that had been mistakenly omitted from the draft chapter.

In Section 2.4.6. Disinfection of eggs and larvae, the Commission clarified that disinfection is of the surface of the eggs.

In Section 2.4.7. General husbandry, the Commission did not agree with the proposal to add that fish can be held 'at permissive temperatures for development of clinical signs' as other factors influence development of clinical signs; the change would imply that two thresholds have been established which is not the case.

In Section 3.1. Selection of populations and individual specimens, the Commission added information on sampling for surveillance and for disease outbreak situations to be consistent with the other updated fish disease chapters.

In Section 3.4. Non-lethal sampling, the Commission did not agree with a proposal to include 'However, serology may be useful to document exposure to KHV if the virus is latent' as serology is not sufficiently validated and not rated in Table 4.1.

In Section 3.5.2. Preservation of samples for molecular detection, the Commission agreed to add a line that repeated freezing and thawing should be avoided.

Section 3.5.3. Samples for histopathology, immunohistochemistry or in-situ hybridisation, the Commission deleted the existing text and replaced it with a cross reference to Chapter 2.3.0. (also see Item 6.2.1.2.).

In Section 3.5.5. Samples for other tests, the Commission deleted the existing texts as reference to blood sampling is not relevant and inserted 'Not applicable'.

For Section 3.6. Pooling of samples, the Commission added the standard text clarifying that pooling of samples from more than one individual animal for a given purpose should only be recommended where robust supporting data on diagnostic sensitivity and diagnostic specificity have been evaluated and found to be suitable. Regarding small life stages, the Commission agreed to delete 'or specimens up to 0.5 g' from the pooling recommendation because 0.5 g of tissue is the minimum amount needed to perform virus isolation, but not necessarily the other test methods referenced in this chapter.

In Table 4.1, the Commission agreed to remove serology (ELISA) from the table as it is not used for KHV. For the real-time PCR, the Commission also agreed to increase the ratings for all categories in purposes A *Surveillance of apparently healthy animals* and B *Presumptive diagnosis of clinically affected animals* to '+++' and the level of validation to '3'. The Commission did not agree to score the conventional PCR for purpose A *Surveillance of apparently healthy animals* because the sensitivity is considered too low. Finally the Commission agreed to modify footnote 2 (also see Item 6.2.1.1.).

In Section 4.3. Cell culture for virus isolation, the Commission deleted 'KFC' from the recommended cell line as it is not preferred for virus isolation. The remaining cell lines (CCB and KF-1) were reordered according to their usefulness. In the subsection 'Confirmatory identification', a few edits were made including correcting a reference and organising all the references to place it at the end of the paragraph.

In Section 4.4. Nucleic acid amplification, the Commission placed all the information on controls in a paragraph at the beginning of the section as they are common to both real-time and conventional PCR.

In Section 4.4.2. Real-time PCR, the Commission agreed to delete a sentence at the end of the first paragraph stating that positive results obtained by real-time PCR should be confirmed by conventional PCR and sequence analysis as this information is more suited to and given in the case definitions in Section 6. Finally, the Commission agreed to add a footnote to Table 4.4.2.1 clarifying that the Gilad *et al.* (2004) assay had been modified by Clouthier *et al*. (2017) by increasing the probe quantity.

In Section 4.4.3. Conventional PCR, the Commission agreed to add a footnote to Table 4.4.3.1. clarifying that the Bercovier *et al*. (2005) assay performed using different cycling conditions has been validated by Clouthier *et al*. (2017). The cycling conditions given for the Bercovier *et al*. assay were also corrected.

In Section 4.4.4. Other nucleic acid amplification methods, the Commission did not agree to a proposal to include a protocol for the LAMP method noting that the cited reference included sufficient details for users to undertake the method.

In Section 4.10. Other methods, the existing text on the ELISA was deleted as serology is not relevant in this chapter. The text was replaced with the words 'none published or validated'.

In response to various comments received on Section 5 Tests recommended for surveillance to demonstrate disease freedom in apparently healthy populations, the Commission clarified that real-time PCR assays are recommended for surveillance in apparently healthy animals but may not detect the KHV variants that were described by Englesma *et al.* (2013). In areas where these variants may be present, the conventional nested PCR assay published by Engelsma *et al*. (2013) should be considered.

In Section 6 Corroborative diagnostic criteria, the Commission did not agree with a general comment not to separate the case definitions into 'apparently healthy' and 'clinically affected' animals. The Commission reminded Members that this approach in the disease chapter template was first provided to Members for comment in the report of the February 2018 meeting before being implemented from February 2019. The template has been consistently applied to all the fish disease-specific chapters, and the case definitions are consistent with the purposes of the diagnostic tests given in Table 4.1 (also see Item 4.1. of the Commission's February 2021 report).

In Section 6.1. Apparently healthy animals or animals of unknown health status, the Commission did not agree to add text stating that healthy populations are sampled 'for early detection of disease' as this purpose is not addressed in Section 5.

In Sections 6.1.1., 6.1.2. and 6.2.2., the Commission edited existing criteria and included new criteria as needed. The Commission did not agree with a comment to delete several criteria from Section 6.2.2. as it is deviating from the harmonised approach adopted in all chapters.

For the Table in Section 6.3.2. For surveillance of apparently healthy animals, the Commission added a footnote to clarify that the diagnostic accuracy study did not include samples that were known to be positive for KHV variants.

The revised Chapter 2.3.6. Infection with koi herpesvirus, is presented in **Annex 20** for Member comments.

6.1.6. Chapter 2.3.7. Infection with red sea bream iridoviral disease

The Aquatic Animals Commission reviewed Chapter 2.3.7. Infection with red sea bream iridovirus, which had been updated by the OIE Reference Laboratory experts and reformatted using the new disease chapter template.

The main amendments include:

- updated the scope of the chapter;
- updated information on the aetiological agent;
- updated sections on disease pattern, biosecurity and disease control strategies, and on specimen selection, sample collection, transportation and handling;
- updated the section on diagnostic methods including completing Table 4.1 and revising the molecular tests, the indirect fluorescent antibody test and immunocytochemistry; and
- revised definitions of suspect and confirmed case in apparently healthy and clinically affected animals.

The Commission is aware that other viruses in the Genus *Megalocytivirus*, for example, infectious spleen and kidney necrosis virus (ISKNV) and turbot reddish body iridovirus (TRBIV), may also cause disease of fish. These viruses are not currently listed by the OIE and are not included within the scope of the infection with red sea bream iridovirus (RSIV) chapter. If ISKNV, TRBIV or other megalocytiviruses were to be listed, the viruses would need to be assessed against the listing criteria in Chapter 1.2. of the *Aquatic Code*. If they were found to fulfil the listing criteria, they could be proposed for listing to the OIE General Assembly. In the meantime, this chapter remains focused on infection with RSIV.

The revised Chapter 2.3.7. Infection with red sea bream iridovirus, is presented as **Annex 21** for Member comments.

6.1.7. Susceptible species of Section 2.4. Diseases of molluscs

6.1.7.1. Sections 2.2.1. and 2.2.2. of Chapter 2.4.1. Infection with abalone herpesvirus (susceptibility of species)

The Aquatic Animals Commission amended Sections 2.2.1. and 2.2.2. of Chapter 2.4. Infection with abalone herpesvirus, in line with the recommendations of the *ad hoc* Group on Susceptibility of mollusc species to infection with OIE listed diseases (also see Item 5.1.9.1.).

The Commission encouraged Members to refer to the *ad hoc* Group's June 2021 report available on the OIE Website (Link to add), for details of the assessments conducted by the *ad hoc* Group.

The revised Sections 2.2.1. and 2.2.2. of Chapter 2.4.3. Infection with abalone herpesvirus, are presented as **Annex 22** for Member comments.

6.1.7.2. Sections 2.2.1. and 2.2.2. of Chapter 2.4.2. Infection with Bonamia exitiosa (susceptible species)

Comments were received from the EU.

Background

At its February 2021 meeting, the Aquatic Animals Commission reviewed the December 2020 report of the *ad hoc* Group on Susceptibility of mollusc species to infection with OIE listed diseases. The *ad hoc* Group 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 *Bonamia exitiosa*.

The Commission had agreed to amend Sections 2.2.1. and 2.2.2. of Chapter 2.4.2. Infection with *Bonamia exitiosa* in line with the recommendations made by the ad hoc Group (report available at: https://www.oie.int/en/what-we-do/standards/standards-setting-process/ad-hoc-groups/).

Previous Commission reports where this item was discussed:

February 2021 (Part B: Item 3.2., page 13).

September 2021

Members agreed with the proposed modifications.

The revised Sections 2.2.1. and 2.2.2. of Chapter 2.4.2. Infection with *Bonamia exitiosa* are presented as **Annex 23** for Member comments.

6.1.7.3. Table 4.1. OIE recommended diagnostic methods and their level of validation for surveillance of apparently health animals and investigation of clinically affected animals

Table 4.1 of the new *Aquatic Manual* chapter template includes a column for the level of validation of each test method (from 1 to 4 in accordance with Chapter 1.1.2. Principles and methods of validation of diagnostic assays for infectious diseases) in addition to rating each test against its purpose of use. Having two different scoring systems for two different components of diagnostic test use and interpretation has led to some confusion. Questions about divergence between the two scores have often arisen (e.g. low level of validation but high rating for purpose of use or vice versa).

To address this issue, the Commission revised the explanatory text for Table 4.1 in the disease chapter template: the key and ratings against purposes of use of each test has been clarified, and a note linking the validation stage to Chapter 1.1.2. has been included. The new key and explanatory text would be sent to the OIE References Laboratories for feedback before it is included in the chapter template. Should be feedback be positive, the new key could be applied to the chapters presented for adoption in May 2022.

The new key and explanatory text in Table 4.1. OIE recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals, are presented as **Annex 24** for Member comments.

6.2. Texts for Members' information

6.2.1. Disease Chapter Template

6.2.1.1. Horizontal amendments

The Commission agreed to amend footnote 2 of Table 4.1 *OIE recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals* from 'Early and juvenile life stages have been defined in Section 2.2.3.' to 'Susceptibility of early and juvenile life stages is described in Section 2.2.3.' and to amend Section 2.2.3. Likelihood of infection by species, host life stage, population or sub-populations, where necessary.

6.2.1.2. Section 3.5.3. Samples for histopathology, immunohistochemistry or in-situ hybridisation

A Member had noted inconsistencies in the information provided in Section 3.5.3. Samples for histopathology, immunohistochemistry or in-situ hybridisation, of the *Aquatic Manual* disease-specific chapters, with some chapters providing many details, while other chapters only providing a reference to Chapter 2.3.0. To address this issue, the Commission agreed that the following standard text would be applied to the section in all the disease chapters:

'Standard sample collection, preservation and processing methods for histological techniques can be found in Section 2.2. of Chapter 2.3.0. General information (diseases of fish).'

Where additional disease-specific information is required, this would be included on a case by case basis.

The text has been added to the template.

6.2.2. Chapter 2.3.1. Infection with *Aphanomyces invadans* (epizootic ulcerative syndrome)

The Aquatic Animals Commission reviewed the first draft of Chapter 2.3.1. Infection with *Aphanomyces invadans* (epizootic ulcerative syndrome), which had been reformatted using the new disease chapter template. The chapter had been reviewed by laboratory experts and a member of the Commission, but the task was challenging because of the absence of an OIE Reference Laboratory for this disease. The Commission agreed to work further on the revision, in particular on Section 4. Diagnostic methods including Table 4.1. OIE recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals, and on Section 6. Corroborative diagnostic criteria. The further revised chapter would be reviewed again at the next meeting in February 2022.

6.2.3. Chapter 2.3.9. Infection with spring viraemia of carp virus

A Member had submitted published references on the validation of real-time RT-PCRs for the detection of spring viraemia of carp virus (SVCV). The Commission reviewed the publications and related literature and noted that considerable efforts had been made to validate the assays by the research teams. However, despite the high analytical sensitivity and good specificity, the validation did not fully cover all the SVCV genotypes known to exist, a mismatch of the primers and probes was found by multiple alignment of the related viral fragments, and poor sensitivity was shown when testing some tissue homogenate samples. Therefore the expert advised that the tests cannot be recommended for inclusion in the *Aquatic Manual* until the assays have been further evaluated.

6.2.4. Establishment of the order for review of the *Aquatic Manual* chapters

The Aquatic Animals Commission noted that all the disease chapters in Section 2.3. Diseases of fish, had now been updated and reformatted using the new disease chapter template. The Commission agreed to next address the chapters in Section 2.2. Diseases of crustaceans, and identified the following four chapters to which the template will now be applied:

- Chapter 2.2.1. Acute hepatopancreatic necrosis disease
- Chapter 2.2.2. Infection with *Aphanomyces astaci* (crayfish plague)
- Chapter 2.2.3. Infection with *Hepatobacter penaei* (necrotising hepatopancreatitis)
- Chapter 2.2.4. Infection with infectious hypodermal and haematopoietic necrosis virus.

The OIE Reference Laboratory experts will be asked to assist in the task of updating the texts.

6.2.5. Diseases of Crustaceans: Chapter 2.2.0. General Information

Following the decision to update and reformat the disease chapters in Section 2.2. of the *Aquatic Manual*, the Commission identified the need to update Chapter 2.2.0. General Information. As previously done for Chapter 2.3.0., the Commission will request the assistance of all OIE Reference Laboratory experts for crustacean diseases in this revision.

7. *AD HOC* GROUPS

7.1. *Ad hoc* Group on Tilapia lake virus

The electronic *ad hoc* Group on Tilapia lake virus (TiLV) was established in November 2017 to assess TiLV diagnostics and validation. The *ad hoc* Group worked from November 2017 to September 2021 to:

- evaluate published and unpublished methods for detection of TiLV;
- describe the level of validation of each method and determine additional validation requirements;
- recommend any additional assays that may need to be developed; and
- facilitate the sourcing and distribution of well-characterised positive control material for method evaluation, implementation and two inter-laboratory comparability studies.

The first round of the OIE Inter-laboratory comparability panel for Tilapia lake virus PCR was carried out in 2019 and the second round was carried out in 2021. The *ad hoc* Group recommended that all four tests evaluated would allow criterion 3 of Chapter 1.2. to be fulfilled (see Item 5.1.3.).

The report of the *ad hoc* Group on Tilapia lake virus can be found at (https://www.oie.int/en/what-we-do/standards/standards-setting-process/ad-hoc-groups/)

7.2. *Ad hoc* Group on Susceptibility of mollusc species to infection with OIE listed diseases report (June 2021)

The *ad hoc* Group on Susceptibility of mollusc species to infection with OIE listed diseases met during June 2021 to conduct assessments for susceptibility of mollusc species to infection with abalone herpes virus (see Item 5.1.9.1.).

The Commission was informed that the *ad hoc* Group is planning to meet in November in 2021 to progress its work assessing species susceptible to listed OIE mollusc diseases.

The report of the *ad hoc* Group on Susceptibility of mollusc species to infection with OIE listed diseases (June 2021) can be found at (https://www.oie.int/en/what-we-do/standards/standards-setting-process/ad-hoc-groups/).

7.3. *Ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases

The Commission agreed to request that the *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases be reconvened to complete its work on the applying the criteria of Chapter 1.5., for listing species as susceptible to infection with a specific pathogen to OIE listed diseases, for the remaining two diseases: infection with red seabream iridovirus and infection with *Aphanomyces invadans*.

7.4. *Ad hoc* Group on new draft Chapters 4.X. Emergency disease preparedness and 4.Y. Disease outbreak management

The Commission requested that an *ad hoc* Group be convened to develop the text of the two new chapters and requested that the *ad hoc* Group start work on the new draft Chapter 4.X. Emergency disease preparedness (also see Item 5.2.2.).

8. OIE REFERENCE CENTRES OR CHANGE OF EXPERTS

8.1. Further develop SOPs to include provisions for suspending laboratories and for handling laboratories that temporarily have no designated expert

The *Procedures for the designation of OIE Reference Laboratories* (SOPs) were adopted in 2017 and have been implemented by this Commission and the Biological Standards Commissions since then. More recently, it has become apparent that parts of the SOPs need to be updated, for example the time line for the achievement of accreditation to a quality management system can be deleted from the SOPs as the final deadline of 31 December 2019 is past. Implementation of the SOPs has also revealed the need for a procedure for temporary suspension of OIE Reference Laboratory status, for example because of temporary lack of diagnostic ability due to construction or restructuring of the laboratory's facilities. The need was also identified for a procedure to be followed when the nomination for a replacement expert is not endorsed by the Specialist Commission leaving a laboratory without a designated expert for a short period of time. The Aquatic Animal Commission reviewed and approved text proposed by the Secretariat to address these issues. The SOPs had been approved by the Biological Standards Commissions in February 2021. They would now be presented to the Director General for endorsement. If endorsed, the amended SOPs would be appended to the report of the February 2022 meeting of the Biological Standards Commission and uploaded to the web page.

8.2. Follow-up on the Biological Standards Commission's consultation with the Council

The Aquatic Animals Commission was updated on a consultation that had taken place between the OIE Council and the Biological Standards Commission on the appointment of OIE Reference Laboratory experts, applications from private companies for Reference Centre status, and lack of testing of international samples by OIE Reference Laboratories (see report of the meeting of the OIE Biological Standards Commission, February 2021 Part B, agenda Item 4.5.).

8.3. Evaluation of applications for OIE Reference Centres for aquatic animal health issues or change of experts

The Aquatic Animals Commission recommended acceptance of the following application for OIE Reference Centre status:

OIE Collaborating Centre for Antimicrobial Stewardship in Aquaculture

Laboratory of Veterinary Pharmacology (FARMAVET) and Laboratory of Food Safety (LIA) and Center for Research and Innovation in Aquaculture (CRIA), University of Chile, Faculty of Veterinary and Animal Sciences, Santa Rosa 1735, La Pintana, Region Metropolitana, CHILE
Tel.: (+56-2) 29.78.55.80 / 29.78.03.52
E-mail: bsmartin@uchile.cl; jcornejo@uchile.cl Website: http://www.veterinaria.uchile.cl
Contact Point: Dr Betty San Martin Nunez.

8.4. Explore candidates as Reference Laboratory for Infection with decapod iridescent virus 1

The Aquatic Animals Commission noted the need to designate an OIE Reference Laboratory for infection with decapod iridescent virus 1 following its listing in May 2021. The Commission considered OIE Members that could potentially host an OIE Reference Laboratory for this disease. The Commission proposed that the Delegates of the Members concerned be asked to consider supporting an application

from suitable institutes in their countries. The Commission also invites applications from any Member with expertise in this disease.

9. Next meeting

The next meeting of the Aquatic Animals Commission is scheduled for 16–23 February 2022.

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…/Annexes

MEETING OF THE OIE
AQUATIC ANIMAL HEALTH STANDARDS COMMISSION

Virtual meeting, 22–29 September 2021

List of participants

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Return to Agenda

WORK PLAN FOR THE AQUATIC ANIMALS COMMISSION

September 2021–May 2022

Aquatic Code – Current work			
Chapter/Subject	**Status**		
	September 2021	**February 2022**	**May GS 2022**
User's Guide	Reviewed amended articles and presented for comments	Review comments (1st round)	Propose for adoption
Glossary definitions: 'Competent Authority', 'Veterinary Authority' and 'Aquatic Animal Health Services'	Reviewed comments (1st round) and presented for comments	Review comments (2nd round)	Propose for adoption
Glossary definitions: 'Basic biosecurity conditions', 'Biosecurity plan', 'Early detection system' and 'Passive surveillance'	Reviewed comments (1st round) and presented for comments	Review comments (2nd round)	Propose for adoption
Chapter 1.3. Listing of infection with tilapia lake virus (TiLV)	Reassessed infection with TiLV for listing	Review comments (1st round)	Propose for adoption
Chapter 1.4. Aquatic Animal Health Surveillance	Reviewed comments (1st round)	Review comments (2nd round)	Propose for adoption
Model Articles X.X.4. to X.X.8. for disease-specific chapters to address declaration of freedom from [Pathogen X]	Reviewed comments (2nd round)	Review comments (3rd round)	Propose for adoption
New emerging diseases	On-going	On-going	
Chapter 4.X. New draft chapter on Emergency disease preparedness	Requested an *ad hoc* Group be convened	Consider *ad hoc* Group report	
Chapter 4.Y. New draft chapter on Disease outbreak management	*Ad hoc* Group to be convened	*Ad hoc* Group to start drafting after completion of Chapter 4.X.	
Articles 8.X.3. – Amphibian		Review amended articles and present for comments	
Articles 9.X.3. – Crustacean	Reviewed comments (1st round)	Review comments (2nd round)	Propose for adoption
Chapter 9.X. Infection with decapod iridescent virus 1	Reviewed draft chapter and presented for comments	Review Comments (1st round)	Propose for adoption
Articles 10.X.1. and 10.X.2. for: - Infection with Red seabream iridovirus/infectious sleen and kidney necrosis virus (ISKNV) - Infection with *Aphanomyces invadans* (Epizootic ulcerative syndrome)	Requested *ad hoc* Group be reconvened		

Aquatic Code – Current work			
Chapter/Subject	**Status**		
	September 2021	**February 2022**	**May GS 2022**
Articles 10.X.3. - Fish	Reviewed amended articles and presented for comments	Review comments (1st round)	Propose for adoption
Chapter 10.1. Infection with epizootic haematopoietic necrosis virus	Reviewed amended articles and presented for Member comments	Review 1st round Member comments	Propose for adoption
Chapter 10.7. Infection with koi herpes virus	Reviewed amended articles and presented for Member comments	Review 1st round Member comments	Propose for adoption
Articles 11.1.1, and 11.1.2, of Chapter 11.1. Infection with abalone herpesvirus	Reviewed *ad hoc* Group report and presented the revised articles for comments	Review comments (1st round)	Propose for adoption
Articles 11.2.1. and 11.2.2. of Chapter 11.2. Infection with *Bonamia exitiosa*	Review comments (1st round)	Review comments (2nd round)	Propose for adoption
Articles 11.X.1. and 11.X.2. for: - Infection with *Xenohaliotis californiensis* - Infection with *Marteilia refringens* - Infection with *Perkinsus marinus* - Infection with *Perkinsus olseni*	Requested *ad hoc* Group continue its assessments	Review *ad hoc* Group report and present amended articles for comment	
Articles 11.X.3. - Mollusc		Review amended articles and present for comments	
The use of environmental DNA methods for aquatic animal disease surveillance	Reviewed comments (1st round)	Review Member comments and upload onto OIE website	
Levels of validation and the test scoring system in Table 4.1	Reviewed comments and sent to Reference Laboratories for feedback	Review feedback from Reference Laboratories	
Chapter 2.3.2. Infection with epizootic haematopoietic necrosis virus	Updated and reformatted and presented for comments	Review comments (1st round)	
Chapter 2.3.1. Infection with *Aphanomyces invadans* (epizootic ulcerative syndrome)	Updated and reformatted	Review further updated draft and present for comments	
Chapter 2.3.4. Infection with HPR-deleted or HPR0 infectious salmon anaemia virus	Reviewed Member comments (1st round) and presented for comment	Review comments (2nd round)	Propose for adoption
Chapter 2.3.6. Infection with koi	Reviewed comments (1st round) and	Review comments	Propose for

Aquatic Code – Current work			
Chapter/Subject	**Status**		
	September 2021	**February 2022**	**May GS 2022**
herpesvirus	presented for comment	(2nd round)	adoption
Chapter 2.3.7. Red sea bream iridoviral disease	Updated and reformatted and presented for comments	Review comments (1st round)	
Sections 2.2.1., 2.2.2. of Chapter 2.4.1. Infection with abalone herpesvirus	Reviewed *ad hoc* Group report and presented revised articles for comments	Review comments (1st round)	Propose for adoption
Sections 2.2.1., 2.2.2. of Chapter 2.4.2. Infection with *Bonamia exitiosa*	Reviewed comments (1st round) and presented for comments	Review comment (2nd round)	Propose for adoption

Return to Agenda

USER'S GUIDE

A. Introduction

1) The OIE *Aquatic Animal Health Code* (hereafter referred to as the *Aquatic Code*) establishes standards for the improvement of aquatic animal health worldwide. The *Aquatic Code* also includes standards for the welfare of farmed fish and use of antimicrobial agents in aquatic animals. The purpose of this guide is to advise the Competent Authorities in OIE Member Countries on how to use the *Aquatic Code*.

2) Competent Authorities should use the standards in the *Aquatic Code* to develop measures for prevention including biosecurity at aquaculture establishments, early detection, internal reporting, notification, control or eradication of pathogenic agents in aquatic animals (amphibians, crustaceans, fish and molluscs) and preventing their spread via international trade in aquatic animals and aquatic animal products, while avoiding unjustified sanitary barriers to trade.

3) The OIE standards in the *Aquatic Code* are based on the most recent scientific and technical information and are adopted by the World Assembly of Delegates. Correctly applied, they protect aquatic animal health during the production and trade in aquatic animals and aquatic animal products as well as the welfare of farmed fish.

4) The absence of chapters, articles or recommendations on particular pathogenic agents or aquatic animal products does not preclude the application of appropriate sanitary measures by the Competent Authorities, provided they are based on risk analyses conducted in accordance with the *Aquatic Code*.

5 The year that a chapter was first adopted and the year of last revision are noted at the end of each chapter.

6) The complete text of the *Aquatic Code* is available on the OIE website at and individual chapters may be downloaded from: http://www.oie.int.

B. *Aquatic Code* content

1) Key terms and expressions used in more than one chapter in the *Aquatic Code* are defined in the Glossary, where common dictionary definitions are not deemed to be adequate. The reader should be aware of definitions given in the Glossary when reading and using the *Aquatic Code*. Defined terms appear in italics. In the online version of the *Aquatic Code*, a hyperlink leads to the relevant definition.

2) The term '(under study)' is found in some rare instances, with reference to an article or part of an article. This means that this part of the text has not been adopted by the World Assembly of OIE Delegates and the particular provisions are thus not part of the *Aquatic Code*.

3) The standards in the chapters of Section 1 are designed for the implementation of measures for the surveillance and notification of pathogenic agents. The section includes the criteria for listing aquatic animal diseases, the diseases which are listed by the OIE, procedures for notification to the OIE, and criteria for listing species as susceptible to infection with a specific pathogenpathogenic agent.

4) The standards in the chapters of Section 2 are designed to guide the importing country in conducting import risk analysis in the absence of OIE standards. The importing country should also use these standards to justify import measures which are more stringent than existing OIE standards.

5) The standards in the chapters of Section 3 are designed for the establishment, maintenance and evaluation of Aquatic Animal Health Services, including communication. These standards are intended to assist the Competent Authorities of Member Countries to meet their objectives of improving aquatic animal health and the welfare of farmed fish, as well as to establish and maintain confidence in their international aquatic animal health certificates.

6) The standards in the chapters of Section 4 are designed for the implementation of measures for the prevention and control of pathogenic agents. Measures in this section include biosecurity for aquaculture establishments, zoning, compartmentalisation, disinfection, contingency planning, fallowing, disposal of aquatic animal waste and control of pathogenic agents in aquatic animal feed.

7) The standards in the chapters of Section 5 are designed for the implementation of general sanitary measures for trade. They address certification and the measures applicable by the exporting, transit and importing countries. A range of model international aquatic animal health certificates is provided to facilitate consistent documentation for international trade.

8) The standards in the chapters of Section 6 are designed to ensure the responsible and prudent use of antimicrobial agents in aquatic animals.

9) The standards in the chapters of Section 7 are designed for the implementation of welfare measures for farmed fish. The standards cover the general principles for welfare of farmed fish, including during transport, stunning and killing for human consumption, and when killing for disease control purposes.

10) The standards in each of the chapters of Sections 8 to 11 are designed to prevent the pathogenic agents of OIE listed diseases from being introduced into an importing country. Each disease chapter includes a list of currently known susceptible species. The standards take into account the nature of the traded commodity, the aquatic animal health status of the exporting country, zone or compartment, and the risk reduction measures applicable to each commodity.

These standards assume that the agent is either not present in the importing country or is the subject of a control or eradication programme. Sections 8 to 11 each relate to amphibian, crustacean, fish and molluscan hosts, respectively.

C. Specific issues

[…]

Return to Agenda

GLOSSARY

BASIC BIOSECURITY CONDITIONS

means a minimum set of conditions, as described in Article 1.4.6., required to ensure *biosecurity* for a ~~particular~~ specific *disease*, in a country, *zone* or *compartment*. ~~that should include:~~

~~a) compulsory notification of the disease or suspicion of the disease to the Competent Authority; and~~

~~b) an early detection system; and~~

~~c) requirements to prevent the introduction of the pathogenic agent into a free country, zone or compartment, or the spread within or from infected zones and protection zones, in accordance with the relevant disease-specific chapter.~~

BIOSECURITY PLAN

means a document that identifies potential pathways for the introduction of *pathogenic agents* into, or spread within, or release from, a *zone*, *compartment* or *aquaculture establishment* and describes the measures applied to mitigate the identified *risk*, in accordance with the recommendations in ~~the Aquatic Code~~ Chapter 4.1.

EARLY DETECTION SYSTEM

means ~~an efficient~~ system, as described in Article 1.4.7., ~~for ensuring~~ which ensures 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~~ an investigation by the *Aquatic Animal Health Services* with minimal delay. ~~Such a system will include the following characteristics:~~

~~a) 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*;~~

~~b) *veterinarians* or *aquatic animal health professionals* trained in recognising and reporting suspicions of *disease* occurrence;~~

~~c) ability of the *Aquatic Animal Health Services* to undertake rapid and effective *disease* investigation based on a national chain of command;~~

~~d) access by the *Aquatic Animal Health Services* to laboratories with the facilities for diagnosing and differentiating *listed diseases* and *emerging diseases*;~~

~~e) the legal obligation of private *veterinarians* or *aquatic animal health professionals* to report suspicions of *disease* occurrence to the *Competent Authority*.~~

PASSIVE SURVEILLANCE

means ~~the generation of observer-initiated~~ *aquatic animal* health ~~data~~ surveillance typically based on observations of clinical or behavioural signs of *disease*, production information, or mortality rates which are generated by an *early detection system*.

Return to Agenda

GLOSSARY CONTINUED

AQUATIC ANIMAL HEALTH SERVICES

means the combination of governmental and non-governmental individuals and organisations that perform activities to implement the standards of the *Aquatic Code* in the territory. The Aquatic Animal Health Services are under the overall control and direction of the *Competent Authority*. Private sector organisations, *veterinarians* or *aquatic animal health professionals* are normally accredited or approved by the *Competent Authority* to deliver the delegated functions.

AQUATIC ANIMAL HEALTH SERVICES (CLEAN VERSION)

means the combination of governmental and non-governmental individuals and organisations that perform activities to implement the standards of the *Aquatic Code*.

COMPETENT AUTHORITY

means the a *Veterinary Authority* or other Governmental Authority of a Member Country having the responsibility and competence for ensuring or supervising the implementation of *aquatic animal* health and welfare measures, international health certification and other in the whole or part of the territory for the implementation of certain standards and recommendations in of the *Aquatic Code* in the whole territory.

COMPETENT AUTHORITY (CLEAN VERSION)

means a Governmental Authority of a Member Country having the responsibility in the whole or part of the territory for the implementation of certain standards of the *Aquatic Code*.

VETERINARY AUTHORITY

means the Governmental Authority of a Member Country, comprising *veterinarians*, other professionals and paraprofessionals, having the primary responsibility and competence for ensuring or supervising in the whole territory for coordinating the implementation of *aquatic animal* health and welfare measures, international *aquatic animal* health certification and other the standards and recommendations in of the *Aquatic Code* by *Competent Authorities* in the whole *territory*. The *Veterinary Authority* is a *Competent Authority*.

VETERINARY AUTHORITY (CLEAN VERSION)

means the Governmental Authority of a Member Country having the primary responsibility in the whole territory for coordinating the implementation of the standards of the *Aquatic Code* by *Competent Authorities*.

Return to Agenda

CHAPTER 1.3.

DISEASES LISTED B Y THE OIE

The *diseases* in this chapter have been assessed in accordance with Chapter 1.2. and constitute the OIE list of *aquatic animal diseases*.

In case of modifications of this list of *aquatic animal diseases* adopted by the World Assembly of Delegates, the new list comes into force on 1 January of the following year.

Article 1.3.1.

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

– Infection with *Aphanomyces invadans* (epizootic ulcerative syndrome)

– Infection with epizootic haematopoietic necrosis virus

– Infection with *Gyrodactylus salaris*

– Infection with HPR-deleted or HPR0 infectious salmon anaemia virus

– Infection with infectious haematopoietic necrosis virus

– Infection with koi herpesvirus

– Infection with red sea bream iridovirus

– Infection with salmonid alphavirus

– Infection with spring viraemia of carp virus

– Infection with tilapia lake virus

– Infection with viral haemorrhagic septicaemia virus.

[…]

Return to Agenda

ASSESSMENT FOR LISTING INFECTION WITH TILAPIA LAKE VIRUS (TiLV) IN THE *AQUATIC CODE*

Overall assessment

The OIE Aquatic Animal Health Standards Commission assessed infection with tilapia lake virus (TiLV) against the criteria for listing aquatic animal diseases in Article 1.2.2. of the *Aquatic Code* (see Table 1 below).

Table 1. Summary of assessment of infection with TiLV

	Listing criteria						Conclusion
	1	2	3	4a	4b	4c	
Infection with TiLV	+	+	+	NA	+	+	The disease meets the criteria for listing

NA = not applicable.

The criteria for the inclusion of a disease in the OIE list are as follows:

1. International spread of the pathogenic agent (via aquatic animals, aquatic animal products, vectors or fomites) is likely.

AND

2. At least one country may demonstrate country or zone freedom from the disease in susceptible aquatic animals, based on provisions of Chapter 1.4.

AND

3. A precise case definition is available and a reliable means of detection and diagnosis exists.

AND

4a. Natural transmission to humans has been proven, and human infection is associated with severe consequences.

OR

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.

OR

4c. The disease has been shown to, or scientific evidence indicates that it would affect the health of wild resulting in significant consequences e.g. morbidity or mortality at a population level, reduced productivity or ecological impacts.

Background

A novel orthomyxo-like virus, named as tilapia lake virus (TiLV), has been identified as the cause of mass die-offs of tilapia (Eyngor *et al.*, 2014) in both farms and the wild environment. The host range is not well known but a number of tilapines are known to be susceptible (Eyngor *et al.*, 2014). Tilapia is the second most imported group of farmed fish after carps. Global production of tilapia, predominantly *Oreochromis niloticus*, is estimated at 4.5 million metric tonnes (FAO data). Farming occurs primarily in tropical and subtropical countries though some production in recirculation systems has started in other regions. *O. niloticus* was first introduced to developing countries to support subsistence farming. However, larger scale commercial production is now important and frozen fillet and other tilapia products are traded globally.

Assessment of TiLV using the new criteria for listing aquatic animal diseases in Chapter 1.2. of the *Aquatic Code*

Criterion No. 1 International spread of the pathogenic agent (via aquatic animals, aquatic animal products, vectors or fomites) is likely.

Assessment

TiLV has been reported in Bangladesh, Chinese Taipei, Colombia, Ecuador, Egypt, India, Indonesia, Israel, Malaysia, Mexico, Peru, Philippines, Tanzania, Thailand, Uganda and the United States of America (Ahasan *et al*., 2020, Amal *et al*., 2018, Bacharach *et al*., 2016; Behera *et al*., 2018; Chaput *et al*., 2020; Dong *et al*., 2017; Fathi *et al*., 2017, Ferguson *et al*., 2014; Koesharyani *et al*., 2018, Mugimba., 2018, OIE, 2018a, OIE, 2018b; OIE, 2018c; Tsofack *et al*., 2016). The Network of Aquaculture Centres in Asia–Pacific (NACA) also have notification requirements for infection with TiLV and this data shows a similar distribution of the disease for that region, as reported to the OIE. Despite geographic separation; strains were highly homologous, suggesting an epidemiological link and international spread. Historically, tilapia has been traded internationally to establish populations for production in new regions, and there is still extensive trade in tilapia. The current driver for international trade is the dissemination of improved genetic strains (though current pattern and volume of trade has not been determined for this assessment). Tilapia products are traded internationally and while a risk of transmission with some product types should be expected, specific risks have not been considered in this assessment.

Given the evidence of spread and the broad distribution of tilapia (Asia, Africa and South America), international spread is likely.

Conclusion

The criterion is met.

Criterion No. 2 At least one country may demonstrate country or zone freedom from the disease in susceptible aquatic animals, based on provisions of Chapter 1.4.

TiLV has been reported in Bangladesh, Chinese Taipei, Colombia, Ecuador, Egypt, India, Indonesia, Israel, Malaysia, Mexico, Peru, Philippines, Tanzania, Thailand, Uganda and the United States of America (Ahasan *et al*., 2020; Amal *et al*., 2018; Bacharach *et al*., 2016; Behera *et al*., 2018; Chaput *et al*., 2020; Dong *et al*., 2017; Fathi *et al*., 2017; Ferguson *et al*., 2014; Koesharyani *et al*., 2018; Mugimba *et al*., 2018; OIE, 2018a; OIE, 2018b; OIE, 2018c; Tsofack *et al*., 2016). The Network of Aquaculture Centres in Asia – Pacific (NACA) also have notification requirements for infection with TiLV and this data shows a similar distribution of the disease for that region, as reported to the OIE. Additional countries in Africa have expressed a wish to declare freedom from infection with TiLV, but report that there is a lack of diagnostic capacity to support such self-declarations.

The distribution of the virus may be wider (mortality may not have been investigated in other regions); however, due to the broad distribution of tilapia (Asia, Africa and South America), virulence of the virus and the extensive trade in tilapia, it is likely that many countries are currently free. The information provided to the OIE and NACA on the disease status of Members for infection with TiLV through immediate notifications, six-monthly reports and annual reports provides support that it is likely countries are currently free of the disease.

Table 2. Outbreaks of infection with TiLV by country and commencement year notified to the OIE through the OIE-WAHIS.

Region or Country	**2017**	**2018**	**2019**	**2020**	**2021***
Americas					
Colombia				1	
Mexico		20	1		
Peru		5	2	1	
USA			3		
Asia					
Chinese Taipei	9				
India			3		
Malaysia	2	2			
Philippines	1		1		
Thailand	1				
Europe					
Israel	16 (Tilapia syncytial hepatitis)				
Total	29	27	10	2	

*No notifications have been notified to the OIE in 2021 to date.

Conclusion

The criterion is met.

Criterion No. 3 A precise case definition is available and a reliable means of detection and diagnosis exists.

An *ad hoc* Group was convened in 2017 on request from the Commission with the objective to assess TiLV diagnostics and validation, and specifically:

- evaluate published and unpublished methods for detection of TiLV;
- describe the level of validation of each method and determine additional validation requirements;
- recommend any additional assays that may need to be developed;
- and facilitate the sourcing and distribution of well-characterised positive control material for method evaluation, implementation and two inter-laboratory comparability studies.

The *ad hoc* Group undertook TiLV inter-laboratory panel testing in two stages. Round 1 involved two laboratories and four molecular assays and Round 2 involved seven laboratories and four molecular assays. The *ad hoc* Group provided recommendations based on results of testing for both rounds.

The *ad hoc* Group evaluated three real-time PCR assays and one conventional nested PCR for their ability to reliably detect TiLV in an inter-laboratory comparison using a panel of 30 samples. All assays performed as expected and could reliably detect TiLV. Based on the recommendations of the *ad hoc* Group, the Commission considered all four tests evaluated would allow criterion 3, a precise case definition is available and a reliable means of detection and diagnosis exist, of Chapter 1.2. of the *Aquatic Code*, to be fulfilled.

Conclusion

The criterion is met.

Criterion No. 4a Natural transmission to humans has been proven, and human infection is associated with severe consequences.

Assessment

There is no evidence of transmission to humans.

Conclusion

Criterion not applicable.

Criterion No. 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.

Assessment

Very high levels of mortality (>80%) have been observed in affected populations (both farmed and wild) (Bacharach *et al*., 2016; Behera *et al*., 2018; Ferguson *et al*., 2014; Gophen *et al*., 2015). Dong *et al*. (2017) reported approximately 90% mortality in red tilapia fingerlings within one month of stocking into cages. Since 2009 episodic losses of tilapia (*Oreochromis niloticus*) were recorded in fish farms all over Israel (Eyngor *et al*., 2014). Mortality in farmed *O. niloticus* in Ecuador have also been attributed to TiLV (Ferguson *et al*., 2014). Losses are significant regionally and at a national level.

Conclusion

The criterion is met.

Criterion No. 4c The disease has been shown to, or scientific evidence indicates that it would affect the health of wild resulting in significant consequences e.g. morbidity or mortality at a population level, reduced productivity or ecological impacts.

Assessment

Very high levels of mortality (>80%) have been observed in affected populations (both farmed and wild) (Bacharach *et al*., 2016; Ferguson *et al*., 2014; Gophen *et al*., 2015). Decreases of catch of tilapines, specifically *Sarotherodon* (Tilapia) *galilaeus*, from the Sea of Galilee have been observed since 2007. In 2017, a mortality event in wild tilapia in Malaysia was reported with an estimated 50% mortality (OIE, 2018c).

Conclusion

The criterion is met.

Conclusion

Infection with TiLV clearly meets the criteria for listing (1, 2, 3, 4b and 4c) and is proposed for inclusion in Chapter 1.3. Diseases listed by the OIE.

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Return to Agenda

CHAPTER 1.4.

AQUATIC ANIMAL DISEASE SURVEILLANCE

Article 1.4.1.

Purpose

This chapter provides guidance on the *surveillance* approaches to be used by a *Competent Authority* to make and maintain a *self-declaration of freedom from disease* or to confirm the occurrence of a *listed disease* or an *emerging disease*.

Article 1.4.2.

Introduction and scope

This chapter supports the *Competent Authority* to meet the requirements for *self-declaration of freedom from disease* at the level of a country, *zone* or *compartment*, and for maintenance of freedom, that are presented in each disease-specific chapter. It also provides the *Competent Authority* with guidance to meet the requirements of *notification* of a *listed disease* or an *emerging disease* in accordance with Chapter 1.1.

This chapter is not intended to provide detailed technical guidance on *surveillance* design or analysis. The *Competent Authority* is encouraged to consult published literature and seek appropriate expertise to design and analyse *surveillance* programmes that meet the requirements of the *Aquatic Code*.

1) The general requirements of a *surveillance* system necessary to support a *self-declaration of freedom from disease* are specified in Article 1.4.5. to Article 1.4.8.

2) The criteria that have been used to set the periods specified in each disease-specific chapter for *basic biosecurity conditions* to be in place, or for *targeted surveillance* that should be undertaken, prior to claiming freedom, are included in Article 1.4.9. and 1.4.10.

3) The requirements for each of the four pathways for claiming freedom, and for maintaining freedom, are introduced in Article 1.4.3. and ~~are~~ described in detail in Article 1.4.11. to Article 1.4.15.

4) Guidance on the design of surveys to demonstrate freedom from *disease*, and for combining multiple sources of *surveillance* information are provided in Article 1.4.16. and

Article 1.4.17., respectively.

5) Article 1.4.18. provides guidance on diagnostic confirmation of *listed diseases* or an *emerging disease*.

The *Competent Authority* should refer to the relevant disease-specific chapter of the *Aquatic Manual* for recommendations on sample collection and appropriate diagnostic methods for *surveillance* and diagnosis of *listed diseases*. The relevant disease-specific chapter of the *Aquatic Manual* should also be consulted for the necessary information on epidemiology and diagnostic performance of assays required for *surveillance* programme design.

Article 1.4.3.

Pathways for demonstrating freedom from disease

The *Competent Authority* may use one of four pathways to make a *self-declaration of freedom from disease*. Each pathway outlines the *aquatic animal* health circumstances and requirements that should be met for a self-declaration to be made. Any one of these four pathways may be utilised; however, the *Competent Authority* should provide evidence that all relevant requirements to demonstrate *disease* freedom have been met as described in this chapter and the relevant disease-specific chapter of the *Aquatic Code*. The four pathways are:

1. Absence of susceptible species

This pathway may be utilised if, as described in Article 1.4.11., it can be demonstrated that no *susceptible species* are present.

2. Historical freedom

This pathway may be utilised if, as described in Article 1.4.12., there is evidence of historical absence of a *disease* that is supported primarily by *passive surveillance* ~~data~~ information generated by a country's *early detection system*. *Targeted surveillance* data may also be used in this pathway, where appropriate.

3. Targeted Surveillance

This pathway may be utilised if the requirements of pathway 1 (absence of *susceptible species*) or pathway 2 (historical freedom) cannot be met. The pathway primarily uses targeted *surveillance* data, but other sources of evidence may be utilised as described in Article 1.4.13. *Passive surveillance* information may also be used in this pathway, where appropriate.

4. Returning to freedom

This pathway may be utilised, as described in Article 1.4.14., in circumstances where a self-declaration had been made, but free status was subsequently lost due to detection of the *disease*.

Table 1.1. A summary of the four pathways for *self-declaration of freedom from disease*, including the types of primary and secondary *surveillance* information, and the applicable level of application for either a country, *zone* or *compartment*.

Pathway	**Primary surveillance evidence to claim *disease* freedom**	**~~Proposed~~ ~~s~~Secondary evidence to claim freedom (if required)**	**Applicable level of application**
1. Absence of susceptible species	~~*Active surveillance*~~*Surveys, historical data, import records, environmental information*	None	Country, *zone*
2. Historical freedom	*Passive surveillance*	*Targeted surveillance* (in populations where *passive surveillance* is not appropriate)	Country, *zone*
3. Targeted surveillance	*Targeted surveillance*	*Passive surveillance* (in appropriate populations)	Country, *zone*, *compartment*
4. Returning to freedom	*Targeted surveillance*	*Passive surveillance* (in appropriate populations)	Country, *zone*, *compartment*

Article 1.4.4.

Publication by the OIE of a self-declaration of freedom from disease by a Member Country

A Member Country may make a *self-declaration of freedom from disease* in a country, *zone* or *compartment*. The Member Country ~~may~~should inform the OIE of the claimed status for a country, *zone* or *compartment* and the OIE may publish the self-declaration.

A Member Country requesting the publication of a self-declaration should follow the Standard Operating Procedure (~~under development~~available on the OIE website) for submission and provide documented information on its compliance with the relevant chapters of the *Aquatic Code.* This information should include, but is not limited to the following:

1) the scope of the declaration, i.e. the specific *disease*, the level of freedom (country, *zone* or *compartment*) and the pathway utilised to claim or return to *disease* freedom;

2) information to confirm that ~~the general requirements of~~ *basic biosecurity conditions* and the requirements of *surveillance* systems have been met;

3) details of the *surveillance* design and assumptions;

4) the *surveillance* analysis and results;

5) the measures implemented to maintain freedom.

The *self-declaration of freedom from disease* ~~may~~will be published only after all the information provided has been received and administrative and technical screening has been performed by the OIE, with a satisfactory outcome. Publication does not however, imply endorsement of the claim of freedom by the OIE and does not reflect the official opinion of the OIE. Responsibility for the accuracy of the information contained in a self-declaration lies entirely with the OIE Delegate of the Member Country concerned.

Except when otherwise provided for in the disease-specific chapter, an *outbreak* in a Member Country, a *zone* or a *compartment* having a self-declared free status results in the loss of the self-declared free status. The notification of an outbreak in a country, *zone* or *compartment* for which a self-declaration of freedom has been made, will result in an update of the OIE website concerning the original declaration. A Member Country wishing to reclaim a lost free status should submit a new self-declaration following the procedure described in this chapter.

Article 1.4.5.

Biosecurity and surveillance system requirements

The following biosecurity and *surveillance* system requirements should be met for any *self-declaration of freedom from* disease in the given *compartment, zone* or country:

1) the quality of *Aquatic Animal Health Services* can be substantiated to meet the requirements of Chapter 3.1.:

2) *basic biosecurity conditions* as described in Article 1.4.6. are in place;

3) an *early detection system* as described in Article 1.4.7. is in place;

4) there has been no vaccination of *susceptible aquatic animals* for the specific *disease* for at least the period that *basic biosecurity conditions* have been applied prior to self-declaration;

5) the *Aquatic Animal Health Services* have sufficient capacity and expertise to investigate and report *disease* events to the *Competent Authority*;

6) the *Competent Authority* has access to appropriate diagnostic capability (from a laboratory with a quality management system that meets requirements of Chapter 1.1.1. of the *Aquatic Manual*) to confirm or exclude cases of *listed diseases* and *emerging diseases* in accordance with Article 1.4.18.

Article 1.4.6.

Basic biosecurity conditions

Basic biosecurity conditions include requirements for preventing the introduction and spread of ~~a~~one specific *disease* and for detection of the *disease* should it occur. The requirements for *basic biosecurity conditions* include:

~~1) a compulsory requirement for *notification* of a specific *disease*, or suspicion of the *disease*, to the *Competent Authority*;~~

~~2~~1) an *early detection system* (as described in Article 1.4.7.);

~~3~~2) measures to prevent the introduction of the *pathogenic agent* into a country, *zone* or *compartment*, or the spread within or from *infected zones* and *protection zones*, in accordance with the relevant disease-specific chapter.

In making a self-declaration of freedom from a specific *disease* for a country, *zone* or *compartment,* the C*ompetent Authority* should describe how all of the requirements for ~~the~~ *basic biosecurity conditions* relevant to its declaration, ~~and ensure all requirements for *basic biosecurity conditions* described in this chapter~~ are continuously met.

Article 1.4.7.

Early detection system

The *early detection system* of the *Competent Authority* ~~underpins~~ is important to ~~any~~collect *passive surveillance* ~~data~~ information utilised by a *Competent Authority* to make a *self-declaration of freedom from disease*.

A *self-declaration of freedom from disease* needs to document that the *early detection system* fulfils each of the~~five characteristics~~ requirements below:

1) broad awareness, ~~e.g.~~ among observers (e.g. the personnel ~~employed at~~of *aquaculture establishments*, ~~or involved in processing~~processors, transportation services) of the characteristic signs of *listed diseases* and *emerging diseases*;

2) *veterinarians* and *aquatic animal health professionals* are trained in recognising and reporting suspicion of *disease* occurrence;

3) the *Aquatic Animal Health Services* have capacity to undertake rapid and effective *disease* investigation based on a national chain of command;

4) the *Aquatic Animal Health Services* have access to sufficient diagnostic capability (from a laboratory with a quality management system that meets requirements of Chapter 1.1.1. of the *Aquatic Manual*) to confirm or exclude cases of *listed diseases* and the capacity and expertise to investigate *emerging diseases* as described in Article 1.4.18.;

5) *veterinarians*, ~~and~~ *aquatic animal health professionals* and others with an occupational role with aquatic animals have a legal obligation to report suspicion~~s~~ of listed or emerging *disease*s occurrence to the *Competent Authority*.

The sensitivity of an *early detection system* is the likelihood that the *disease* will be detected if present. Of fundamental importance is *disease* reporting by farmers, *aquatic animal health professionals* and *veterinarians* and others to initiate the necessary steps of *passive surveillance*. Specifically, the *Competent Authority* should be able to demonstrate that efforts have been made to make ~~farmers~~ relevant observers (e.g. farmers and fishers) aware of signs of *listed diseases* and *emerging diseases*, and secondly the obligation of farmers, *aquatic animal health professionals* and others with an occupational role with aquatic animals to report suspicion. The underpinning legal instruments should be cited.

The capacity of the *Aquatic Animal Health Services* to respond to suspicion of a *listed disease* can be evidenced by response plans, and a descriptive chain of command that will result in an official declaration that the *pathogenic agent* has been detected. Standard operating procedures for diagnostic assays for *listed diseases* and accreditation to internationally recognised laboratory standards can demonstrate the capacity of the *Aquatic Animal Health Services* to detect *listed diseases*. In addition, the effective functioning of the *early detection system* is best illustrated through examples of investigations in response to reported suspicion of *disease*. ~~Ideally, t~~The sensitivity of an *early detection system* (i.e. the likelihood of *pathogenic agent* detection following introduction) ~~should~~can be quantified, for example, by use of a scenario tree model; however, in most circumstances a qualitative assessment will be sufficient.

Article 1.4.8.

Requirements for passive surveillance

1) In addition to the characteristics of an *early detection system* described in Article 1.4.7., the conditions described in this article should be met for *passive surveillance* ~~data~~ information to be utilised for a *self-declaration of freedom from disease.* The conditions, which apply to each defined *study population* of *susceptible species* of a specific *disease*, are that:

   a) conditions (biotic and abiotic) are conducive to clinical expression of the *infection*, such that if the *pathogenic agent* were present within the population of *susceptible species*, it would produce ~~clinical~~ signs of the *disease* at least seasonally;

   b) there should be sufficient awareness by potential observers of the *study population*, such that observation of ~~clinical~~ signs of the *disease*, which may include increased mortality, would lead to ~~reporting~~ investigation and where appropriate, reporting to the *Competent Authority*;

   c) populations of susceptible farmed *aquatic animals* should be under sufficient observation ~~in all relevant production systems~~, such that, if ~~clinical~~ signs of the *disease* were to occur, they would be observed;

   d) for populations of susceptible wild *aquatic animals*, they should:

      i) be under sufficient observation, such that if ~~clinical~~ signs of the *disease* were to occur, they would be observed and reported, or

      ii) be epidemiologically linked to farmed populations, such that if the *disease* were to occur in wild *aquatic animal* populations ~~would occur and~~ it would be observed and reported in adjacent farmed populations ~~if it were to occur in adjacent wild *aquatic animal* populations~~.

2) *Passive surveillance* depends primarily on observers (e.g. farmers, *aquatic animal health professionals*, *veterinarians* and others) recognizing signs of *disease* that are suspicious of a *listed disease* ~~reporting suspicion of *disease*~~ or unexplained increased mortality and reporting them to the *Competent Authority*. For wild populations, the requirements of point ~~4 a)~~ 1 d) i) above ~~are unlikely to be~~ may not be met under most circumstances and, therefore, *passive surveillance* will be insufficiently sensitive. If a *Competent Authority* utilises *passive surveillance* ~~data~~ information for defined populations of wild *aquatic animals*, it should demonstrate that the conditions of this article have been met, and that the *early detection system* ~~provides appropriate *sensitivity* for~~will result in detection of the *disease* should it occur.

3) Awareness of ~~clinical~~ signs of *disease* and the necessary level of observation is best demonstrated through examples of reporting by farmers, *aquatic animal health professionals* and others to the *Competent Authority*. In addition to reporting, information for *passive surveillance* may originate from inspections at processing plants, routine visits by government officials and surveys (e.g. of wild populations), submissions to laboratories, *aquaculture establishment* records (e.g. mortality, medicine use, etc.).

4) *Passive surveillance* is only effective if conditions are conducive to clinical expressions of *disease*, which include:

   a) environmental conditions (e.g. water temperatures) being permissive for the development of clinical signs, at least seasonally~~during at least a period of the year~~; and

   b) the presence of *susceptible species* in which *infection* results in clinical signs.

5) Evidence from published literature will generally be sufficient to demonstrate the environmental conditions in ~~over~~which ~~clinical signs appear, and in which~~ *infection* of *susceptible species* will result in clinical signs. This information should be supplemented with data on the environmental conditions for the *target populations*.

6) *Passive surveillance* only contributes to the *early detection system* if observations and investigations that lead to suspicion of *listed diseases* or *emerging diseases* are rapidly reported, to allow ~~by~~ the *Competent Authority* ~~follow reports of *disease*~~to undertake their own investigation.

Article 1.4.9.

Required periods for basic biosecurity conditions

1) Prior to a Member Country making a *self-declaration of freedom from disease*, *basic biosecurity conditions* should be in place for a ~~defined period. *Basic biosecurity conditions* should be applied for~~ sufficient duration ~~prior to a self-declaration~~, so that, by the end of the period, should the *disease* have been introduced before the *basic biosecurity conditions* began:

   a) ~~no~~ the specific *pathogenic agent* would not remain present in the environment (see pathway 1 – absence of *susceptible species*),

   b) the *disease* would manifest clinically and be detected by the country's *early detection system* (see pathway 2 – historical freedom), and

   c) by the time *targeted surveillance* commenced (see pathway 3 – *surveillance*), *infection* levels would have reached the minimum *prevalence* estimate (i.e. the design *prevalence*) used in the survey design to calculate the sample sizes (e.g. number of *aquaculture establishments* and *aquatic animals* needed to demonstrate freedom).

2) Each disease-specific chapter of the *Aquatic Code* includes minimum periods that *basic biosecurity conditions* should be in place prior to a *self-declaration of freedom from disease*. These periods ~~are determined based on the factors described below.~~ reference a default minimum period or a longer period if determined necessary based on the factors described below:

   a) For pathway 1, the default minimum period ~~that~~ of *basic biosecurity conditions* required ~~should be in place~~prior to a self-declaration, for all *listed diseases,* ~~*of freedom from disease*~~ is six months. It is expected that this period will be sufficient for most *diseases* to ensure that no viable *pathogenic agent* introduced via *aquatic animal* commodities has remained present in the environment, and the *early detection system* was well established and demonstrated to be functioning. The required period that *basic biosecurity conditions* should be in place prior to making a self-declaration*,* using this pathway, is determined for each ~~*pathogenic agent*~~*listed disease* based on its epidemiology (e.g. agent stability in the environment, presence of resistant life stages, *vectors*), and ~~is~~ a period longer than the default minimum may be specified in the relevant disease-specific chapter of the *Aquatic Code*.

   b) For pathway 2, the default minimum period ~~that~~of *basic biosecurity conditions* required ~~should be in place~~prior to a self-declaration, for all *listed diseases,* is ten years. This period is the minimum required to achieve 95% likelihood of freedom, if the annual likelihood of detection is approximately 30%. However, if the average annual likelihood of detection ~~by a country's *early detection system*~~ is considered to be less than 30% ~~in the period preceding declaration~~ (following consideration of the factors below), the minimum period required for *basic biosecurity conditions* defined in the relevant disease-specific chapter of the *Aquatic Code* will be set to a period ~~greater~~ longer than ten years, as appropriate. An evaluation of the following factors will determine whether a period longer than ten years is required:

      i) the maximum duration of the production cycle for the *susceptible species*;

      ii) the life stages at which *aquatic animals* are susceptible;

      iii) the variation in predilection to clinical *disease* among *susceptible species*;

      iv) the expected severity and duration of clinical signs in the *susceptible species* (and therefore the likelihood of detection);

      v) 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);

      vi) factors specific to the *pathogenic agent* (e.g. production of spores);

vii) production systems and management practices that would affect observation of clinical signs if they were to occur;

viii) any other relevant factors that may influence presentation of clinical signs and observation of the *disease* should it be present.

c) For pathway 3, the default minimum period ~~that~~ of *basic biosecurity conditions* ~~should be in place~~ required 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 survey designed in accordance with the recommendations of this chapter. However, ~~different recommendations are provided in the disease-specific chapters of the *Aquatic Code* for some *diseases* where~~ the epidemiology of a *disease* and nature of production systems ~~would~~ may affect ~~limit~~ the ~~expected transmission, and thus~~ increase in *prevalence* and intensity of *infection* in the *susceptible species* following introduction of the *disease*. In these instances, the minimum period required for *basic biosecurity conditions* defined in the relevant disease-specific chapter of the *Aquatic Code* will be set to a period longer than one year, as appropriate. An evaluation of the following factors will determine whether a period longer than one year is required:

i) the maximum duration of the production cycle for the *susceptible species*;

ii) the life stages at which *aquatic animals* are susceptible;

iii) seasonality of the *disease* (periods of the year when *prevalence* and intensity of *infection* is highest and most conducive to detection);

iv) production systems and management practices that would affect occurrence of *infection*;

v) 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*.

d) Pathway 4 is only applicable following the loss of *disease* freedom due to a *disease outbreak*. This circumstance implies a failure of *basic biosecurity conditions* to prevent the introduction of the *disease*. The pathway of *disease* introduction should be investigated and *basic biosecurity conditions* should be reviewed and modified as necessary following eradication of the *disease*, and prior to commencement of any *targeted surveillance* that will be utilised as evidence for a subsequent self-declaration.

Article 1.4.10.

Required periods for targeted surveillance

Prior to a *Competent Authority* making a *self-declaration of freedom from disease* utilising pathway 3 or pathway 4, *targeted surveillance* should be conducted for a defined period, as described in the relevant disease-specific chapter of the *Aquatic Code*. The period of *targeted surveillance* is determined for each disease-specific chapter of the *Aquatic Code,* based on the factors described below:

1) the maximum duration of the production cycle for the *susceptible species*;

2) the life stages at which *aquatic animals* are susceptible;

3) seasonality of the *disease* (periods of the year when *prevalence* and intensity of *infection* is highest and most conducive to detection);

4) production systems and management practices that would affect the seasonal occurrence of *infection*.

For a country or *zone*, the minimum default period for which *targeted surveillance* should occur prior to a *self-declaration of freedom from disease* is two years. During the period of *targeted surveillance,* surveys should occur during defined time periods when conditions are optimal for detection of the *pathogenic agent* (e.g. seasons, temperatures, and life stages). All populations of *susceptible species* in the country or *zone* should be considered in the design of each survey

(i.e. included in the sampling frame). Populations with higher likelihood of *infection* can be preferentially sampled. Article 3.1. of the relevant disease-specific chapter of the *Aquatic Manual* should be used to inform sampling at the farm level.~~should be included in the scope of each survey.~~ There should be a gap of at least three months between surveys and, if there are breaks in production, the surveys should also ideally span two production cycles.

For a country or *zone* to regain freedom in accordance with pathway 4, the required period of *targeted surveillance* specified in the disease-specific chapter of the *Aquatic Code* will be consistent with the original self-declaration of freedom.

For *compartments*, the minimum default period that *targeted surveillance* should occur prior to a *self-declaration of freedom from disease* is one year. This shorter period for a *compartment* reflects the more clearly defined populations, the *biosecurity* required to maintain its population's health status and a likely narrower variation in environmental variables. However, a different period (more ~~or less~~ than one year) may be stipulated in the *disease*-specific chapter of the *Aquatic Code* if warranted by the epidemiology of the *disease* and the criteria proposed above. For example, different requirements may be appropriate where *susceptible species* have 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 near the end of the production cycle.

For *compartments* to regain freedom in accordance with pathway 4, the required period of *targeted surveillance* specified in the disease-specific chapter of the *Aquatic Code* may be less than the original declaration of freedom (dependent on the nature of the specific *disease* and as specified in the relevant disease-specific chapter). However, at least one ~~round of testing~~survey in the compartment is required to demonstrate that eradication has been successful and to ~~test~~ensure the reviewed *basic biosecurity conditions* are effective.

Article 1.4.11.

Pathway 1 – Absence of susceptible species

Unless otherwise specified in the relevant disease-specific chapter of the *Aquatic Code*, a self-declaration of freedom from a specific *disease* may be made for a country or *zone* without applying *targeted surveillance* if there are no *susceptible species* (as listed in Article X.X.2. of the relevant disease-specific chapter of the *Aquatic Code*) present in that country or *zone*.

Basic biosecurity conditions should be in place for a period of time prior to a *self-declaration of freedom from disease*.

This pathway relies on confidence that *susceptible species* are in fact absent from a country or *zone*. To be confident that *susceptible species* are absent there should be:

1) sound knowledge of the range of *susceptible species* of a *pathogenic agent;* and

2) sufficient knowledge, ~~based on active *surveillance*,~~ of the local *aquatic animal* fauna (including wild populations) demonstrated by the following forms of evidence:~~.~~

~~The forms of evidence that may be required to demonstrate absence of *susceptible species* include:~~

~~1~~a) the absence of reports of the existence of the *susceptible species* in the country or *zone* from structured surveys (e.g. of fisheries and aquatic fauna surveys, historical fisheries data);

~~2~~b) documentation from the relevant *Competent Authority* showing that those *susceptible species* have not been imported into the country or *zone*;

~~3~~c) provision of documentation which sets out scientific evidence indicating that the likelihood of the presence of *susceptible species* in the country or *zone* is negligible (e.g. data on physiological requirements, oceanographic information, biodiversity databases).

This pathway cannot be used for *diseases* where there is uncertainty regarding the full range of *susceptible species* (e.g. *diseases* with a broad host range), or where the *pathogenic agent* may not be obligate (e.g. able to survive indefinitely outside the host). In these cases, the pathway will be absent from the relevant disease-specific chapter of the *Aquatic Code*, and alternative pathways to demonstrate freedom should be utilised.

The pathway is intended primarily to be used by the *Competent Authority* wishing to establish freedom ahead of farming a new species.

Article 1.4.12.

Pathway 2 – Historical~~ly~~ freedom

Unless otherwise specified in the relevant disease-specific chapter of the *Aquatic Code*, a *self-declaration of freedom from disease* may be made for a country or *zone* on the basis of historical freedom. The primary evidence for historical freedom is *passive surveillance* ~~data~~ information generated by a country's *early detection system*. For this pathway to be utilised, the following conditions should be met:

1) 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 conditions of Article 1.4.8.are met;

2) the *disease* has not been reported in the country or *zone* (including in wild *aquatic animal* populations) for the minimum period specified in the relevant disease-specific chapter of the *Aquatic Code*.

Requirements for passive surveillance

The level of confidence provided by *passive surveillance* ~~data~~information (generated by the *early detection system* of the *Competent Authority*) to demonstrate historical freedom should be set at 95%, equivalent to that of other pathways for which the evidence is provided by *targeted surveillance*. If a combination of *surveillance* data sources is to be used (e.g. *passive surveillance* and *targeted surveillance*), the level of confidence should also be set at 95% that the *disease* is absent. ~~The data sources for *passive surveillance* are described in Article 1.4.8. of this chapter.~~

A *Competent Authority* making a *self-declaration of freedom from disease* 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. Specifically, the *Competent Authority* needs to provide evidence that its *early detection system* meets the conditions ~~as~~ described in Article 1.4.7. ~~(and ideally a quantitative assessment of *sensitivity* would be included)~~ and the requirements for passive surveillance in Article 1.4.8. The *early detection system* needs to cover all the *susceptible species* populations in the country or *zone*. If the *Competent Authority* cannot demonstrate that the required characteristics are fulfilled, due to a country's circumstances (e.g. nature of the *early detection system*, environmental conditions, nature of the *aquaculture* industry), this pathway is not considered valid. Instead, an alternative pathway that utilises *targeted surveillance* data will be required, or the *passive surveillance* ~~data~~information will need to be supplemented with *targeted surveillance* data (see below).

Need for targeted surveillance

If the requirements for *passive surveillance* specified in points 1 and 2 above would not be met for some defined populations of *susceptible species* (e.g. for wild populations), *targeted surveillance* may be used to provide additional evidence of freedom for those populations. ~~However, for this~~ This pathway should only ~~pathway to~~ be utilised as the basis of a *self-declaration of freedom from disease*, if it is ~~it should be~~ based primarily on *passive surveillance* ~~data~~ information to demonstrate historical freedom; alternatively, pathway 3, as described in Article 1.4.13., should be used.

Article 1.4.13.

Pathway 3 – Targeted Surveillance

As specified in the relevant disease-specific chapter of the *Aquatic Code*, a *self-declaration of freedom from disease* may be made for a country, a *zone* or a *compartment* where the primary evidence for freedom is *targeted surveillance* data. For this pathway to be utilised, the following conditions should be met:

1) prior to the commencement of *targeted surveillance* *basic biosecurity conditions* have been in place for a default minimum period as specified in the relevant disease-specific chapter of the *Aquatic Code*;

2) the *disease* has not been reported in the country, *zone* or *compartment,* despite *targeted surveillance* that has been conducted for a period as specified in the relevant disease-specific chapter of the *Aquatic Code,* and in accordance with the requirements below.

~~Requirements for basic biosecurity conditions~~

~~*Targeted surveillance* surveys should only commence following a period of time that *basic biosecurity conditions* have been in place, as specified in the relevant disease-specific chapter of the *Aquatic Code*.~~

Requirements 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 ~~the rate~~ *pathogenic agent* replication and transmission are at their highest.

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 should 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. Based on an assessment of potential pathways of introduction of the *diseases*, high risk regions or *aquaculture establishments* should be identified and preferentially included in the *surveillance* programmes. For example, establishments near ports or processing facilities may have higher likelihood of exposure to introduced *pathogenic agents*.

To maximise the likelihood of *pathogenic agent* detection, surveys should select 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 *aquaculture establishments* and *aquatic animals* sampled should be sufficient to generate ~~an overall~~ at least 95% confidence or greater that the *pathogenic agent* is at or below the design *prevalence*. Design *prevalence* at the animal and higher levels of aggregation (i.e. pond, *aquaculture establishment*, village, etc.) should be set to a maximum of 2% ~~or lower~~ (a higher design *prevalence* can only be used if justified by epidemiological evidence). Surveys should be designed in accordance with the recommendations provided in Article 1.4.1.

~~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 *Competent Authority*, to generate an annual 95% confidence of detection.~~

Other sources of data

This pathway to *disease* freedom should be based primarily on the results of ~~structured~~ *targeted surveillance*, however, the submission may also include an analysis of the *passive surveillance* ~~data~~information to provide supplemental evidence. This evidence may be used for defined populations of *susceptible species* where the ~~*sensitivity* of~~ *passive surveillance* is demonstrated to be sufficiently sensitive (as described in Article 1.4.8.).

Article 1.4.14.

Pathway 4 – Returning to freedom

As specified in the relevant disease-specific chapter of the *Aquatic Code*, a *self-declaration of freedom from disease* may be made for a country, a *zone* or a *compartment* for which a self-declaration had previously been made, but subsequently lost due to an *outbreak* of the *disease*.

For a *country or a zone,* the default minimum period of *surveillance* to regain freedom is consistent with the requirements for pathway 3. However, a self-declaration of freedom can be made sooner if the relevant *Competent Authority* can demonstrate that the approach would provide an appropriate standard of evidence for the circumstances of the *outbreak* and the *disease*.

Compartments are able to return to freedom relatively rapidly; however, a minimum period of time is required as specified in each disease-specific chapter of the *Aquatic Code* to ~~test~~ demonstrate that eradication has been successful and to ensure the reviewed *basic biosecurity conditions* are effective~~, and to undertake sufficient testing to demonstrate that eradication has been successful~~.

For a country, *zone* or *compartment*, a self-declaration utilising this pathway should provide information on the process employed to review and update *basic biosecurity conditions*. This information should also address the outcomes of the review and any relevant *sanitary measures* implemented to strengthen *basic biosecurity conditions*.

1. Infected zone and protection zone

Infected and *protection zones* should be established through exposure contact tracing from known infected *aquaculture establishments* (e.g. by following movements of *aquatic animals* or equipment to and from infected establishments) to identify all known infected establishments. Once contact tracing is complete and no new cases are being reported or detected through tracing, the boundaries of *infected zones* and *protection zones* can be finalised. The geographic extent of an *infected zone* should be based on the spatial distributions of infected and non-infected establishments within a region (e.g. river, estuary or bay). The *zone* should be defined to encompass geographically clustered infected populations.

The geographic extent of a *protection zone* needs to provide a very high level of confidence that measures implemented within the *zone* will prevent spread from the *zone* and should be based on the epidemiology of the transmissible *pathogenic agent*, the potential for exposure of neighbouring *aquaculture establishments*, the type of aquaculture productions systems (e.g. open or closed systems), the influence of wild populations, and the local hydrology. In the marine environment, local hydrology (including tidal excursion), the distribution of suitable habitats for *susceptible species* and the movement of wild *susceptible species* or vectors should be considered. In the freshwater environment, the boundaries of the *protection zone* should be determined by the distance downstream that viable *pathogenic agent* is likely to spread on currents. If susceptible wild populations or vectors are present, their migratory patterns and ranges should be used.

Once *infected zones* and *protection zones* have been established, and no new cases have been detected for a period equal to or greater than the incubation period of the *pathogenic agent* (but no shorter than one month), the region outside of the *infected zones* and *protection zones* can be declared a *disease free zone*. Re-establishing *disease* freedom in the *infected* and *protection zones* requires *targeted surveillance*.

2. Requirements for targeted surveillance in a country or zone

Once all infected populations have been depopulated and affected *aquaculture establishments* have been disinfected, as described in Chapter 4.3~~4~~4., and synchronously fallowed as described in Chapter 4.6~~7~~7., for a period determined by the biophysical properties of the *pathogenic agent* (i.e. 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 *infected zones*. A *risk*-based approach to the design of the survey is recommended (refer to Article 1.4.6.). The following *aquaculture establishments* or populations should be preferentially selected for sampling:

a) establishments which ~~were depopulated (following restocking)~~ have been restocked following depopulation;

b) establishments and wild populations at greatest *risk* of exposure to *infection* during the *outbreak*, i.e. in close ~~geographic~~ hydrographical proximity to infected establishments or with other epidemiological contacts such as sharing equipment or movements of *aquatic animals*;

c) wild populations of *susceptible species* downstream or in the immediate vicinity of previously infected establishments.

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. Surveys should take place during optimum seasons, temperatures, and priority life stages to optimise *pathogenic agent* detection. If there are breaks in production, the surveys should also ideally span two production cycles. The number of *aquaculture establishments* and the samples taken per establishment in each 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). If disease is detected in wild populations of susceptible species and eradication is not possible, the country or *zone* remains infected.

3. Requirements for targeted surveillance in a compartment

Once the infected populations have been depopulated and affected *aquaculture establishments* disinfected, ~~and fallowed~~ as described in Chapter 4.~~3~~4. and fallowed as described in Chapter 4.~~6~~7., for a period determined by the biophysical properties of the *pathogenic agent* (i.e. survival in the environment), the *compartment* can be restocked. A single survey is required following restocking to demonstrate that eradication has been successful. The survey should be undertaken at least 6 months, or at the maximum length of time allowed by the production cycle of species, after the *aquaculture establishment* has been restocked to ensure that the reviewed *basic biosecurity conditions* are effective. The survey~~; and~~ should take place during optimum seasons, temperatures, and priority life stages to optimise *pathogenic agent* detection. The number of holding units (e.g. ponds, tanks) and the animals per holding unit sampled 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).

Article 1.4.15.

Maintenance of disease free status

A country, *zone* or *compartment* that is declared free may maintain its free status provided that the biosecurity and *surveillance* requirements described in Article 1.4.5. are continuously maintained and the following requirements are met, as relevant:

1) For a country or *zone* with shared water bodies extending across the territory of other countries, free status can only be maintained if the requirements to maintain freedom are in place across all epidemiologically linked shared water bodies.

2) A country, *zone* or *compartment* declared free may maintain its free status without *targeted surveillance* provided that the requirements for *passive surveillance* in Article 1.4.8. are met for the entire country, *zone* or *compartment*, and in the case of:

   a) a declared free *zone*, the *zone* occurs within the territory of a country declared free;

   b) a declared free *compartment,* the *compartment* occurs within the territory of a country declared free.

3) If the conditions of point 2 are not met, ongoing *targeted surveillance* for the *pathogenic agent,* as described in Article 1.4.16., is required at a level determined by the *Competent Authority,* to generate an annual 95% confidence of detection, taking into account the likelihood of infection.

4) *Competent Authorities* should ensure prompt investigation of any health events or other information that may raise suspicion of the occurrence of a listed disease from which a country, *zone* or *compartment* has been declared free. The investigation should be undertaken in accordance with Article 1.4.18. and the requirements of Chapters 1.1. and 5.1. should be met at all times.

~~For maintenance of *disease* free status achieved via pathways 2, 3 and 4, the *Competent Authority* should provide evidence that *basic biosecurity conditions* are continuously maintained.~~

~~If *targeted surveillance*, that was required for initial demonstration of freedom, is to be discontinued for any identified population, evidence should be provided to demonstrate that conditions remain conducive to clinical expression of *disease*, and that *passive surveillance*, as provided by the country's *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*.~~

Article 1.4.16.

Design of surveys to demonstrate freedom from disease

Surveys to demonstrate freedom from a specified *disease* (i.e. *targeted surveillance*) are required for pathway 3 as described in Article 1.4.13. to achieve a *disease* free status, and to regain a *disease* free status following detection of

the *pathogenic agent* as described in Article 1.4.14. and to maintain *disease* freedom). Surveys may be required to supplement *passive surveillance* datainformation generated by the *early detection system* required for pathway 2 as described in Article 1.4.12. In addition, where conditions are not conducive to clinical expression of *disease*, and, therefore, the *early detection system* cannot provide evidence for the maintenance of freedom, ongoing *targeted surveillance* is required.

It is not possible to provide absolute certainty of the absence of *disease*. Surveys can demonstrate freedom from *disease* by generating evidence that a *disease* is not present in a population at or above a predetermined *prevalence* (the design *prevalence*) and to an acceptable level of confidence. Apparent *disease* at any level in the *target population* automatically invalidates any freedom from *disease* claim, unless, on the basis of further testing, positive test results are accepted as false positives. A survey to demonstrate freedom from *disease* should meet the following requirements set out in this article:

1. Population

   The population of *epidemiological units* should be clearly defined. *Aquaculture establishments* and holding *units* (e.g. ponds, tanks) within establishments are the most commonly used *epidemiological unit* in surveys to demonstrate *disease* freedom. It is, therefore, important that *Competent Authorities* should keep registries of *aquaculture establishments*, which include geographic location and species held.

   The *target population* consists of all individuals within the selected population of all *susceptible species* to the *disease* in a country, *zone* or *compartment,* to which the *surveillance* results apply. Exotic *disease* introduction may be more likely to occur in some components of the *target population* than others. In these cases, it is advisable to focus *surveillance* efforts on this part of the population.

   The design of the survey will depend on the size and structure of the population being studied. If the population is relatively small, and can be considered to be homogenous with regards to *risk* of *infection*, a single-stage survey can be used.

   Farmed *aquatic animals* are not individually identified and usually kept in holding *units* (e.g. ponds, tanks) which can lead to clusters of *infection* within *aquaculture establishments*. Similarly, wild aquatic animal populations are not evenly distributed within a zone. For these reasons, multi-stage sampling is recommended. In two-stage sampling, at the first stage of sampling, groups of animals (e.g. ponds, *aquaculture establishments* or villages) are selected. At the second stage, animals are selected for testing from each of the selectedfirst-stage sampling groups.

   In the case of a complex (e.g. multi-level) population structure, multi-stage sampling may be used, and the data analysed accordingly.

2. Dossier of evidence

   The sources of evidence should be fully described. A survey should include a description of the sampling strategy used for the selection of units for testing. For complex *surveillance* systems, a full description of the system is required, including consideration of any *biases* that may be inherent in the system. Evidence to support claims of freedom from *disease* can use non-random sources of information, provided that, overall, any *biases* introduced subsequently favour the detection.

3. Statistical methodology

   The analysis and interpretation of test results from a survey shall be in accordance with the provisions of this chapter and consider the following factors:

   a) the survey design;

   b) the diagnostic *sensitivity* and *specificity* of the test or test system;

   c) the design *prevalence* (or *prevalences* where a multi-stage design is used).

Analysis of data for evidence of freedom from *disease* involves estimating the probability (alpha) that the evidence observed (i.e. negative results for *disease* detection from *surveillance*) could have been produced assuming that *infection* is present in the population at or below the minimum specified *prevalence* (the design *prevalence*). The confidence in (or, equivalently, the *sensitivity* of) the survey that produced the evidence is equal to 1–alpha. If the confidence level exceeds a pre-set threshold, the evidence is deemed adequate to demonstrate freedom from *infection*. The required level of confidence (that the survey would detect *infection* if *infection* were present at or above the specified level) should be equal to or greater than ~~or equal to~~ 95%.

The power (probability that the survey would report that no *infection* is present if *infection* is truly not present) is by convention set to 80%, but may be adjusted in accordance with the country's or *zone*'s requirements.

Statistical analysis of *surveillance* data often requires assumptions about population parameters or test characteristics. These are usually based on expert opinion, previous studies on the same or similar populations, and epidemiology of the *disease*.

The values for design *prevalence* used in calculations should be those specified in the relevant disease-specific ~~*disease*~~ chapter ~~(if present)~~ of the *Aquatic Manual*. If not specified for the particular *disease*, justification for the selection of design *prevalence* values should be provided, and should be based on the following recommendations:

a) At the individual animal level (e.g. *prevalence* of infected animals in a pond, tank or net pen, or cages), the design *prevalence* is based on the epidemiology of the *infection* in the population. It is equal to the minimum expected *prevalence* of *infection* in the *study population*, if the *infection* had become established in that population. A suitable design *prevalence* value at the animal level may be:

   i) between 1% and 5% for *infections* that are present in a small part of the population, e.g. are transmitted slowly or have been recently introduced, etc.;

   ii) over 5% for highly transmissible and persistent *infections*;

   iii) if reliable information, including expert opinion, on the expected *prevalence* in an infected population is not available, a value of 2% should be used for the design *prevalence*.

b) At higher levels (e.g. net pen or cage, pond, *aquaculture establishments*, village, etc.) the design *prevalence* should be based on empirical evidence and reflect the expected behaviour of the *infection*. A higher establishment-level design *prevalence* can be used for diseases which spread rapidly between pens or cages, and establishments. *Diseases* which are transient or that can remain sub-clinical require lower design *prevalences*:

   i) a suitable design *prevalence* value for the first level of clustering (e.g. proportion of infected establishments in a *zone*) is normally not greater than 2%. If a higher design *prevalence* is selected, it should be justified.

4. Risk based sampling

Risk-based sampling is an approach to identify and sample populations that have the greatest likelihood of *infection*. It can be applied to the design of surveys to demonstrate freedom from *disease* for a country, *zone* or *compartment*. A key advantage of *risk*-based sampling is that it can improve the efficiency of *surveillance* to demonstrate freedom from *disease* compared to random sampling approaches.

Risk-based sampling requires the identification of *risk*-factors that are applied to *bias* sample collection to populations of *aquatic animals* considered most likely to be infected if the specific *disease* had been introduced and had established. Where *risk*-based sampling is used for demonstration of freedom, the *risk* factors that underpin survey design, and the evidence or assumptions for their selection, should be documented. Where existing *risk assessments* are available, these may be utilised to identify *risk* factors associated with introduction, exposure and establishment. The identification of appropriate *risk* factors may include consideration of:

a) the possible pathways of *disease* introduction (e.g. through ~~imported~~ *aquatic animals*, ~~imported~~ *aquatic animal products*, feed, fomites, *vectors* and ~~ship ballast~~ water~~or biofouling~~);

b) proximity of susceptible populations to sources of exposure (e.g. to ~~*quarantine* facilities,~~ *aquatic animal* processing facilities, or ports);

c) environmental or husbandry conditions that are permissive for establishment (e.g. temperature, salinity, production system type, habitat type);

d) conditions that are conducive for development of clinical *disease*; including the species or life stages that are most susceptible to clinical *disease*.

5. Test characteristics

All *surveillance* involves performing one or more tests for evidence of the presence of current or past *infection*, ranging from laboratory assays to farmer observations. The performance level of a test is described in terms of its diagnostic *sensitivity* and *specificity*. Imperfect *sensitivity* or *specificity* impact on the interpretation of *surveillance* results, and should be taken into account in the analysis of *surveillance* data. For example, in the case of a test with imperfect diagnostic *specificity*, if the population is free of *disease* or has a very low *prevalence* of *infection*, all or a large proportion of positive tests will be false. Samples that test positive should be confirmed or refuted using a second highly specific test. Where more than one test is used (sometimes called using tests in series or parallel), the *sensitivity* and *specificity* of the test combination should be calculated.

All calculations should take the performance level (*sensitivity* and *specificity*) of any tests used into account. Information on test characteristics provided in the relevant disease-specific chapter of the *Aquatic Manual* should be used unless more appropriate information is available. The estimate of test *sensitivity* when the test was used in apparently healthy *aquatic animals* should be used. Samples should not be pooled before testing, unless approved in the relevant disease-specific chapter of the *Aquatic Manual*. If pooled testing is used, the results of testing should be interpreted using *sensitivity* and *specificity* values that have been determined or estimated for that particular pooled testing procedure, and for the applicable pool sizes being used.

6. Sample size

The number of units to be sampled from a population should be calculated, using a statistically valid technique that takes at least the following factors into account:

a) the *sensitivity* and *specificity* of the diagnostic test,

b) the design *prevalence* (or *prevalences* where a multi-stage design is used),

c) the level of confidence that is desired of the survey results.

Additionally, other factors may be considered in sample size calculations, including (but not limited to):

a) the size of the population (but it is acceptable to assume that the population is infinitely large),

b) the desired power of the survey.

Software for the calculation of sample sizes at varying parameter values are available. Table 1.~~1~~ 2 provides examples of sample sizes generated by the software for a type I and type II error of 5% (i.e. 95% confidence and 95% statistical power). However, this does not mean that a type 1 and type 2 error of 0.05 should always be used. For example, using a test with *sensitivity* and *specificity* of 99%, 528 units should be sampled. If nine or less of those units test positive, the population can still be considered free of the *infection* at a design *prevalence* of 2%, provided that all efforts are made to ensure that all presumed false positives are indeed false (i.e. by use of a second highly specific assay). This means that there is a 95% confidence that the *prevalence* is 2% or lower, which reflects the fact that false negative results can occur. Incorrectly concluding that a population is free can be reduced by increasing the sample size and using more than one assay but cannot be completely eliminated.

In the case in which the values of *sensitivity* and *specificity* are not known (e.g. no information is available in the relevant disease-specific chapter of the *Aquatic Manual*), they should not automatically be assumed to be 100%. All positive results should be included and discussed in any report regarding that particular survey, and all efforts should be made to ensure that all presumed false positives are indeed false.

7. Multi-stage structured survey design

In general, a survey to demonstrate freedom at *zone* or *country* level should use a multi-stage design. The first sampling level is often *aquaculture establishments* (or villages) or discrete populations of wild susceptible species, and the second stage may be ponds or individual animals within the establishment (or village) or defined stocks within a wild population. At each level, design levels need to be set and sample sizes calculated.

8. Discounting

Where conditions are not conducive to clinical expression of *disease* in a population, ongoing *surveillance* is required. Regions and *aquaculture establishments* at high risk of introduction of *pathogenic agent* should be regularly sampled. *Targeted surveillance* required to maintain confidence in *disease* freedom at 95% can be determined based on estimates of the likelihood of introduction of *pathogenic agent* (low due to basic *biosecurity* measures) and the discounting of historic *surveillance*. Methods for using historical *surveillance* data have been developed.

9. Quality assurance

Surveys should include a documented quality assurance system, to ensure that field and other procedures conform to the specified survey design. Acceptable systems may be quite simple, as long as they provide verifiable documentation of procedures and basic checks to detect significant deviations of procedures from those documented in the survey design.

Table 1.2. Sample sizes for different design *prevalences* and test characteristics.

Design prevalence (%)	**Sensitivity (%)**	**Specificity (%)**	**Sample size**	**Maximum number of false positive if the population is free**
2	100	100	149	0
2	100	99	524	9
2	100	95	1,671	98
2	99	100	150	0
2	99	99	528	9
2	99	95	1,707	100
2	95	100	157	0
2	95	99	542	9
2	95	95	1,854	108
2	90	100	165	0
2	90	99	607	10
2	90	95	2,059	119
2	80	100	186	0
2	80	99	750	12
2	80	95	2,599	148
5	100	100	59	0
5	100	99	128	3
5	100	95	330	23

5	99	100	59	0
5	99	99	129	3
5	99	95	331	23
5	95	100	62	0
5	95	99	134	3
5	95	95	351	24
5	90	100	66	0
5	90	99	166	4
5	90	95	398	27
5	80	100	74	0
5	80	99	183	4
5	80	95	486	32

Article 1.4.17.

Combining multiple sources of information

Pathway 1 to achieving *disease* freedom (absence of *susceptible species*) relies on a range of data sources. Pathway 2 to achieving *disease* freedom (historical freedom) will primarily use evidence from *passive surveillance,* which may come from multiple sources (as described in Article 1.4.8.). *Passive surveillance* ~~data~~ information can also be used to provide additional support ~~to case~~ for *disease* freedom, ~~primarily~~ based on *targeted surveillance* (i.e. pathway 3). Estimates of the confidence in each data source may be combined to provide an overall level of confidence of freedom from *disease* for the combined data sources. The methodology used to combine the estimates from multiple data sources:

1) should be scientifically valid and fully documented, including references to published material; and

2) should, where possible, take into account any lack of statistical independence between different data sources.

A scenario tree modelling approach can be used to combine evidence from different sources including *passive* and *targeted surveillance*.

Article 1.4.18.

Diagnostic confirmation of a listed disease or an emerging disease

A *Competent Authority* is required to provide *disease notifications* as described in Chapter 1.1.

The relevant disease-specific chapter of the *Aquatic Manual* provide recommendations for the appropriate diagnostic methods for presumptive and confirmatory diagnostic purposes. The assays recommended for these purposes are presented in Table 4.1 of the relevant disease-specific chapter of the *Aquatic Manual*.

The recommended standards of diagnostic evidence to confirm *infection* in either apparently healthy or clinically diseased animals are provided in Section 6 of the relevant disease-specific chapter of the *Aquatic Manual*. These case definitions for suspect and confirmed cases have been developed to support decision making in relation to trade and for confirmation of *disease* status at the level of a country, *zone* or *compartment*. A *Competent Authority* may choose to apply a lower standard of evidence for *disease* confirmation within its *territory* for known endemic *diseases*.

If standards of evidence are not met to confirm a suspect case of *disease* in accordance with the case definitions in Section 6 of the relevant *disease*-specific chapter of the *Aquatic Manual*, ongoing investigation is required until sufficient evidence is obtained to either:

1) exclude the presence of a *listed disease* or an *emerging disease*, or;

2) to confirm the presence of a *listed disease* or an *emerging disease*.

If a Member Country does not have access to a laboratory with ~~does not have~~ the capability to undertake the necessary diagnostic tests and which meets the requirements of Chapter 1.1.1. of the *Aquatic Manual*~~,~~ it should seek advice from the relevant OIE Reference Laboratory.

In all circumstances, Member Countries should comply with the requirements described in Chapter 1.1. to provide transparent and timely *notification* to allow Member Countries to take appropriate action to prevent the transboundary spread of important *diseases* of *aquatic animals*.

Return to Agenda

Model Articles X.X.4 to X.X.8 for disease-specific chapters to address declaration of freedom from [Pathogen X]

Note: time periods in these model articles will be determined by the Aquatic Animals Commission for each disease-specific chapter based on criteria that will be included in the revised Chapter 1.4. For this reason, periods are shown as [X] to indicate that the period is yet to be determined for each specific disease. Where a period is shown (e.g. 'the last [X] years') this indicates an intended default period that may vary depending on the circumstances of each disease.

Article X.X.4.

[**Note**: this is a new article that will outline general requirements for making a self-declaration of freedom for a country, zone or compartment.]

Requirements for self-declaration of freedom from infection with [PATHOGEN X]

A Member Country may make a self-declaration of freedom from infection with [PATHOGEN X] for the entire country, a *zone* or a *compartment* in accordance with the provisions of Articles X.X.5. to X.X.8., as relevant. The self-declaration of freedom must be made in accordance with other relevant requirements of the *Aquatic Code*, including that the Member Country meet the following conditions:

1) complies with the provisions of Chapter 3.1.; and

2) uses appropriate methods of diagnosis, as recommended in the *Aquatic Manual*; and

3) meets all requirements of Chapter 1.4. that are relevant to the self-declaration of freedom.

Article X.X.5.

[**Note**: this article will replace the existing Article X.X.4.]

Country free from infection with [PATHOGEN X]

If a country shares water bodies ~~a *zone* with one or more~~ other countries, it can only make a self-declaration of freedom from infection with [PATHOGEN X] if ~~the~~ all shared water bodies are within countries or *zones* declared free from infection with [PATHOGEN X] (see Article X.X.6.).

As described in Article 1.4.X., a Member Country may make a self-declaration of freedom from infection with [PATHOGEN X] for its entire *territory* if:

1) none of the *susceptible species* referred to in Article X.X.2. are present and *basic biosecurity conditions* have been continuously met for at least the last ~~[two] years~~ [six] months;

OR

2) there has been no occurrence of infection with [PATHOGEN X] for at least the last [ten] years, and:

   a) the Member Country can demonstrate that conditions are conducive to the clinical expression of infection with [PATHOGEN X], as described in the corresponding chapter of the *Aquatic Manual*; and

   b) *basic biosecurity conditions* as described in Chapter 1.4. have been continuously met for at least the last [ten] years;

OR

3) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last [two] years without detection of [PATHOGEN X], and:

a) *basic biosecurity conditions* have been continuously met ~~from~~ for at least [one] year prior to commencement of *targeted surveillance*;

OR

4) it previously made a self-declaration of freedom from infection with [PATHOGEN X] and subsequently lost its free status due to the detection of [PATHOGEN X] but the following conditions have been met:

a) on detection of [PATHOGEN X], the affected area was declared an *infected zone* and a *protection zone* was established; and

b) infected populations within the *infected zone* have been killed and disposed of by means that minimise the likelihood of further transmission of [PATHOGEN X], and the appropriate *disinfection* procedures (as described in Chapter 4.~~3~~4.) have been completed followed by fallowing as described in Chapter 4.~~6~~7.; and

c) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place since eradication of infection with [PATHOGEN X]; and

d) *targeted surveillance*, as described in Chapter 1.4., has been in place for i) at least the last [two] years in wild and farmed *susceptible species* without detection of [PATHOGEN X] or ii) at least the last [one] year without detection of [PATHOGEN X] if affected ~~farms~~ *aquaculture establishments* were not epidemiologically connected to wild populations of *susceptible species*.

In the meantime, part or all of the country, apart from the *infected* and *protection zones,* may be declared a free *zone* provided that such a part meets the conditions in point 2 of Article X.X.6.

Article X.X.6.

[**Note**: this new article for zone freedom is based on the existing Article X.X.5.]

Zone free from infection with [PATHOGEN X]

If a *zone* extends over the *territory* of more than one country, it can only be declared a *zone* free from infection with [PATHOGEN X] if all of the relevant *Competent Authorities* confirm that all relevant conditions have been met.

As described in Article 1.4.X., a Member Country may make a self-declaration of freedom from infection with [PATHOGEN X] for a *zone* within its *territory* if:

1) none of the *susceptible species* referred to in Article X.X.2. ~~10.6.2~~. are present and *basic biosecurity conditions* have been continuously met for at least the last ~~[two] years~~ [six] months;

OR

2) there has been no occurrence of infection with [PATHOGEN X] for at least the last [ten] years, and;

a) the Member Country can demonstrate that conditions are conducive to the clinical expression of infection with [PATHOGEN X], as described in Article 1.4.8. of Chapter 1.4. ~~the corresponding chapter of the *Aquatic Manual*~~; and

b) *basic biosecurity conditions* as described in Chapter 1.4. have been continuously met for the *zone* for at least the last [ten] years;

OR

3) *targeted surveillance*, as described in Chapter 1.4., has been in place in the *zone* for at least the last [two] years without detection of [PATHOGEN X], and:

a) *basic biosecurity conditions* have been continuously met for at least [one] year prior to commencement of *targeted surveillance*;

OR

4) it previously made a self-declaration of freedom for a *zone* from infection with [PATHOGEN X] and subsequently lost its free status due to the detection of [PATHOGEN X] in the *zone* but the following conditions have been met:

   a) on detection of [PATHOGEN X], the affected area was declared an *infected zone* and a *protection zone* was established; and

   b) infected populations within the *infected zone* have been killed and disposed of by means that minimise the likelihood of further transmission of [PATHOGEN X], and the appropriate *disinfection* procedures (as described in Chapter 4.4.) have been completed followed by fallowing as described in Chapter 4.7.; and

   c) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place since eradication of infection with [PATHOGEN X]; and

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

Article X.X.7.

[**Note**: this is a new article to address free compartments].

Compartment free from infection with [PATHOGEN X]

As described in Article 1.4.X., a Member Country may make a self-declaration of freedom from infection with [PATHOGEN X] for a *compartment* within its *territory* if:

1) *targeted surveillance*, as described in Chapter 1.4., has been in place in the *compartment* for at least the last [two] years without detection of [PATHOGEN X], and:

   ~~a)~~ *basic biosecurity conditions* have been continuously met for at least [one] year prior to commencement of *targeted surveillance*;

OR

2) it previously made a self-declaration of freedom for a *compartment* from infection with [PATHOGEN X] and subsequently lost its free status due to the detection of [PATHOGEN X] in the *compartment* ~~*zone*~~ but the following conditions have been met:

   a) all *aquatic animals* within the *compartment* have been killed and disposed of by means that minimise the likelihood of further transmission of [PATHOGEN X], the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed, and the *compartment* has been fallowed as described in Chapter 4.6. ~~for at least [X] weeks~~; and

   b) previously existing *basic biosecurity conditions*, including the *compartment biosecurity plan*, have been reviewed and modified as necessary and have continuously been in place from the time of restocking with animals from an approved pathogen free source in accordance with the requirements of Articles X.X.9. and X.X.10., as appropriate; and

   c) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last [one] year without detection of [PATHOGEN X].

Article X.X.8.

[**Note**: this article is based on the current Article X.X.6.]

Maintenance of free status

A country, *zone* or *compartment* that is declared free from infection with [PATHOGEN X] following the provisions of Articles X.X.4. to X.X.7. (as relevant) may maintain its status as free from infection with [PATHOGEN X] provided that the requirements described in Article 1.4.15. are continuously maintained.

A country or *zone* that is declared free from infection with [PATHOGEN X] following the provisions of point 1 of in Articles X.X.5. or X.X.6. (as relevant) may maintain its status as free from infection with [PATHOGEN X] provided that *basic biosecurity conditions* are continuously maintained.

A country or *zone* that is declared free from infection with [PATHOGEN X] following the provisions of point 2 of in Article X.X.5. may discontinue *targeted surveillance* and maintain its free status provided that conditions are conducive to clinical expression of infection with [PATHOGEN X], as described in the corresponding chapter of the *Aquatic Manual*, and *basic biosecurity conditions* are continuously maintained.

For declared free *zones* or *compartments* within the *territory* of a country not declared free, *targeted surveillance* should be continued at a level determined by the *Aquatic Animal Health Service* on the basis of the likelihood of *infection*.

In all cases where conditions are not conducive to clinical expression of infection with [PATHOGEN X], ongoing *targeted surveillance*, as described in Chapter 1.4., is required at a level that maintains the level of confidence in freedom from infection with [PATHOGEN X] that was required for the initial declaration of freedom.

Return to Agenda

ARTICLES 9.X.3 FOR CRUSTACEAN DISEASE-SPECIFIC CHAPTERS (TRACK CHANGES AND CLEAN VERSIONS)

(TRACK CHANGES VERSION)

CHAPTER 9.1.

ACUTE HEPATOPANCREATIC NECROSIS DISEASE

[…]

Article 9.1.3.

Measures for the i~~I~~mportation or transit of aquatic animal products for any purpose regardless of the AHPND status of the exporting country, zone or compartment

~~1)~~ The ~~following~~ *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of the these *aquatic animal products* ~~listed below.~~ *Competent Authorities* should not require any *sanitary measures* ~~conditions~~ related to AHPND, regardless of the AHPND status of the *exporting country*, *zone* or *compartment*~~:, when authorising the importation or transit of the following *aquatic animal products* derived from a species referred to in Article 9.1.2. that are intended for any purpose and comply with Article 5.4.1.:~~

~~a~~1) cooked or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 100°C for at least ~~one minute~~ 60 seconds, ~~(~~or a time/temperature equivalent that ~~has been demonstrated to~~ inactivates Vp_{AHPND}~~);~~;

~~b) heat sterilised hermetically sealed crustacean products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent that has been demonstrated to inactivate Vp_{AHPND});~~

~~c) cooked crustacean products that have been subjected to heat treatment at 100°C for at least one minute (or any time/temperature equivalent that has been demonstrated to inactivate Vp_{AHPND});~~

2) crustacean *meal* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 100°C for at least 60 seconds, or a time/temperature equivalent that inactivates Vp_{AHPND};

~~d)~~~~b)~~3) crustacean oil;

~~e)~~~~c)~~ ~~crustacean *meal* that has been heat treated at a core temperature of at least 100°C for at least one minute (or a time/temperature equivalent that has been demonstrated to inactivate Vp_{AHPND});~~

~~f)~~~~d~~4) chemically extracted chitin.

~~2) When authorising the importation or transit of *aquatic animal products* derived from a species referred to in Article 9.1.2., other than those referred to in point 1 of Article 9.1.3., *Competent Authorities* should require the conditions prescribed in Articles 9.1.7. to 9.1.12. relevant to the AHPND status of the *exporting country*, *zone* or *compartment*.~~

~~3) When considering the importation or transit of *aquatic animal products* derived from a species not referred to in Article 9.1.2. but which could reasonably be expected to pose a *risk* of transmission of Vp_{AHPND}, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this analysis.~~

[…]

(CLEAN VERSION)

CHAPTER 9.1.

ACUTE HEPATOPANCREATIC NECROSIS DISEASE

[…]

Article 9.1.3.

Measures for the importation or transit of aquatic animal products for any purpose regardless of the AHPND status of the exporting country, zone or compartment

The *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of these *aquatic animal products*, *Competent Authorities* should not require any *sanitary measures* related to AHPND, regardless of the AHPND status of the *exporting country*, *zone* or *compartment*:

1) cooked or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 100°C for at least 60 seconds, or a time/temperature equivalent that inactivates Vp_{AHPND};

2) crustacean *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 100°C for at least 60 seconds, or a time/temperature equivalent that inactivates Vp_{AHPND};

3) crustacean oil;

4) chemically extracted chitin.

[…]

(TRACK CHANGES VERSION)

CHAPTER 9.2.

INFECTION WITH *APHANOMYCES ASTACI* (CRAYFISH PLAGUE)

[…]

Article 9.2.3.

Measures for the i~~I~~mportation or transit of aquatic animal products for any purpose regardless of the infection with *A. astaci* status of the exporting country, zone or compartment

~~1)~~ The ~~following~~ *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of ~~the~~ these *aquatic animal products* ~~listed below~~, *Competent Authorities* should not require any *sanitary measures*~~conditions~~ related to *A. astaci*, regardless of the infection with *A. astaci* status of the *exporting country*, *zone* or *compartment*~~:, when authorising the importation or transit of the following *aquatic animal products* derived from a species referred to in Article 9.2.2. that are intended for any purpose and comply with Article 5.4.1.:~~

~~a~~1) cooked, pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 100°C for at least ~~one minute~~ 60 seconds, ~~(~~or a time/temperature equivalent that ~~has been demonstrated to~~ inactivates *A. astaci*~~)~~;

~~a) heat sterilised hermetically sealed crayfish products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent that has been demonstrated to inactivate *A. astaci*);~~

~~b) cooked crayfish products that have been subjected to heat treatment at 100°C for at least one minute (or any time/temperature equivalent that has been demonstrated to inactivate *A. astaci*);~~

~~c) pasteurised crayfish products that have been subjected to heat treatment at 90°C for at least ten minutes (or any time/temperature equivalent that has been demonstrated to inactivate *A. astaci*);~~

~~d)~~~~b~~2) frozen crayfish products that have been subjected to minus 20°C or lower temperatures for at least 72 hours;

3) crayfish *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 100°C for at least 60 seconds, or a time/temperature equivalent that inactivates *A.astaci*;

~~e)~~~~c)~~4) crayfish oil;

~~f)~~~~d)~~ ~~crayfish *meal* that has been heat treated at a core temperature of at least 100°C for at least one minute (or a time/temperature equivalent that has been demonstrated to inactivate *A.astaci*);~~

~~g)~~~~e~~5) chemically extracted chitin.

~~2) When authorising the importation or transit of *aquatic animal products* derived from a species referred to in Article 9.2.2., other than those referred to in point 1 of Article 9.2.3., *Competent Authorities* should require the conditions prescribed in Articles 9.2.7. to 9.2.12. relevant to the infection with *A. astaci* status of the *exporting country*, *zone* or *compartment*.~~

~~3) When considering the importation or transit of *aquatic animal products* derived from a species not referred to in Article 9.2.2. but which could reasonably be expected to pose a *risk* of transmission of *A. astaci*, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this analysis.~~

[…]

(CLEAN VERSION)

CHAPTER 9.2.

INFECTION WITH *APHANOMYCES ASTACI* (CRAYFISH PLAGUE)

[…]

Article 9.2.3.

Measures for the importation or transit of aquatic animal products for any purpose regardless of the infection with *A. astaci* status of the exporting country, zone or compartment

The *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of these *aquatic animal products*, *Competent Authorities* should not require any *sanitary measures* related to *A. astaci*, regardless of the infection with *A. astaci* status of the *exporting country*, *zone* or *compartment:*

1) cooked, pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 100°C for at least 60 seconds, or a time/temperature equivalent that inactivates *A. astaci*;

2) frozen crayfish products that have been subjected to minus 20°C or lower temperatures for at least 72 hours;

3) crayfish *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 100°C for at least 60 seconds, or a time/temperature equivalent that inactivates *A. astaci*;

4) crayfish oil;

5) chemically extracted chitin.

[…]

(TRACK CHANGES VERSION)

CHAPTER 9.3.

INFECTION WITH *HEPATOBACTER PENAEI* (NECROTISING HEPATOPANCREATITIS)

[…]

Article 9.3.3

Measures for the i~~I~~mportation or transit of aquatic animal products for any purpose regardless of the infection with *H. penaei* status of the exporting country, zone or compartment

~~1)~~ The ~~following~~ *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of ~~the~~ these *aquatic animal products* ~~listed below.~~ *Competent Authorities* should not require any *sanitary measures*~~conditions~~ related to *H. penaei*, regardless of the infection with *H. penaei* status of the *exporting country*, *zone* or *compartment*~~:, when authorising the importation or transit of the following *aquatic animal products* derived from a species referred to in Article 9.3.2. that are intended for any purpose and comply with Article 5.4.1.:~~

~~a~~1) cooked, pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 63°C for at least 30 minutes~~, (~~or a time/temperature equivalent that ~~has been demonstrated to~~ inactivate~~s~~ *H. penaei*);

~~a) heat sterilised hermetically sealed crustacean products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent that has been demonstrated to inactivate *H. penaei*);~~

~~b) cooked crustacean products that have been subjected to heat treatment at 100°C for at least three minutes (or any time/temperature equivalent that has been demonstrated to inactivate *H. penaei*);~~

~~c) pasteurised crustacean products that have been subjected to heat treatment at 63°C for at least 30 minutes (or any time/temperature equivalent that has been demonstrated to inactivate *H. penaei*);~~

2) crustacean *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 63°C for at least 30 minutes, or a time/temperature equivalent that has been demonstrated to inactivate *H. penaei*;

~~d)~~~~b)~~3) crustacean oil;

~~e)~~~~c)~~ ~~crustacean *meal* that has been heat treated at a core temperature of at least 63°C for at least 30 minutes (or a time/temperature equivalent that has been demonstrated to inactivate *H. penaei*);~~

~~f)~~~~d~~4) chemically extracted chitin.

~~2) When authorising the importation or transit of *aquatic animal products* derived from a species referred to in Article 9.3.2., other than those referred to in point 1 of Article 9.3.3., *Competent Authorities* should require the conditions prescribed in Articles 9.3.7. to 9.3.12. relevant to the infection with *H. penaei* status of the *exporting country*, *zone* or *compartment*.~~

~~3) When considering the importation or transit of *aquatic animal products* derived from a species not referred to in Article 9.3.2. but which could reasonably be expected to pose a *risk* of transmission of *H. penaei*, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this analysis.~~

[…]

(CLEAN VERSION)

CHAPTER 9.3.

INFECTION WITH *HEPATOBACTER PENAEI* (NECROTISING HEPATOPANCREATITIS)

[…]

Article 9.3.3

Measures for the importation or transit of aquatic animal products for any purpose regardless of the infection with *H. penaei* status of the exporting country, zone or compartment

The *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of these *aquatic animal products*, *Competent Authorities* should not require any *sanitary measures* related to *H. penaei*, regardless of the infection with *H. penaei* status of the *exporting country*, *zone* or *compartment*:

1) cooked, pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 63°C for at least 30 minutes, or a time/temperature equivalent inactivates *H. penaei*;

2) crustacean *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 63°C for at least 30 minutes, or a time/temperature equivalent that inactivates *H. penaei*;

3) crustacean oil;

4) chemically extracted chitin.

[…]

(TRACK CHANGES VERSION)

CHAPTER 9.4.

INFECTION WITH INFECTIOUS HYPODERMAL AND HAEMATOPOIETIC NECROSIS VIRUS

[…]

Article 9.4.3.

Measures for the i~~I~~mportation or transit of aquatic animal products for any purpose regardless of the infection with IHHNV status of the exporting country, zone or compartment

~~1)~~ The ~~following~~ *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of ~~the~~ these *aquatic animal products* ~~listed below~~, *Competent Authorities* should not require any *sanitary measures*~~conditions~~ related to IHHNV, regardless of the infection with IHHNV status of the *exporting country*, *zone* or *compartment*~~:, when authorising the importation or transit of the following *aquatic animal products* derived from a species referred to in Article 9.4.2. that are intended for any purpose and comply with Article 5.4.1.:~~

~~a~~1) cooked or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 100°C for at least two minutes~~, (~~or a time/temperature equivalent that ~~has been demonstrated to~~ inactivates IHHNV~~)~~;

~~a) heat sterilised hermetically sealed crustacean products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent that has been demonstrated to inactivate IHHNV);~~

~~b) cooked crustacean products that have been subjected to heat treatment at 90°C for at least 20 minutes (or any time/temperature equivalent that has been demonstrated to inactivate IHHNV);~~

2) crustacean *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 100°C for at least two minutes, or a time/temperature equivalent that inactivates IHHNV;

~~c)b)~~3) crustacean oil.

~~d)c) crustacean *meal* that has been heat treated at a core temperature of at least 100°C for t least two minutes (or a time/temperature equivalent that has been demonstrated to inactivate IHHNV).~~

~~2) When authorising the importation or transit of *aquatic animal products* derived from a species referred to in Article 9.4.2., other than those referred to in point 1 of Article 9.4.3., *Competent Authorities* should require the conditions prescribed in Articles 9.4.7. to 9.4.12. relevant to the infection with IHHNV status of the *exporting country*, *zone* or *compartment*.~~

~~3) When considering the importation or transit of *aquatic animal products* derived from a species not referred to in Article 9.4.2. but which could reasonably be expected to pose a *risk* of transmission of IHHNV, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this analysis.~~

[…]

(CLEAN VERSION)

CHAPTER 9.4.

INFECTION WITH INFECTIOUS HYPODERMAL AND HAEMATOPOIETIC NECROSIS VIRUS

[…]

Article 9.4.3.

Measures for the importation or transit of aquatic animal products for any purpose regardless of the infection with IHHNV status of the exporting country, zone or compartment

The *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of these *aquatic animal products*, *Competent Authorities* should not require any *sanitary measures* related to IHHNV, regardless of the infection with IHHNV status of the *exporting country*, *zone* or *compartment*:

a) cooked or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 100°C for at least two minutes, or a time/temperature equivalent that inactivates IHHNV;

b) crustacean *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 100°C for at least two minutes, or a time/temperature equivalent that inactivates IHHNV;

c) crustacean oil.

[…]

(TRACK CHANGES VERSION)

CHAPTER 9.5.

INFECTION WITH INFECTIOUS MYONECROSIS VIRUS

[…]

Article 9.5.3.

Measures for the i~~I~~mportation or transit of aquatic animal products for any purpose regardless of the infection with IMNV status of the exporting country, zone or compartment

~~1)~~ The ~~following~~ *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of ~~the~~ these *aquatic animal products* ~~listed below~~, *Competent Authorities* should not require any *sanitary measures*~~conditions~~ related to IMNV, regardless of the infection with IMNV status of the *exporting country*, *zone* or *compartment*~~:, when authorising the importation or transit of the following *aquatic animal products* derived from a species referred to in Article 9.5.2. that are intended for any purpose and comply with Article 5.4.1.:~~

~~a~~1) cooked or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least 60 minutes~~, (~~or a time/temperature equivalent that ~~has been demonstrated to~~ inactivate~~s~~ IMNV~~)~~;

~~a) heat sterilised hermetically sealed crustacean products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent that has been demonstrated to inactivate IMNV);~~

~~c) cooked crustacean products that have been subjected to heat treatment at 60°C for at least three minutes (or any time/temperature equivalent that has been demonstrated to inactivate IMNV);~~

2) crustacean *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least 60 minutes, or a time/temperature equivalent that inactivates IMNV;

~~d)~~~~b)~~3) crustacean oil;

~~e)~~~~c)~~ ~~crustacean *meal* that has been heat treated at a core temperature of at least 60°C for at least 60 minutes (or a time/temperature equivalent that has been demonstrated to inactivate IMNV);~~

~~f)~~~~d)~~4) chemically extracted chitin.

~~2) When authorising the importation or transit of *aquatic animal products* derived from a species referred to in Article 9.5.2., other than those referred to in point 1 of Article 9.5.3., *Competent Authorities* should require the conditions prescribed in Articles 9.5.7. to 9.5.12. relevant to the infection with IMNV status of the *exporting country*, *zone* or *compartment*.~~

~~3) When considering the importation or transit of *aquatic animal products* derived from a species not referred to in Article 9.5.2. but which could reasonably be expected to pose a *risk* of transmission of IMNV, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this analysis.~~

[…]

(CLEAN VERSION)

CHAPTER 9.5.

INFECTION WITH INFECTIOUS MYONECROSIS VIRUS

[…]

Article 9.5.3.

Measures for the importation or transit of aquatic animal products for any purpose regardless of the infection with IMNV status of the exporting country, zone or compartment

The *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of these *aquatic animal products*, *Competent Authorities* should not require any *sanitary measures* related to IMNV, regardless of the infection with IMNV status of the *exporting country*, *zone* or *compartment*:

1) cooked or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least 60 minutes, or a time/temperature equivalent that inactivates IMNV;

2) crustacean *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least 60 minutes, or a time/temperature equivalent that inactivates IMNV;

3) crustacean oil;

4) chemically extracted chitin.

[…]

(TRACK CHANGES VERSION)

CHAPTER 9.6.

INFECTION WITH *MACROBRACHIUM ROSENBERGII* NODAVIRUS (WHITE TAIL DISEASE)

[…]

Article 9.6.3.

Measures for the i~~I~~mportation or transit of aquatic animal products for any purpose regardless of the infection with MrNV status of the exporting country, zone or compartment

~~1)~~ The ~~following~~ *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of ~~the~~ these *aquatic animal products* listed below, *Competent Authorities* should not require any *sanitary measures*~~conditions~~ related to MrNV, regardless of the infection with MrNV status of the *exporting country*, *zone* or *compartment*:~~, when authorising the importation or transit of the following *aquatic animal products* derived from a species referred to in Article 9.6.2. that are intended for any purpose and comply with Article 5.4.1.:~~

~~a~~1) cooked, pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least 60 minutes~~, (~~or a time/temperature equivalent that ~~has been demonstrated to~~ inactivates MrNV~~)~~;

~~a) heat sterilised hermetically sealed crustacean products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent that has been demonstrated to inactivate MrNV);~~

~~b) cooked crustacean products that have been subjected to heat treatment at 60°C for at least 60 minutes (or any time/temperature equivalent that has been demonstrated to inactivate MrNV);~~

~~c) pasteurised crustacean products that have been subjected to heat treatment at 90°C for at least ten minutes (or any time/temperature equivalent that has been shown to inactivate MrNV);~~

2) crustacean *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least 60 minutes, or a time/temperature equivalent that inactivates MrNV;

~~d)~~~~b)~~3) crustacean oil;

~~e)~~c) ~~crustacean *meal* that has been heat treated at a core temperature of at least 60°C for at least 60 minutes (or a time/temperature equivalent that has been demonstrated to inactivate MrNV);~~

~~f)~~~~d~~4) chemically extracted chitin.

~~2) When authorising the importation or transit of *aquatic animal products* derived from a species referred to in Article 9.6.2., other than those referred to in point 1 of Article 9.6.3., *Competent Authorities* should require the conditions prescribed in Articles 9.6.7. to 9.6.12. relevant to the infection with MrNV status of the *exporting country*, *zone* or *compartment*.~~

~~3) When considering the importation or transit of *aquatic animal products* derived from a species not referred to in Article 9.6.2. but which could reasonably be expected to pose a *risk* of transmission of MrNV, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this analysis.~~

[…]

(CLEAN VERSION)

CHAPTER 9.6.

INFECTION WITH *MACROBRACHIUM ROSENBERGII* NODAVIRUS (WHITE TAIL DISEASE)

[…]

Article 9.6.3.

Measures for the importation or transit of aquatic animal products for any purpose regardless of the infection with MrNV status of the exporting country, zone or compartment

The *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of these *aquatic animal products*, *Competent Authorities* should not require any *sanitary measures* related to MrNV, regardless of the infection with MrNV status of the *exporting country*, *zone* or *compartment*:

1) cooked, pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least 60 minutes, or a time/temperature equivalent that inactivates MrNV;

2) crustacean *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least 60 minutes, or a time/temperature equivalent that inactivates MrNV;

3) crustacean oil;

4) chemically extracted chitin.

[…]

(TRACK CHANGES VERSION)

CHAPTER 9.7.

INFECTION WITH TAURA SYNDROME VIRUS

[…]

Article 9.7.3.

Measures for the i~~I~~mportation or transit of aquatic animal products for any purpose regardless of the infection with TSV status of the exporting country, zone or compartment

~~1)~~ The ~~following~~ *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of ~~the~~ these *aquatic animal products* ~~listed below~~. *Competent Authorities* should not require any *sanitary measures* ~~conditions~~ related to TSV, regardless of the infection with TSV status of the *exporting country*, *zone* or *compartment*:~~, when authorising the importation or transit of the following *aquatic animal products* derived from a species referred to in Article 9.7.2. that are intended for any purpose and comply with Article 5.4.1.:~~

~~a~~1) cooked, pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 70°C for at least 30 minutes, ~~(~~or a time/temperature equivalent that ~~has been demonstrated to~~ inactivates TSV~~)~~;

~~a) heat sterilised hermetically sealed crustacean products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent that has been demonstrated to inactivate TSV);~~

~~b) cooked crustacean products that have been subjected to heat treatment at 70°C for at least 30 minutes (or any time/temperature equivalent that has been demonstrated to inactivate TSV);~~

~~c) pasteurised crustacean products that have been subjected to heat treatment at 90°C for at least ten minutes (or any time/temperature equivalent that has been demonstrated to inactivate TSV);~~

2) crustacean *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 70°C for at least 30 minutes, or a time/temperature equivalent that inactivates TSV;

~~d)~~~~b)~~3) crustacean oil;

~~e)~~~~c)~~ ~~crustacean *meal* that has been heat treated at a core temperature of at least 70°C for at least 30 minutes (or a time/temperature equivalent that has been demonstrated to inactivate TSV);~~

~~f)~~~~d~~4) chemically extracted chitin.

~~2) When authorising the importation or transit of *aquatic animal products* derived from a species referred to in Article 9.7.2., other than those referred to in point 1 of Article 9.7.3., *Competent Authorities* should require the conditions prescribed in Articles 9.7.7. to 9.7.12. relevant to the infection with TSV status of the *exporting country*, *zone* or *compartment*.~~

~~3) When considering the importation or transit of *aquatic animal products* derived from a species not referred to in Article 9.7.2. but which could reasonably be expected to pose a *risk* of transmission of TSV, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this analysis.~~

[…]

(CLEAN VERSION)

CHAPTER 9.7.

INFECTION WITH TAURA SYNDROME VIRUS

[…]

Article 9.7.3.

Measures for the importation or transit of aquatic animal products for any purpose regardless of the infection with TSV status of the exporting country, zone or compartment

The *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of these *aquatic animal products*, *Competent Authorities* should not require any *sanitary measures* related to TSV, regardless of the infection with TSV status of the *exporting country*, *zone* or *compartment*:

1) cooked, pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 70°C for at least 30 minutes, or a time/temperature equivalent that inactivates TSV;

2) crustacean *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 70°C for at least 30 minutes, or a time/temperature equivalent that inactivates TSV;

3) crustacean oil;

4) chemically extracted chitin.

[…]

(TRACK CH ANGES VERSION)

CHAPTER 9.8.

INFECTION WITH WHITE SPOT SYNDROME VIRUS

[…]

Article 9.8.3.

Measures for the ~~I~~importation or transit of aquatic animal products for any purpose regardless of the infection with WSSV status of the exporting country, zone or compartment

~~1)~~ The ~~following~~ *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of ~~the~~ these *aquatic animal products* ~~listed below~~, *Competent Authorities* should not require any *sanitary measures* ~~conditions~~ related to WSSV, regardless of the infection with WSSV status of the *exporting country*, *zone* or *compartment*: ~~when authorising the importation or transit of the following *aquatic animal products* derived from a species referred to in Article 9.8.2. that are intended for any purpose and comply with Article 5.4.1.:~~

~~a~~1) cooked, ~~canned,~~ pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least ~~one minute~~ 60 seconds, ~~(~~or a time/temperature equivalent that ~~has been demonstrated to~~ inactivates WSSV~~)~~;

~~a) heat sterilised hermetically sealed crustacean products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent that has been demonstrated to inactivate WSSV);~~

~~b) cooked crustacean products that have been subjected to heat treatment at 60°C for at least one minute (or any time/temperature equivalent that has been demonstrated to inactivate WSSV);~~

~~c) pasteurised crustacean products that have been subjected to heat treatment at 90°C for at least ten minutes (or any time/temperature equivalent that has been demonstrated to inactivate WSSV);~~

2) crustacean *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least 60 seconds, or a time/temperature equivalent that inactivates WSSV;

~~d)~~~~b)~~3) crustacean oil;

~~e)~~~~c)~~ ~~crustacean *meal* that has been heat treated to a core temperature of at least 60°C for at least one minute (or a time/temperature equivalent that has been demonstrated to inactivate WSSV);~~

~~f)~~~~d~~4) chemically extracted chitin.

~~2) When authorising the importation or transit of *aquatic animal products* derived from a species referred to in Article 9.8.2., other than those referred to in point 1 of Article 9.8.3., *Competent Authorities* should require the conditions prescribed in Articles 9.8.7. to 9.8.12. relevant to the infection with WSSV status of the *exporting country*, *zone* or *compartment*.~~

~~3) When considering the importation or transit of *aquatic animal products* derived from a species not referred to in Article 9.8.2. but which could reasonably be expected to pose a risk of transmission of WSSV, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this analysis.~~

[…]

(CLEAN VERSION)

CHAPTER 9.8.

INFECTION WITH WHITE SPOT SYNDROME VIRUS

[…]

Article 9.8.3.

Measures for the importation or transit of aquatic animal products for any purpose regardless of the infection with WSSV status of the exporting country, zone or compartment

The *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of these *aquatic animal products*, *Competent Authorities* should not require any *sanitary measures* related to WSSV, regardless of the infection with WSSV status of the *exporting country*, *zone* or *compartment*:

1) cooked, pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least 60 seconds, or a time/temperature equivalent that inactivates WSSV;

2) crustacean *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least 60 seconds, or a time/temperature equivalent that inactivates WSSV;

3) crustacean oil;

4) chemically extracted chitin.

[…]

(TRACK CHANGES VERSION)

CHAPTER 9.9.

INFECTION WITH YELLOW HEAD VIRUS GENOTYPE 1

[…]

Article 9.9.3.

Measures for the i~~I~~mportation or transit of aquatic animal products for any purpose regardless of the infection with YHV1 status of the exporting country, zone or compartment

~~1)~~ The ~~following~~ *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of ~~the~~ these *aquatic animal products* ~~listed below~~, *Competent Authorities* should not require any *sanitary measures*~~conditions~~ related to YHV1, regardless of the infection with YHV1 status of the *exporting country*, *zone* or *compartment*~~:, when authorising the importation or transit of the following *aquatic animal products* derived from a species referred to in Article 9.9.2. that are intended for any purpose and comply with Article 5.4.1.:~~

~~a~~1) cooked, pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least 15 minutes, ~~(~~or a time/temperature equivalent that ~~has been demonstrated to~~ inactivates YHV1);

~~a) heat sterilised hermetically sealed crustacean products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent that has been demonstrated to inactivate YHV1);~~

~~b) cooked crustacean products that have been subjected to heat treatment at 60°C for at least 15 minutes (or any time/temperature equivalent that has been demonstrated to inactivate YHV1);~~

~~c) pasteurised crustacean products that have been subjected to heat treatment at 90°C for at least ten minutes (or any time/temperature equivalent that has been demonstrated to inactivate YHV1);~~

2) crustacean *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least 15 minutes, or a time/temperature equivalent that inactivates YHV1;

~~d)~~~~b)~~3) crustacean oil;

~~e)~~~~c)~~ ~~crustacean *meal* that has been heat treated at a core temperature of at least 60°C for at least 15 minutes (or a time/temperature equivalent that has been demonstrated to inactivate YHV1);~~

~~f)~~~~d~~4) chemically extracted chitin.

~~2) When authorising the importation or transit of *aquatic animal products* derived from a species referred to in Article 9.9.2., other than those referred to in point 1 of Article 9.9.3., *Competent Authorities* should require the conditions prescribed in Articles 9.9.7. to 9.9.12. relevant to the infection with YHV1 status of the *exporting country*, *zone* or *compartment*.~~

~~3) When considering the importation or transit of *aquatic animal products* derived from a species not referred to in Article 9.9.2. but which could reasonably be expected to pose a *risk* of transmission of YHV1, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this analysis.~~

[…]

(CLEAN VERSION)

CHAPTER 9.9.

INFECTION WITH YELLOW HEAD VIRUS GENOTYPE 1

[…]

Article 9.9.3.

Measures for the importation or transit of aquatic animal products for any purpose regardless of the infection with YHV1 status of the exporting country, zone or compartment

The *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of these *aquatic animal products*, *Competent Authorities* should not require any *sanitary measures* related to YHV1, regardless of the infection with YHV1 status of the *exporting country*, *zone* or *compartment*:

1) cooked, pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least 15 minutes, or a time/temperature equivalent that inactivates YHV1;

2) crustacean *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least 15 minutes, or a time/temperature equivalent that inactivates YHV1;

3) crustacean oil;

4) chemically extracted chitin.

[…]

Return to Agenda

ARTICLES 10.X.3 FOR FISH DISEASE-SPECIFIC CHAPTERS (TRACK CHANGES AND CLEAN VERSIONS)

(TRACK CHANGES VERSION)

CHAPTER 10.1.

INFECTION WITH EPIZOOTIC HAEMATOPOIETIC NECROSIS VIRUS

[…]

Article 10.1.3.

Measures for the i~~I~~mportation or transit of aquatic animal products for any purpose regardless of the infection with EHNV status of the exporting country, zone or compartment

~~1)~~ The *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of these *aquatic animal products*, *Competent Authorities* should not require any sanitary measures~~conditions~~ related to EHNV, regardless of the infection with EHNV status of the *exporting country*, *zone* or *compartment*:~~, when authorising the importation or transit of the following *aquatic animal products* derived from a species referred to in Article 10.1.2. that are intended for any purpose and comply with Article 5.4.1.:~~

1) pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least 15 minutes, or a time/temperature equivalent that inactivates EHNV;

~~a) heat sterilised hermetically sealed fish products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent that has been demonstrated to inactivate EHNV);~~

~~b) pasteurised fish products that have been subjected to heat treatment at 90°C for ten minutes (or any time/temperature equivalent that has been demonstrated to inactivate EHNV);~~

~~c)~~2) mechanically dried eviscerated fish that has been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least 15 minutes, ~~(i.e. a heat treatment at 100°C for at least 30 minutes~~ or ~~any~~a time/temperature equivalent that ~~has been demonstrated to~~ inactivates EHNV~~)~~;

3) fish *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least 15 minutes, or a time/temperature equivalent that inactivates EHNV;

~~d~~4) fish oil;

~~e) fish *meal*;~~

~~f)~~5) fish skin leather.

~~2) When authorising the importation or transit of *aquatic animal products* derived from a species referred to in Article 10.1.2., other than those referred to in point 1 of Article 10.1.3., *Competent Authorities* should require the conditions prescribed in Articles 10.1.7. to 10.1.12. relevant to the infection with EHNV status of the *exporting country*, *zone* or *compartment*.~~

~~3) When considering the importation or transit of *aquatic animal products* derived from a species not referred to in Article 10.1.2. but which could reasonably be expected to pose a *risk* of transmission of EHNV, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this analysis.~~

[…]

(CLEAN VERSION)

CHAPTER 10.1.

INFECTION WITH EPIZOOTIC HAEMATOPOIETIC NECROSIS VIRUS

[…]

Article 10.1.3.

Measures for the importation or transit of aquatic animal products for any purpose regardless of the infection with EHNV status of the exporting country, zone or compartment

The *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of these *aquatic animal products*, *Competent Authorities* should not require any sanitary measures related to EHNV, regardless of the infection with EHNV status of the *exporting country*, *zone* or *compartment*:

1) pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least 15 minutes, or a time/temperature equivalent that inactivates EHNV;

2) mechanically dried eviscerated fish that has been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least 15 minutes, or a time/temperature equivalent that inactivates EHNV;

3) fish *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least 15 minutes, or a time/temperature equivalent that inactivates EHNV;

4) fish oil;

5) fish skin leather.

[…]

(TRACK CHANGES VERSION)

CHAPTER 10.2.

INFECTION WITH *APHANOMYCES INVADANS* (EPIZOOTIC ULCERATIVE SYNDROME)

[…]

Article 10.2.3.

Measures for the i~~I~~mportation or transit of aquatic animal products for any purpose regardless of the infection with *A. invadans* status of the exporting country, zone or compartment

~~1)~~ The *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of these *aquatic animal products*, *Competent Authorities* should not require any sanitary measures~~conditions~~ related to *A. invadans*, regardless of the infection with *A. invadans* status of the *exporting country*, *zone* or *compartment:*~~, when authorising the importation or transit of the following *aquatic animal products* derived from a species referred to in Article 10.2.2. that are intended for any purpose and comply with Article 5.4.1.:~~

1) pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least five minutes, or a time/temperature equivalent that inactivates *A.invadans*;

~~a) heat sterilised hermetically sealed fish products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent that has been demonstrated to inactivate *A. invadans*);~~

~~b) pasteurised fish products that have been subjected to heat treatment at 90°C for at least ten minutes (or any time/temperature equivalent that has been demonstrated to inactivate *A. invadans*);~~

~~c)~~2) mechanically dried eviscerated fish that have been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least five minutes, ~~(i.e. a heat treatment at 100°C for at least 30 minutes~~ or ~~any~~a time/temperature equivalent that ~~has been demonstrated to~~ inactivate~~s~~ *A. invadans*~~)~~;

3) fish *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least five minutes, or a time/temperature equivalent that inactivates *A.invadans*;

~~d~~4) fish oil;

~~e) fish *meal*~~;

~~f)~~5) frozen eviscerated fish;

~~g)~~6) frozen fish fillets or steaks.

~~2) When authorising the importation or transit of *aquatic animal products* derived from a species referred to in Article 10.2.2., other than those referred to in point 1 of Article 10.2.3., *Competent Authorities* should require the conditions prescribed in Articles 10.2.7. to 10.2.12. relevant to infection with *A. invadans* status of the *exporting country*, *zone* or *compartment*.~~

~~3) When considering the importation or transit of *aquatic animal products* derived from a species not referred to in Article 10.2.2. but which could reasonably be expected to pose a *risk* of transmission of *A. invadans*, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *exporting country* should be informed of the outcome of this analysis.~~

[…]

(CLEAN VERSION)

CHAPTER 10.2.

INFECTION WITH *APHANOMYCES INVADANS* (EPIZOOTIC ULCERATIVE SYNDROME)

[…]

Article 10.2.3.

Measures for the importation or transit of aquatic animal products for any purpose regardless of the infection with *A. invadans* status of the exporting country, zone or compartment

The *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of these *aquatic animal products*, *Competent Authorities* should not require any sanitary measures related to *A. invadans*, regardless of the infection with *A. invadans* status of the *exporting country*, *zone* or *compartment*:

1) pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least five minutes, or a time/temperature equivalent that inactivates *A. invadans*;

2) mechanically dried eviscerated fish that have been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least five minutes, or a time/temperature equivalent that inactivates *A. invadans*;

3) fish *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least five minutes, or a time/temperature equivalent that inactivates *A. invadans*;

4) fish oil;

5) frozen eviscerated fish;

6) frozen fish fillets or steaks.

[…]

(TRACK CHANGES VERSION)

CHAPTER 10.3.

INFECTION WITH *GYRODACTYLUS SALARIS*

[…]

Article 10.3.3.

Measures for the i~~I~~mportation or transit of aquatic animal products for any purpose regardless of the infection with *G. salaris* status of the exporting country, zone or compartment

~~1)~~ The *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of these *aquatic animal products*, *Competent Authorities* should not require any sanitary measures~~conditions~~ related to *G. salaris*, regardless of the infection with *G. salaris* status of the *exporting country*, *zone* or *compartment*:~~,~~ ~~when authorising the importation or transit of the following *aquatic animal products* derived from a species referred to in Article 10.3.2. that are intended for any purpose and comply with Article 5.4.1.:~~

1) pasteurised or retorted *aquatic animal products*;

~~a) heat sterilised hermetically sealed fish products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent that has been demonstrated to inactivate *G. salaris*);~~

~~b) pasteurised fish products that have been subjected to a heat treatment at 63°C for at least 30 minutes (or any time/temperature equivalent that has been demonstrated to inactivate *G. salaris*);~~

~~c)~~2) mechanically dried eviscerated fish;

~~d)~~3) naturally dried eviscerated fish (i.e. sun-dried or wind-dried);

~~e)~~4) frozen eviscerated fish that have been subjected to minus 18°C or lower temperatures;

~~f)~~5) frozen fish fillets or steaks that have been subjected to minus 18°C or lower temperatures;

~~g)~~6) chilled eviscerated fish that have been harvested from seawater with a salinity of at least 25 parts per thousand (ppt);

~~h)~~7) chilled fish fillets or steaks derived from fish that have been harvested from seawater with a salinity of at least 25 ppt;

~~i)~~8) chilled fish products from which the skin, fins and gills have been removed;

~~j)~~9) non-viable fish roe;

~~k)~~10) fish oil;

~~l)~~11) fish *meal*;

~~m)~~12) fish skin leather.

~~2) When authorising the importation or transit of *aquatic animal products* derived from a species referred to in Article 10.3.2., other than those referred to in point 1 of Article 10.3.3., *Competent Authorities* should require the conditions prescribed in Articles 10.3.7. to 10.3.12. relevant to the infection with *G. salaris* status of the *exporting country*, *zone* or *compartment*.~~

~~3) When considering the importation or transit of *aquatic animal products* derived from a species not referred to in Article 10.3.2. but which could reasonably be expected to pose a *risk* of transmission of *G. salaris*, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this analysis.~~

[…]

(CLEAN VERSION)

CHAPTER 10.3.

INFECTION WITH *GYRODACTYLUS SALARIS*

[…]

Article 10.3.3.

Measures for the importation or transit of aquatic animal products for any purpose regardless of the infection with *G. salaris* status of the exporting country, zone or compartment

The *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of these *aquatic animal products*, *Competent Authorities* should not require any sanitary measures related to *G. salaris*, regardless of the infection with *G. salaris* status of the *exporting country*, *zone* or *compartment:*

1) pasteurised or retorted *aquatic animal products*;

2) mechanically dried eviscerated fish;

3) naturally dried eviscerated fish (i.e. sun-dried or wind-dried);

4) frozen eviscerated fish that have been subjected to minus 18°C or lower temperatures;

5) frozen fish fillets or steaks that have been subjected to minus 18°C or lower temperatures;

6) chilled eviscerated fish that have been harvested from seawater with a salinity of at least 25 parts per thousand (ppt);

7) chilled fish fillets or steaks derived from fish that have been harvested from seawater with a salinity of at least 25 ppt;

8) chilled fish products from which the skin, fins and gills have been removed;

9) non-viable fish roe;

10) fish oil;

11) fish *meal*;

12) fish skin leather.

[…]

(TRACK CHANGES VERSION)

CHAPTER 10.4.

INFECTION WITH INFECTIOUS SALMON ANAEMIA VIRUS

[…]

Article 10.4.3.

Measures for the i~~I~~mportation or transit of aquatic animal products for any purpose regardless of the infection with ISAV status of the exporting country, zone or compartment

In this article, all statements referring to ISAV include HPR deleted ISAV and HPR0 ISAV.

~~1)~~ The *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of these *aquatic animal products*, *Competent Authorities* should not require any sanitary measures ~~conditions~~ related to ISAV, regardless of the infection with ISAV status of the *exporting country*, *zone* or *compartment:*~~,~~ ~~when authorising the importation or transit of the following *aquatic animal products* derived from a species referred to in Article 10.4.2. that are intended for any purpose and comply with Article 5.4.1.:~~

1) pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 56°C for at least five minutes, or a time/temperature equivalent that inactivates ISAV;

~~a) heat sterilised hermetically sealed fish products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent that has been demonstrated to inactivate ISAV);~~

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

~~c)~~2) mechanically dried eviscerated fish that have been subjected to a heat treatment sufficient to attain a core temperature of at least 56°C for at least five minutes, ~~(i.e. a heat treatment at 100°C for 30 minutes or anyc~~a time/temperature equivalent that ~~has been demonstrated to~~ inactivate~~s~~ ISAV~~)~~;

3) fish *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 56°C for at least five minutes, or a time/temperature equivalent that to inactivates ISAV;

~~D~~4) fish oil;

~~e) fish *meal*;~~

~~f)~~5) fish skin leather.

~~2) When authorising the importation or transit of *aquatic animal products* derived from a species referred to in Article 10.4.2., other than those referred to in point 1 of Article 10.4.3., *Competent Authorities* should require the conditions prescribed in Articles 10.4.10. to 10.4.17. relevant to the infection with ISAV status of the *exporting country*, *zone* or *compartment*.~~

~~3) When considering the importation or transit of *aquatic animal products* derived from a species not referred to in Article 10.4.2. but which could reasonably be expected to pose a *risk* of transmission of ISAV, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this analysis.~~

[…]

(CLEAN VERSION)

CHAPTER 10.4.

INFECTION WITH INFECTIOUS SALMON ANAEMIA VIRUS

[…]

Article 10.4.3.

Measures for the importation or transit of aquatic animal products for any purpose regardless of the infection with ISAV status of the exporting country, zone or compartment

In this article, all statements referring to ISAV include HPR deleted ISAV and HPR0 ISAV.

The *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of these *aquatic animal products*, *Competent Authorities* should not require any sanitary measures related to ISAV, regardless of the infection with ISAV status of the *exporting country*, *zone* or *compartment:*

1) pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 56°C for at least five minutes, or a time/temperature equivalent that inactivates ISAV;

2) mechanically dried eviscerated fish that have been subjected to a heat treatment sufficient to attain a core temperature of at least 56°C for at least five minutes, or a time/temperature equivalent that inactivates ISAV;

3) fish *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 56°C for at least five minutes, or a time/temperature equivalent that inactivates ISAV;

4) fish oil;

5) fish skin leather.

[…]

(TRACK CHANGES VERSION)

CHAPTER 10.5.

INFECTION WITH SALMONID ALPHAVIRUS

[…]

Article 10.5.3.

Measures for the i~~I~~mportation or transit of aquatic animal products for any purpose regardless of the infection with SAV status of the exporting country, zone or compartment

~~1)~~ The *aquatic animal product*s listed below have been assessed as meeting the criteria for safety of *aquatic animal product*s in accordance with Article 5.4.1. When authorising the importation or transit of these *aquatic animal product*s, *Competent Authorities* should not require any sanitary measures ~~conditions~~ related to SAV, regardless of the infection with SAV status of the *exporting country*, *zone* or *compartment*:~~,~~ ~~when authorising the importation or transit of the following *aquatic animal products* derived from a species referred to in Article 10.5.2. that are intended for any purpose and comply with Article 5.4.1.:~~

1) pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least 60 minutes, or a time/temperature equivalent inactivates SAV;

~~a) heat sterilised hermetically sealed fish products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent that has been demonstrated to inactivate SAV);~~

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

~~c)~~2) mechanically dried eviscerated fish that have been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least 60 minutes, ~~(i.e. a heat treatment at 100°C for 30 minutes or any~~ a time/temperature equivalent that ~~has been demonstrated to~~ inactivate~~s~~ SAV);

3) fish *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least 60 minutes or a time/temperature equivalent that inactivates SAV;

~~d~~4) fish oil;

~~e) fish *meal*;~~

~~f)~~5) fish skin leather.

~~2) When authorising the importation or transit of *aquatic animal products* derived from a species referred to in Article 10.5.2., other than those referred to in point 1 of Article 10.5.3., *Competent Authorities* should require the conditions prescribed in Articles 10.5.7. to 10.5.13. relevant to the infection with SAV status of the *exporting country*, *zone* or *compartment*.~~

~~3) When considering the importation or transit of *aquatic animal products* derived from a species not referred to in Article 10.5.2. but which could reasonably be expected to pose a *risk* of transmission of SAV, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this analysis.~~

[…]

(CLEAN VERSION)

CHAPTER 10.5.

INFECTION WITH SALMONID ALPHAVIRUS

[…]

Article 10.5.3.

Measures for the importation or transit of aquatic animal products for any purpose regardless of the infection with SAV status of the exporting country, zone or compartment

The *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of these *aquatic animal products*, *Competent Authorities* should not require any sanitary measures related to SAV, regardless of the infection with SAV status of the *exporting country*, *zone* or *compartment*:

1) pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least 60 minutes, or a time/temperature equivalent that inactivates SAV;

2) mechanically dried eviscerated fish that have been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least 60 minutes, or a time/temperature equivalent that inactivates SAV;

3) fish *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least 60 minutes or a time/temperature equivalent that inactivates SAV;

4) fish oil;

5) fish skin leather.

[…]

(TRACK CHANGES VERSION)

CHAPTER 10.6.

INFECTION WITH INFECTIOUS HAEMATOPOIETIC NECROSIS VIRUS

[…]

Article 10.6.3.

Measures for the i~~I~~mportation or transit of aquatic animal products for any purpose regardless of the infection with IHNV status of the exporting country, zone or compartment

~~1)~~ The *aquatic animal product*s listed below have been assessed as meeting the criteria for safety of *aquatic animal product*s in accordance with Article 5.4.1. When authorising the importation or transit of these *aquatic animal products*, *Competent Authorities* should not require any sanitary measures~~conditions~~ related to IHNV, regardless of the infection with IHNV status of the *exporting country*, *zone* or *compartment*~~:, when authorising the importation or transit of the following *aquatic animal products* derived from a species referred to in Article 10.6.2. that are intended for any purpose and comply with Article 5.4.1.:~~

1) pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 90°C for at least 30 seconds, or a time/temperature equivalent that inactivates IHNV;

~~a) heat sterilised hermetically sealed fish products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent that has been demonstrated to inactivate IHNV);~~

~~b) pasteurised fish products that have been subjected to a heat treatment at 90°C for at least ten minutes (or any time/temperature equivalent that has been demonstrated to inactivate IHNV);~~

~~c)~~2) mechanically dried eviscerated fish that have been subjected to a heat treatment sufficient to attain a core temperature of at least 90°C for at least 30 seconds, ~~(i.e. a heat treatment at 100°C for at least 30 minutes~~ or ~~any~~ a time/temperature equivalent that ~~has been demonstrated to~~ inactivates IHNV~~)~~;

3) fish *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 90°C for at least 30 seconds, or a time/temperature equivalent that inactivates IHNV;

~~d~~4) fish oil;

~~e) fish *meal*;~~

~~f)~~5) fish skin leather.

~~2) When authorising the importation or transit of *aquatic animal products* derived from a species referred to in Article 10.6.2., other than those referred to in point 1 of Article 10.6.3., *Competent Authorities* should require the conditions prescribed in Articles 10.6.7. to 10.6.13. relevant to the infection with IHNV status of the *exporting country*, *zone* or *compartment*.~~

~~3) When considering the importation or transit of *aquatic animal products* derived from a species not referred to in Article 10.6.2. but which could reasonably be expected to pose a *risk* of transmission of IHNV, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this analysis.~~

[…]

(CLEAN VERSION)

CHAPTER 10.6.

INFECTION WITH INFECTIOUS HAEMATOPOIETIC NECROSIS VIRUS

[…]

Article 10.6.3.

Measures for the importation or transit of aquatic animal products for any purpose regardless of the infection with IHNV status of the exporting country, zone or compartment

The *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of these *aquatic animal products*, *Competent Authorities* should not require any sanitary measures related to IHNV, regardless of the infection with IHNV status of the *exporting country*, *zone* or *compartment*:

1) pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 90°C for at least 30 seconds, or a time/temperature equivalent that inactivates IHNV;

2) mechanically dried eviscerated fish that have been subjected to a heat treatment sufficient to attain a core temperature of at least 90°C for at least 30 seconds, or a time/temperature equivalent that inactivates IHNV;

3) fish *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 90°C for at least 30 seconds, or a time/temperature equivalent that inactivates IHNV;

4) fish oil;

5) fish skin leather.

[…]

(TRACK CHANGES VERSION)

CHAPTER 10.7.

INFECTION WITH KOI HERPES VIRUS

[…]

Article 10.7.3.

Measures for the i~~I~~mportation or transit of aquatic animal products for any purpose regardless of the infection with KHV status of the exporting country, zone or compartment

~~1)~~ The *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of these *aquatic animal products*, *Competent Authorities* should not require any sanitary measures ~~conditions~~ related to KHV, regardless of the infection with KHV status of the *exporting country*, *zone* or *compartment*:~~, when authorising the importation or transit of the following *aquatic animal products* derived from a species referred to in Article 10.7.2. that are intended for any purpose and comply with Article 5.4.1.~~:

1) pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 50°C for at least three minutes, or a time/temperature equivalent that inactivates KHV;

~~a) heat sterilised hermetically sealed fish products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent that has been demonstrated to inactivate KHV);~~

~~c) pasteurised fish products that have been subjected to heat treatment at 90°C for at least ten minutes (or any time/temperature equivalent that has been demonstrated to inactivate KHV);~~

~~d)~~2) mechanically dried eviscerated fish that have been subjected to a heat treatment sufficient to attain a core temperature of at least 50°C for at least three minutes, ~~(~~i.e. a heat treatment at 100°C for at least ~~30 minutes~~ or ~~any~~ a time/temperature equivalent that ~~has been demonstrated to~~ inactivates KHV~~)~~;

3) fish *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 50°C for at least three minutes, or a time/temperature equivalent that inactivates KHV;

~~e~~4) fish oil~~;~~.

~~f) fish *meal*.~~

~~2) When authorising the importation or transit of *aquatic animal products* derived from a species referred to in Article 10.7.2., other than those referred to in point 1 of Article 10.7.3., *Competent Authorities* should require the conditions prescribed in Articles 10.7.7. to 10.7.12. relevant to the infection with KHV status of the *exporting country*, *zone* or *compartment*.~~

~~3) When considering the importation or transit of *aquatic animal products* derived from a species not referred to in Article 10.7.2. but which could reasonably be expected to pose a *risk* of transmission of KHV, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this analysis.~~

[…]

(CLEAN VERSION)

CHAPTER 10.7.

INFECTION WITH KOI HERPES VIRUS

[…]

Article 10.7.3.

Measures for the importation or transit of aquatic animal products for any purpose regardless of the infection with KHV status of the exporting country, zone or compartment

The *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of these *aquatic animal products*, *Competent Authorities* should not require any sanitary measures related to KHV, regardless of the infection with KHV status of the *exporting country*, *zone* or *compartment*:

1) pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 50°C for at least three minutes, or a time/temperature equivalent that inactivates KHV;

2) mechanically dried eviscerated fish that have been subjected to a heat treatment sufficient to attain a core temperature of at least 50°C for at least three minutes, or a time/temperature equivalent that inactivates KHV;

3) fish *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 50°C for at least three minutes, or a time/temperature equivalent that inactivates KHV;

4) fish oil.

[…]

(TRACK CHANGES VERSION)

CHAPTER 10.8.

INFECTION WITH RED SEA BREAM IRIDOVIRUS

[…]

Article 10.8.3.

Measures for the i~~I~~mportation or transit of aquatic animal products for any purpose regardless of the infection with RSIV status of the exporting country, zone or compartment

~~1)~~ The *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of these *aquatic animal products*, *Competent Authorities* should not require any sanitary measures ~~conditions~~ related to RSIV, regardless of the infection with RSIV status of the *exporting country*, *zone* or *compartment:*~~, when authorising the importation or transit of the following *aquatic animal products* derived from a species referred to in Article 10.8.2. that are intended for any purpose and comply with Article 5.4.1.:~~

1) pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 56°C for at least 30 minutes, or a time/temperature equivalent that inactivates RSIV;

~~a) heat sterilised hermetically sealed fish products (i.e. a heat treatment at 121ºC for at least 3.6 minutes or any time/temperature equivalent that has been demonstrated to inactivate RSIV);~~

~~b) pasteurised fish products that have been subjected to heat treatment at 90ºC for at least ten minutes (or any time/temperature equivalent that has been demonstrated to inactivate RSIV);~~

~~c)~~2) mechanically dried eviscerated fish that have been subjected to a heat treatment sufficient to attain a core temperature of at least 56°C for at least 30 minutes, ~~(i.e. a heat treatment at 100ºC for at least 30 minutes~~ or ~~any~~a time/temperature equivalent that ~~has been demonstrated to~~ inactivates RSIV~~)~~;

3) fish *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 56°C for at least 30 minutes, or a time/temperature equivalent that inactivates RSIV;

~~d~~4) fish oil;

~~e) fish *meal*;~~

~~f)~~5) fish skin leather.

~~2) When authorising the importation or transit of *aquatic animal products* derived from a species referred to in Article 10.8.2., other than those referred to in point 1 of Article 10.8.3., *Competent Authorities* should require the conditions prescribed in Articles 10.8.7. to 10.8.12. relevant to the infection with RSIV status of the *exporting country*, *zone* or *compartment*.~~

~~3) When considering the importation or transit of *aquatic animal products* derived from a species not referred to in Article 10.8.2. but which could reasonably be expected to pose a *risk* of transmission of RSIV, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this analysis.~~

[…]

(CLEAN VERSION)

CHAPTER 10.8.

INFECTION WITH RED SEA BREAM IRIDOVIRUS

[…]

Article 10.8.3.

Measures for the importation or transit of aquatic animal products for any purpose regardless of the infection with RSIV status of the exporting country, zone or compartment

The *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of these *aquatic animal products*, *Competent Authorities* should not require any sanitary measures related to RSIV, regardless of the infection with RSIV status of the *exporting country*, *zone* or *compartment*:

1) pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 56°C for at least 30 minutes, or a time/temperature equivalent that inactivates RSIV;

2) mechanically dried eviscerated fish that have been subjected to a heat treatment sufficient to attain a core temperature of at least 56°C for at least 30 minutes, or a time/temperature equivalent that inactivates RSIV;

3) fish *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 56°C for at least 30 minutes, or a time/temperature equivalent that inactivates RSIV;

4) fish oil;

5) fish skin leather.

[…]

(TRACK CHANGES VERSION)

CHAPTER 10.9.

INFECTION WITH SPRING VIRAEMIA OF CARP VIRUS

[…]

Article 10.9.3.

Measures for the i~~I~~mportation or transit of aquatic animal products for any purpose regardless of the infection with SVCV status of the exporting country, zone or compartment

~~1)~~ The *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of these *aquatic animal products*, *Competent Authorities* should not require any sanitary measures ~~conditions~~ related to SVCV, regardless of the infection with SVCV status of the *exporting country*, *zone* or *compartment:*~~, when authorising the importation or transit of the following *aquatic animal products* derived from a species referred to in Article 10.9.2. that are intended for any purpose and comply with Article 5.4.1.:~~

1) pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 90°C for at least 60 seconds, or a time/temperature equivalent that inactivates SVCV;

~~a) heat sterilised hermetically sealed fish products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent that has been demonstrated to inactivate SVCV);~~

~~b) pasteurised fish products that have been subjected to heat treatment at 90°C for at least ten minutes (or any time/temperature equivalent that has been demonstrated to inactivate SVCV);~~

~~c)~~2) mechanically dried eviscerated fish that have been subjected to a heat treatment sufficient to attain a core temperature of at least 90°C for at least 60 seconds, (or ~~any~~ a time/temperature equivalent that ~~has been demonstrated to~~ inactivate~~s~~ SVCV);

3) fish *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 90°C for at least 60 seconds, or a time/temperature equivalent that inactivates SVCV;

~~d~~4) fish oil~~;~~.

~~e) fish *meal*.~~

~~2) When authorising the importation or transit of *aquatic animal products* derived from a species referred to in Article 10.9.2., other than those referred to in point 1 of Article 10.9.3., *Competent Authorities* should require the conditions prescribed in Articles 10.9.7. to 10.9.12. relevant to the infection with SVCV status of the *exporting country*, *zone* or *compartment*.~~

~~3) When considering the importation or transit of *aquatic animal products* derived from a species not referred to in Article 10.9.2. but which could reasonably be expected to pose a *risk* of transmission of SVCV, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this analysis.~~

[…]

(CLEAN VERSION)

CHAPTER 10.9.

INFECTION WITH SPRING VIRAEMIA OF CARP VIRUS

[…]

Article 10.9.3.

Measures for the importation or transit of aquatic animal products for any purpose regardless of the infection with SVCV status of the exporting country, zone or compartment

The *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of these *aquatic animal products*, *Competent Authorities* should not require any sanitary measures related to SVCV, regardless of the infection with SVCV status of the *exporting country*, *zone* or *compartment*:

1) pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 90°C for at least 60 seconds, or a time/temperature equivalent inactivates SVCV;

2) mechanically dried eviscerated fish that have been subjected to a heat treatment sufficient to attain a core temperature of at least 90°C for at least 60 seconds, or a time/temperature equivalent that inactivates SVCV;

3) fish *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 90°C for at least 60 seconds, or a time/temperature equivalent that inactivates SVCV;

4) fish oil.

[…]

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(TRACK CHANGES VERSION)

CHAPTER 10.10.

INFECTION WITH VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUS

[…]

Article 10.10.3.

Measures for the i~~I~~mportation or transit of aquatic animal products for any purpose regardless of the infection with VHSV status of the exporting country, zone or compartment

~~1)~~ The *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of these *aquatic animal products*, *Competent Authorities* should not require any sanitary measures~~conditions~~ related to VHSV, regardless of the infection with VHSV status of the *exporting country*, *zone* or *compartment*~~:, when authorising the importation or transit of the following *aquatic animal products* derived from a species referred to in Article 10.10.2. that are intended for any purpose and comply with Article 5.4.1.:~~

1) pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 90°C for at least 60 seconds, or a time/temperature equivalent that inactivates VHSV;

~~a) heat sterilised hermetically sealed fish products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent that has been demonstrated to inactivate VHSV);~~

~~b) pasteurised fish products that have been subjected to a heat treatment at 90°C for at least ten minutes (or any time/temperature equivalent that has been demonstrated to inactivate VHSV);~~

~~c)~~2) mechanically dried eviscerated fish that have been subjected to a heat treatment sufficient to attain a core temperature of at least 90°C for at least 60 seconds, ~~(i.e. a heat treatment at 100°C for at least 30 minutes or any~~ a time/temperature equivalent that ~~has been demonstrated to~~ inactivates VHSV~~)~~;

3) fish *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 90°C for at least 60 seconds, or a time/temperature equivalent that inactivates VHSV;

~~d~~4) naturally dried, eviscerated fish (i.e. sun-dried or wind-dried);

~~e~~5) fish oil;

~~f) fish *meal*;~~

~~g)~~6) fish skin leather.

~~2) When authorising the importation or transit of *aquatic animal products* derived from a species referred to in Article 10.10.2., other than those referred to in point 1 of Article 10.10.3., *Competent Authorities* should require the conditions prescribed in Articles 10.10.7. to 10.10.13. relevant to the infection with VHSV status of the *exporting country*, *zone* or *compartment*.~~

~~3) When considering the importation or transit of *aquatic animal products* derived from a species not referred to in Article 10.10.2. but which could reasonably be expected to pose a *risk* of transmission of VHSV, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this analysis.~~

[…]

(CLEAN VERSION)

CHAPTER 10.10.

INFECTION WITH VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUS

[…]

Article 10.10.3.

Measures for the importation or transit of aquatic animal products for any purpose regardless of the infection with VHSV status of the exporting country, zone or compartment

The *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of these *aquatic animal products*, *Competent Authorities* should not require any sanitary measures related to VHSV, regardless of the infection with VHSV status of the *exporting country*, *zone* or *compartment*:

1) pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 90°C for at least 60 seconds, or a time/temperature equivalent that inactivates VHSV;

2) mechanically dried eviscerated fish that have been subjected to a heat treatment sufficient to attain a core temperature of at least 90°C for at least 60 seconds, or any a time/temperature equivalent that inactivates VHSV;

3) fish *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 90°C for at least 60 seconds, or a time/temperature equivalent that inactivates VHSV;

4) naturally dried, eviscerated fish (i.e. sun-dried or wind-dried);

5) fish oil;

6) fish skin leather.

[…]

Return to Agenda

CHAPTER 9.X.

INFECTION WITH DECAPOD IRIDESCENT VIRUS 1

Article 9.X.1.

For the purposes of the *Aquatic Code*, infection with decapod iridescent virus 1 means *infection* with the *pathogenic agent Decapod iridescent virus 1* (DIV1), of the Genus *Decapodiridovirus* and the Family *Iridoviridae*. Information on methods for *diagnosis* is provided in the *Aquatic Manual*.

Article 9.X.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. [white-leg shrimp (*Penaeus vannamei)*, giant tiger prawn (*Penaeus monodon)*, red claw crayfish (*Cherax quadricarinatus*), giant freshwater prawn (*Macrobrachium rosenbergii*), red swamp crayfish (*Procambarus clarkii*), oriental river prawn (*Macrobrachium nipponense*) and ridgetail white prawn (*Exopalaemon carinicauda*)] (under study).

Article 9.X.3.

Measures for the importation or transit of aquatic animal products for any purpose regardless of the infection with DIV1 status of the exporting country, zone or compartment

The *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of these *aquatic animal products*, C*ompetent Authorities* should not require any conditions related to DIV1, regardless of the infection with DIV1 status of the *exporting country*, *zone* or *compartment*:

1) [cooked, pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 56°C for at least 30 minutes, or a time/temperature equivalent that inactivates DIV1;
2) crustacean *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 56°C for at least 30 minutes, or a time/temperature equivalent that inactivates DIV1;
3) crustacean oil;
4) chemically extracted chitin.] (under study)

Article 9.X.4.

Requirements for self-declaration of freedom from infection with DIV1

A Member Country may make a self-declaration of freedom from infection with DIV1 for the entire country, a *zone* or a *compartment* in accordance with the provisions of Articles 9.X.5. to 9.X.8., as relevant. The self-declaration of freedom must be made in accordance with other relevant requirements of the *Aquatic Code,* including that the Member Country meet the following conditions:

1) complies with the provisions of Chapter 3.1.; and
2) uses appropriate methods of *diagnosis*, as recommended in the *Aquatic Manual*; and
3) meets all requirements of Chapter 1.4. that are relevant to the self-declaration of freedom.

Article 9.X.5.

Country free from infection with DIV1

If a country shares water bodies with other countries, it can only make a self-declaration of freedom from infection with DIV1 if all shared water bodies are within countries or *zones* declared free from infection with DIV1 (see Article 9.X.6.).

As described in Article 1.4.X., a Member Country may make a self-declaration of freedom from infection with DIV1 for its entire *territory* if:

1) none of the *susceptible species* referred to in Article 9.X.2. are present and *basic biosecurity conditions* have been continuously met for at least the last [six] months;

OR

2) there has been no occurrence of infection with DIV1 for at least the last [ten] years, and:

   a) the Member Country can demonstrate that conditions are conducive to the clinical expression of infection with DIV1, as described in the corresponding chapter of the *Aquatic Manual*; and

   b) *basic biosecurity conditions* as described in Chapter 1.4. have been continuously met for at least the last [ten] years;

OR

3) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last [two] years without detection of DIV1, and *basic biosecurity conditions* have been continuously met for at least [one] year prior to commencement of *targeted surveillance*;

OR

4) it previously made a self-declaration of freedom from infection with DIV1 and subsequently lost its free status due to the detection of DIV1 but the following conditions have been met:

   a) on detection of DIV1, the affected area was declared an *infected zone* and a *protection zone* was established; and

   b) infected populations within the *infected zone* have been killed and disposed of by means that minimise the likelihood of further transmission of DIV1, and the appropriate *disinfection* procedures (as described in Chapter 4.4.) have been completed followed by fallowing as described in Chapter 4.7.; and

   c) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place since eradication of infection with DIV1; and

   d) *targeted surveillance*, as described in Chapter 1.4., has been in place for i) at least the last [two] years in wild or farmed *susceptible species* without detection of DIV1 or ii) at least the last [one] year without detection of DIV1 if affected *aquaculture establishments* were not epidemiologically connected to wild populations of *susceptible species*.

   In the meantime, part or all of the country, apart from the *infected* and *protection zones,* may be declared a free *zone* provided that such a part meets the conditions in point 2 of Article 9.X.6.

Article 9.X.6.

Zone free from infection with DIV1

If a *zone* extends over the *territory* of more than one country, it can only be declared a *zone* free from infection with DIV1 if all of the relevant *Competent Authorities* confirm that all relevant conditions have been met.

As described in Article 1.4.X., a Member Country may make a self-declaration of freedom from infection with DIV1 for a *zone* within its *territory* if:

1) none of the *susceptible species* referred to in Article 9.X.2. are present and *basic biosecurity conditions* have been continuously met for at least the last [six] months;

OR

2) there has been no occurrence of infection with DIV1 for at least the last [ten] years, and;

   a) the Member Country can demonstrate that conditions are conducive to the clinical expression of

infection with DIV1, as described in Article 1.4.8. of Chapter 1.4.; and

b) *basic biosecurity conditions* as described in Chapter 1.4. have been continuously met for the *zone* for at least the last [ten] years;

OR

3) *targeted surveillance*, as described in Chapter 1.4., has been in place in the *zone* for at least the last [two] years without detection of DIV1, and *basic biosecurity conditions* have been continuously met for at least [one] year prior to commencement of *targeted surveillance*;

OR

4) it previously made a self-declaration of freedom for a *zone* from infection with DIV1 and subsequently lost its free status due to the detection of DIV1 in the *zone* but the following conditions have been met:

   a) on detection of DIV1, the affected area was declared an *infected zone* and a *protection zone* was established; and

   b) infected populations within the *infected zone* have been killed and disposed of by means that minimise the likelihood of further transmission of DIV1, and the appropriate *disinfection* procedures (as described in Chapter 4.4.) have been completed followed by fallowing as described in Chapter 4.7.; and

   c) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place since eradication of infection with DIV1; and

   d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last [two] years without detection of DIV1.

Article 9.X.7.

Compartment free from infection with DIV1

As described in Article 1.4.X., a Member Country may make a self-declaration of freedom from infection with DIV1 for a *compartment* within its *territory* if:

1) *targeted surveillance*, as described in Chapter 1.4., has been in place in the *compartment* for at least the last [two] years without detection of DIV1, and:

   a) *basic biosecurity conditions* have been continuously met for at least [one] year prior to commencement of *targeted surveillance*;

OR

2) it previously made a self-declaration of freedom for a *compartment* from infection with DIV1 and subsequently lost its free status due to the detection of DIV1 in the *compartment* but the following conditions have been met:

   a) all *aquatic animals* within the *compartment* have been killed and disposed of by means that minimise the likelihood of further transmission of DIV1, the appropriate *disinfection* procedures (as described in Chapter 4.4.) have been completed, and the *compartment* has been fallowed as described in Chapter 4.7.; and

   b) previously existing *basic biosecurity conditions*, including the *compartment biosecurity plan*, have been reviewed and modified as necessary and have continuously been in place from the time of restocking with animals from an approved pathogen free source in accordance with the requirements of Articles 9.X.9. and 9.X.10. as appropriate; and

   c) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last [one] year without detection of DIV1.

Article 9.X.8.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from infection with DIV1 following the provisions of Articles

9.X.4. to 9.X.7. (as relevant) may maintain its status as free from infection with DIV1 provided that the requirements described in Article 1.4.15. are continuously maintained.

Article 9.X.9.

Importation of aquatic animals or aquatic animal products from a country, zone or compartment declared free from infection with DIV1

When importing *aquatic animals* of a species referred to in Article 9.X.2., or *aquatic animal products* derived thereof, from a country, *zone* or *compartment* declared free from infection with DIV1, 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*. The *international aquatic animal health certificate* should state that, on the basis of the procedures described in Articles 9.X.5. or 9.X.6. (as applicable) and 9.X.7., the place of production of the *aquatic animals* or *aquatic animal products* is a country, *zone* or *compartment* declared free from infection with DIV1.

The *international aquatic animal health certificate* should be in accordance with the Model Certificate in Chapter 5.11. This article does not apply to *aquatic animal products* listed in Article 9.X.3.

Article 9.X.10.

Importation of aquatic animals for aquaculture from a country, zone or compartment not declared free from infection with DIV1

When importing, for *aquaculture*, *aquatic animals* of a species referred to in Article 9.X.2. from a country, *zone* or *compartment* not declared free from infection with DIV1, the *Competent Authority* of the *importing country* should assess the *risk* in accordance with Chapter 2.1. and consider the *risk* mitigation measures in points 1 and 2 below.

1) If the intention is to grow out and harvest the imported *aquatic animals*, consider applying the following:

   a) the direct delivery to and lifelong holding of the imported *aquatic animals* in a *quarantine* facility; and

   b) before leaving *quarantine* (either in the original facility or following biosecure transport to another *quarantine* facility) the *aquatic animals* are killed and processed into one or more of the *aquatic animal products* referred to in Article 9.X.3. or other products authorised by the *Competent Authority*; and

   c) the treatment of all transport water, equipment, effluent and waste materials to inactive DIV1 in accordance with Chapters 4.4., 4.8. and 5.5.

OR

2) If the intention is to establish a new stock for *aquaculture*, consider applying the following:

   a) In the *exporting country*:

      i) identify potential source populations and evaluate their *aquatic animal* health records;

      ii) test source populations in accordance with Chapter 1.4. and select a founder population (F-0) of *aquatic animals* with a high health status for infection with DIV1.

   b) In the *importing country*:

      i) import the F-0 population into a *quarantine* facility;

      ii) test the F-0 population for DIV1 in accordance with Chapter 1.4. to determine their suitability as broodstock;

      iii) produce a first generation (F-1) population in *quarantine*;

      iv) culture the F-1 population in *quarantine* for a duration sufficient for, and under conditions that are conducive to, the clinical expression of infection with DIV1, and sample and test for DIV1 in accordance with Chapter 1.4. of the *Aquatic Code* and Chapter X.X.6. of the *Aquatic Manual*;

      v) if DIV1 is not detected in the F-1 population, it may be defined as free from infection with DIV1 and may be released from *quarantine*;

vi) if DIV1 is detected in the F-1 population, those animals should not be released from *quarantine* and should be killed and disposed of in a biosecure manner in accordance with Chapter 4.7.

Article 9.X.11.

Importation of aquatic animals or aquatic animal products for processing for human consumption from a country, zone or compartment not declared free from infection with DIV1

When importing, for processing for human consumption, *aquatic animals* of a species referred to in Article 9.X.2., or *aquatic animal products* derived thereof, from a country, *zone* or *compartment* not declared free from infection with DIV1, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, require that:

1) the consignment is delivered directly to, and held in, *quarantine* or containment facilities until processing into one of the products referred to in Article 9.X.3. or in point 1 of Article 9.X.12., or other products authorised by the *Competent Authority*; and

2) all water (including ice), equipment, *containers* and packaging material used in transport are treated to ensure inactivation of DIV1 or disposed of in a biosecure manner in accordance with Chapters 4.4., 4.8. and 5.5.; and

3) all effluent and waste materials are treated to ensure inactivation of DIV1 or disposed of in a biosecure manner in accordance with Chapters 4.4. and 4.8.

For these *aquatic animals* or *aquatic animal products* Member Countries may wish to consider introducing internal measures to address the *risks* associated with the *aquatic animal* or *aquatic animal product* being used for any purpose other than for human consumption.

Article 9.X.12.

Importation of aquatic animals or aquatic animal products intended for uses other than human consumption, including animal feed and agricultural, industrial, research or pharmaceutical use, from a country, zone or compartment not declared free from infection with DIV1

When importing *aquatic animals* of a species referred to in Article 9.X.2., or *aquatic animal products* derived thereof, intended for uses other than human consumption, including animal *feed* and agricultural, industrial, research or pharmaceutical use, from a country, *zone* or *compartment* not declared free from infection with DIV1, the *Competent Authority* of the *importing country* should require that:

1) the consignment is delivered directly to, and held in, *quarantine* or containment facilities until processed into one of the products referred to in Article 9.X.3. or other products authorised by the *Competent Authority*; and

2) all water (including ice), equipment, *containers* and packaging material used in transport are treated to ensure inactivation of DIV1 or disposed of in a biosecure manner in accordance with Chapters 4.4., 4.8. and 5.5.; and

3) all effluent and waste materials are treated to ensure inactivation of DIV1 or disposed of in a biosecure manner in accordance with Chapters 4.4. and 4.8.

Article 9.X.13.

Importation of aquatic animals intended for use in laboratories or zoos from a country, zone or compartment not declared free from infection with DIV1

When importing, for use in laboratories or zoos, *aquatic animals* of a species referred to in Article 9.X.2. from a country, *zone* or *compartment* not declared free from infection with DIV1, the *Competent Authority* of the *importing country* should ensure:

1) the consignment is delivered directly to, and held in, *quarantine* facilities authorised by the *Competent Authority*; and

2) all water (including ice), equipment, *containers* and packaging material used in transport are treated to

ensure inactivation of DIV1 or disposed of in a biosecure manner in accordance with Chapters 4.4., 4.8. and 5.5.; and

3) all effluent and waste materials from the *quarantine* facilities in the laboratories or zoos are treated to ensure inactivation of DIV1 or disposed of in a biosecure manner in accordance with Chapters 4.4. and 4.8.; and

4) the carcasses are disposed of in accordance with Chapter 4.8.

Article 9.X.14.

Importation (or transit) of aquatic animal products for retail trade for human consumption regardless of the infection with DIV1 status of the exporting country, zone or compartment

1) [*Competent Authorities* should not require any conditions related to DIV1, regardless of the infection with DIV1 status of the *exporting country*, *zone* or *compartment*, when authorising the importation (or transit) of frozen crustaceans of the *susceptible species* in Article 9.X.2. (shell off, head off) that have been prepared and packaged for retail trade and comply with Article 5.4.2.] (under study)

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

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

2) When importing *aquatic animal products*, other than those referred to in point 1 above, derived from a species referred to in Article 9.X.2. from a country, *zone* or *compartment* not declared free from infection with DIV1, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

Return to Agenda

CHAPTER 10.1.

INFECTION WITH EPIZOOTIC HAEMATOPOIETIC NECROSIS VIRUS

Article 10.1.2.

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.: ~~black bullhead *(Ameiurus melas)*, crimson spotted rainbow fish *(Melanotaenia fluviatilis)*, eastern mosquito fish *(Gambusia holbrooki)*, European perch *(Perca fluviatilis)*, macquarie perch *(Macquaria australasica)*, mosquito fish *(Gambusia affinis)*, mountain galaxias *(Galaxias olidus)*, northern pike *(Esox lucius)*, pike-perch *(Sander lucioperca)*, rainbow trout *(Oncorhynchus mykiss)* and silver perch *(Bidyanus bidyanus)*.~~

Family	**Scientific name**	**Common name**
Esocidae	*Esox lucius*	Northern pike
Galaxiidae	*Galaxias olidus*	Mountain galaxias
Ictaluridae	*Ameiurus melas*	Black bullhead
Melanotaeniidae	*Melanotaenia fluviatilis*	Crimson spotted rainbow fish
Percidae	*Perca fluviatilis*	European perch
	Sander lucioperca	Pike-perch
Percichthyidae	*Macquaria australasica*	Macquarie perch
Poeciliidae	*Gambusia holbrooki*	Eastern mosquito fish
	Gambusia affinis	Mosquito fish
Salmonidae	*Oncorhynchus mykiss*	Rainbow trout
Terapontidae	*Bidyanus bidyanus*	Silver perch

[…]

Return to Agenda

CHAPTER 10.7.

INFECTION WITH KOI HERPESVIRUS

[…]

Article 10.7.2.

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)* and common carp hybrids (e.g. *Cyprinus carpio* x *Carassius auratus* and *Cyprinus carpio* x *Carassius carassius*).

[…]

Return to Agenda

CHAPTER 11.1.

INFECTION WITH ABALONE HERPESVIRUS

[…]

Article 11.1.1.

For the purposes of the *Aquatic Code*, infection with abalone herpesvirus (AbHV) means *infection* with the *pathogenic agent* Haliotid herpesvirus 1 (HaHV-1), of the Genus *Aurivirus* and Family *Malacoherpesviridae*. ~~herpesvirus known to cause *disease* in abalone.~~

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

Article 11.1.2.

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.: blacklip abalone (*Haliotis rubra),* greenlip abalone (*Haliotis laevigata),* hybrids of greenlip x blacklip abalone (*Haliotis laevigata* x *Haliotis rubra) and* small abalone (*Haliotis diversicolor).* ~~*Haliotis diversicolor* (subspecies *aquatilis* and *supertexta*), *Haliotis laevegata*, *Haliotis rubra* and hybrids of *Haliotis laevegata* x *Haliotis rubra*. These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.~~

[…]

Return to Agenda

CHAPTER 11.2.

INFECTION WITH *BONAMIA EXITIOSA*

[…]

Article 11.2.1.

For the purposes of the *Aquatic Code*, infection with *Bonamia exitiosa* means *infection* with the *pathogenic agent* ~~B.~~ *Bonamia exitiosa* of the Family Haplosporidiidae.

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

Article 11.2.2.

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.: Argentinean flat oyster (*Ostrea puelchana*), Australian mud oyster *(Ostrea angasi)*, ~~and~~ Chilean flat oyster *(Ostrea chilensis)*, dwarf oyster *(Ostrea stentina)*, eastern oyster *(Crassostrea virginica)*, European flat oyster *(Ostrea edulis)*, Olympia oyster *(Ostrea lurida)* and Suminoe oyster *(Crassostrea ariakensis)*. ~~These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.~~

[…]

Return to Agenda

THE USE OF ENVIRONMENTAL DNA METHODS FOR DETECTION OF OIE LISTED AQUATIC ANIMAL DISEASES

A discussion paper developed by the OIE Aquatic Animal Health Standards Commission (Aquatic Animals Commission) for Member comments.

Version: 28 September 2021

1. Summary

The monitoring of aquatic systems using environmental DNA (eDNA) is a rapidly advancing research field that will provide opportunities for cost-effective, non-destructive methods to screen for pathogenic agents, including those of wild aquatic populations where samples may be difficult or undesirable to obtain.

The Aquatic Animals Commission is aware that eDNA methods are being applied for detecting the causative agents of several OIE listed diseases. As these methods are available and currently in use, the Commission has agreed that it would be advisable for guidance to be provided on appropriate application of eDNA methods and potential limitations.

The Commission notes that, as accurate estimates of diagnostic performance are not available for designing surveillance programmes using eDNA assays, data obtained from eDNA methods are unlikely to be suitable to support declarations of freedom from listed diseases. Confirmation of infection with listed diseases could also not be made using eDNA methods because a positive result does not demonstrate that a susceptible host animal(s) is infected.

Positive eDNA results could, however, provide evidence amounting to suspicion of infection such as presence of the pathogen in the sample, perhaps in a different lifecycle stage, or different host. This application of eDNA methods may be particularly useful for the monitoring of high-value or rare animals as an alternative to collection of tissue samples. It has a potential role in early detection of disease incursion in wild populations or under circumstances when infection is not likely to result in observable clinical signs. However, following suspicion, based on positive eDNA, samples obtained directly from aquatic animals need to be tested – described in the relevant disease-specific chapters of the *Manual of Diagnostic Tests for Aquatic Animals* (*Aquatic Manual*) to confirm or exclude the case.

The application of eDNA methods for a given purpose should be considered carefully. Methods should be chosen with consideration given to all relevant factors including the surveillance objective, the target pathogen, the reliability of the method, and the environment to be sampled. It is important that the implications of positive results be considered in advance of applying an eDNA method as any positive results may require that surveys involving direct sampling and testing of susceptible animal be conducted to confirm or exclude a suspect case. eDNA methods will not be an appropriate choice for many aquatic animal disease surveillance purposes.

This document is intended to explore the potential use of eDNA methods with respect to the standards of the OIE *Aquatic Animal Health Code* (*Aquatic Code*) and *Aquatic Manual* and to outline benefits and limitations.

The use of an eDNA method for the detection of *Gyrodactylus salaris* has been included in *Aquatic Manual* Chapter 2.3.3 Infection with *Gyrodactylus salaris*[1]. The inclusion of this method conforms with the conclusions of this discussion paper.

2. Definitions for eDNA

Numerous definitions for eDNA exist (e.g. Bass *et al*., 2015; Diaz-Ferguson & Moyer, 2014; Thomsen & Willerslev, 2015). Most definitions regard eDNA as detectable short DNA fragments from a living

[1] https://www.oie.int/fileadmin/Home/eng/Health_standards/aahm/current/2.3.03_G_salaris.pdf

organism derived from cellular components or fluids secreted into the abiotic components of surrounding environment (i.e. water, air, sediments).

For the purposes of this document we define eDNA as: "nucleic acids of pathogenic agents extracted from 'true' environmental samples (such as water, soil, sediment, biofilm)". Directly host-derived material such as faeces, sloughed cells, and mucous, are excluded from this definition. Once extracted from the environmental sample, target eDNA fragments can be detected using a variety of molecular methods (Diaz-Ferguson & Moyer, 2014). Furthermore, eDNA can be sequenced directly as metagenetic libraries or after PCR amplification of specific target gene regions (Bass *et al*., 2015).

The actual performance of eDNA based detection depends on the sample collection and processing methodology (e.g. volume filtered, presence and removal of PCR inhibitors), biological processes (e.g. rates of shedding, temporal variation) and abiotic factors (analyte degradation, hydrodynamic factors). It is important to evaluate these factors empirically so that the results can be properly interpreted. It is only with a clear understanding of how these factors influence the probability of pathogenic agent detection that eDNA-based detection can be used reliably in a variety of settings (Brunner, 2020).

3. Objectives

This paper considers i) the benefits and ii) limitations of eDNA pathogenic agent detection methods, iii) validation of eDNA methods, iv) the conditions for inclusion of an eDNA method in a disease-specific chapter of the *Aquatic Manual* and v) use of eDNA evidence as diagnostic criteria.

4. Review of published eDNA methods for the detection of aquatic animal pathogenic agents

A literature review was undertaken to assess the application of eDNA methods for the detection and study of pathogens and parasites of aquatic animals. Thirty-three publications reporting the use of eDNA to detect thirteen OIE listed pathogenic agents were identified (see Appendix 1, Table 1 for details). Methods have been developed for the detection of the causative agents of OIE listed pathogenic agents of amphibians, crustaceans, fish and molluscs. The majority of publications concern the detection of the listed pathogenic agents in wild aquatic animal populations, notably infection with *Aphanomyces astaci*, infection with *Batrachochytrium dendrobatidis*, infection with *B. salamandrivorans*, infection with *Ranavirus* species, infection with *G. salaris.*

A further thirteen publications were found that targeted other specific pathogenic agents (e.g. *Microcytos mackini*), groups of pathogenic agents (e.g. of ornamental fish) or applied eDNA methods to broader areas of study (e.g. water-borne transmission of viruses) (see Appendix 1, Table 2 for details).

5. Benefits eDNA methods for the detection of aquatic animal pathogenic agents

eDNA detection is a promising tool that can be used to complement direct sampling of aquatic animals for surveillance. eDNA methods offer some benefits compared to direct sampling and testing of aquatic animals, including, but not limited, to the following:

1. eDNA methods do not require destructive sampling of aquatic animal hosts. They may be particularly useful for rare or valuable aquatic animals, or difficult to collect wild animals (e.g. Rusch *et al*., 2018).

2. eDNA methods do not require handling of animals, avoiding the stress associated with obtaining non-destructive tissue samples (Brunner, 2020).

3. Sample collection and sample processing time and associated costs may be reduced substantially compared to collection and processing of individual animal samples (Rusch *et al*., 2018).

4. As environmental samples may contain analyte from the entire, or a large percentage of a target captive population, many fewer samples may be required to detect a pathogenic agent (compared to individual animal samples), even when diagnostic sensitivity of the eDNA method is low (Brunner, 2020).

5. The same environmental sample can be analysed for the presence of hosts (e.g. see Rusch *et al*., 2018) and multiple pathogens.

6. eDNA methods could be used for assessment of potential introduction pathways where sampling of hosts is not possible (e.g. ballast water).

6. Limitations of eDNA methods

Limitations to the application of eDNA based pathogenic agent detection include, but are not limited to, the following:

1. Very little target pathogen DNA may be available in the environmental sample due to dilution in the environment and degradation of nucleic acids. This may negatively impact the sensitivity of the method (Brunner, 2020).

2. The concentration of target DNA in an environmental sample will vary due a range of factors such as host density, prevalence and intensity of infection, sampling method (e.g. for water volume sampled, filter pore size, storage conditions) and environmental conditions (e.g. amount of organic matter). Sensitivity of eDNA methods may, therefore, vary more between localities, surveys undertaken at different time points and target taxa than direct sampling and testing of animal tissues (Brunner, 2020).

3. There are formal frameworks to assess diagnostic performance of tests using animal-derived samples, but these have not been developed for eDNA methods. This means that the design of surveys to demonstrate freedom from infection using eDNA methods is problematic.

4. A positive detection of target pathogen DNA in an environmental sample may be more likely to result from a source of contamination not representative of viable pathogen (e.g. inactivated pathogen from heat treated products) compared with animal-derived samples. Similarly, it may not indicate infection of a host animal with the target pathogenic agent.

7. Validation of eDNA methods

There is an increasing likelihood that disease management decisions will be made based on results from eDNA studies. It is thus imperative that data generated by eDNA studies is reliable, defendable and executed with high quality assurance standards (Klymus *et al*., 2019). Empirical validation of eDNA-based pathogen detection should focus on understanding the causes and consequences of variation in test characteristics across sampling conditions and needs to take into consideration a clear understanding of what is being sampled/assayed for in the case of each pathogen of interest.

Chapter 1.1.2. of the *Aquatic Manual* describes the principles and methods of validation of diagnostic assays for infectious diseases. The recommendations of this chapter are intended for diagnostic testing of animal-derived samples; however, the principles and many of the methods are applicable to eDNA methods. It is recommended that the general principles and methods of Chapter 1.1.2. be applied to the validation of eDNA detection methods for OIE listed diseases. It should be noted that the process of sample collection, the concentration of target DNA, the DNA extraction, the sensitivity and other performance (indicators) should be emphasised and validated.

Design and reporting standards are available for diagnostic accuracy studies for methods utilising aquatic animal-derived samples (e.g. Laurin *et al*., 2018). Many of the design and reporting considerations are also applicable to eDNA methods and it is recommended that these standards be applied for eDNA diagnostic accuracy studies.

Additional to the guidance described above, design and reporting considerations have been published specifically for eDNA methods (e.g. Doyle & Uthicke, 2020; Goldberg *et al*., 2016; Klymus *et al*., 2019). Many of these studies report on considerations for detection of macro-organisms rather than pathogenic agents; however, the considerations are generally relevant for eDNA detection methods for pathogenic agents. This guidance will be of particular use for the field collection, processing and preservation of eDNA samples.

8. Minimum requirements for inclusion of an eDNA method in the *Aquatic Manual*

It is recognised that the validation pathway described in Chapter 1.1.2. of the *Aquatic Manual* and the design and reporting standards described by Laurin *et al*., 2018 (see above) are not met by many diagnostic methods currently included in the *Aquatic Manual*. Indeed, many assays included in the *Aquatic Manual* may be validated only to level 1 or 2 of the validation pathway described in Chapter 1.1.2. of the *Aquatic Manual*.

For this reason, the Commission proposes that the following minimum reporting requirements be met for an eDNA method to be considered for inclusion in the *Aquatic Manual* [Adapted from Goldberg *et al*., (2016)]:

1. The intended purpose or application of the assay or protocol needs to be clearly defined (note that appropriate purposes of use for eDNA methods in the context of OIE standards are discussed further in section 9).

2. Description of sample collection methods and precautions taken to eliminate contamination, including collection volume, container material, negative controls, number of replicates and sampling locations/depth.

3. Description of the methods used to concentrate the target DNA (precipitation/filtration), filter type (if applicable) and filtering location (e.g. in the field).

4. Description of sample preservation and storage (method, temperature, duration).

5. Description of the DNA extraction process including protocol adjustments, contamination precautions, negative controls, and internal positive controls.

6. Description of the molecular detection method and optimisation according to (Bustin *et al*., 2009). Furthermore, assays should be validated (Level 1) in an environmental matrix according to its purpose of use.

9. Potential application of eDNA detection methods in the disease-specific chapters of the *Aquatic Manual*

The disease-specific chapters of the *Aquatic Manual* recommend tests to identify suspect cases and to confirm suspicion for apparently healthy (or those of unknown health status) and clinically affected animals. 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 aquatic animals or aquatic animal products or equipment, etc., from a known infected population equate to an epidemiological link. Alternatively, healthy populations are sampled in surveys to demonstrate freedom.

The following points describe the suitability of evidence from eDNA detection methods for inclusion as case definition criteria in section 6 of the disease-specific chapters of the *Aquatic Manual*.

a) Apparently healthy animals

i) Definition of suspect case in a population of apparently healthy animals

Suitable as a criterion. A positive result obtained from an eDNA method recommended in the *Aquatic Manual* is considered to provide adequate evidence to be included as a criterion for a suspect case when known susceptible species exist in the environment from which the sample was taken.

ii) Definition of confirmed case in apparently healthy animals

Not suitable as a criterion. A positive result obtained from an eDNA method recommended in the *Aquatic Manual* is not considered to provide appropriate evidence to confirm a case in apparently healthy animals. Methods utilising animal derived samples are considered more appropriate for criteria to confirm a case. Evidence to confirm a case in apparently healthy animals must meet the requirements of Section 6.1.2. of the relevant disease-specific chapter of the *Aquatic Manual*. eDNA evidence will not be included as a criterion within this section.

b) Clinically affected animals

i) Definition of a suspect case in clinically affected animals

Suitable as a criterion. Taking an environmental sample to investigate the cause of disease in a population of clinically affected animals is not generally recommended as samples from clinically

affected animals are more likely to lead to pathogenic agent detection and are more suitable for disease

investigation. However, under some circumstances, an eDNA method may detect a pathogenic agent and lead to the recognition of previously unobserved or unassociated clinical signs of disease. In these circumstances, a positive result obtained from an eDNA method recommended in the *Aquatic Manual* is considered to provide adequate evidence to be included as a criterion for a suspect case.

ii) Definition of confirmed case

Not suitable as a criterion. A positive result from an eDNA method recommended in the *Aquatic Manual* would not be included as a criterion for the confirmation of a pathogenic agent in clinically affected animals (or apparently healthy animals, see point 9.a.ii above). Any positive eDNA test would require further investigation involving the collection and testing of animal tissues as stipulated in the relevant disease-specific chapter of the *Aquatic Manual*. Evidence to confirm a case in clinically affected animals must meet the requirements of Section 6.2.2. of the relevant disease-specific chapter of the *Aquatic Manual*. eDNA evidence will not be included as a criterion within this section.

10. Discussion

The key limitations of eDNA is the lack of validation and diagnostic performance data, meaning that negative results cannot be used to demonstrate disease freedom and positive results always require confirmation using animal samples (Brunner, 2020). Nevertheless, there are circumstances where the advantages of environmental, over animal, sampling means that eDNA approaches can be usefully integrated into a surveillance programme.

A country or zone claiming freedom from a specified pathogenic agent(s) are required to have in place an early detection system for disease incursion. Farmer reporting of morbidity and mortality is a key component of an early detection system. Farmed populations can act as sentinels for wild populations only if they are epidemiologically connected (i.e. through shared water). Otherwise active surveillance in wild populations is required as morbidity or mortality is unlikely to be reported (especially as dead or dying animals are likely to be quickly scavenged or predated). Animal sampling of wild populations can present considerable logistical challenges, especially if populations are remote, sparse or if low numbers make destructive sampling undesirable. eDNA based pathogenic agent detection methods overcome many of the challenges of sampling wild aquatic animals (Kamoroff & Goldberg, 2017; Trebitz *et al*., 2017).

Infection with some listed pathogenic agents, under certain conditions or in some host species, will not invariably cause detectable clinical signs. Early detection systems that rely on observations by farmers (or others) of mortality or morbidity are ineffective in these circumstances and active surveillance would be required. Sampling farmed animals on a frequent basis, and at a level to detect a low prevalence, presents considerable logistical challenges and the cost is likely to be unacceptable. eDNA methods can offer a viable alternative (Trujillo-Gonzalez *et al*., 2019a) for active surveillance for pathogens which may not reliably cause observable clinical signs. They have the additional advantage that the sample will contain analyte from a large percentage, if not the entire, captive population. Thus relatively few environmental, compared with animal samples, are needed (provided sufficient DNA can be extracted).

11. Conclusions

1. eDNA methods may have utility for enhancing passive surveillance systems for early detection; particularly in circumstances where conditions are not conducive to clinical expression of disease, or populations are not under sufficient observation to detect clinical disease should it occur.

2. eDNA methods may have utility for rare, valuable or difficult to collect wild aquatic animals, where direct sampling of animals is undesirable or cost prohibitive. They may also provide cost advantages for disease monitoring programs in production environments.

3. There are currently no frameworks to allow evaluation of diagnostic performance of eDNA methods in a manner similar to animal-derived samples. For this reason, evidence from eDNA detection methods cannot be utilised as evidence for self-declaration of freedom from disease.

4. eDNA methods will be considered for inclusion in disease-specific chapters of the *Aquatic Manual*, if minimum disease and reporting standards as described in this paper are met.

5. Positive results from an eDNA method that has been included in the *Aquatic Manual* will be considered as an appropriate criterion for a suspect case of a disease.

6. The application of eDNA methods for a given purpose should be considered carefully with respect to the pathogen to be tested, the environment to be sampled, the reliability of the method and the implications of positive results that may require surveys of susceptible animal populations to confirm or exclude a suspect case.

7. Positive results from an eDNA methods that has been included in the *Aquatic Manual* will not be considered as an appropriate criterion for a confirmed case of a disease in either apparently healthy or clinically affected animals.

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Appendix 1. Publications describing eDNA methods for aquatic animal pathogenic agents

Table 1. Published applications of eDNA methods for the detection of OIE listed pathogenic agents of aquatic animals

OIE LISTED DISEASE	PUBLICATION
Amphibian diseases	
Infection with *Batrachochytrium dendrobatidis*	Brannelly *et al*., 2020; Julian *et al*., 2019; Kamoroff & Goldberg, 2017; Mosher *et al*., 2017; Pierson & Horner, 2016; Walker *et al*., 2007
Infection with *Batrachochytrium salamandrivorans*	Brunner, 2020; Spitzen-van der Sluijs *et al*., 2020
Infection with Ranavirus species	Hall *et al*., 2016; Julian *et al*., 2019; Miaud *et al*., 2019; Pierson & Horner, 2016; Vilaca *et al*., 2020
Fish diseases	
Infection with *Gyrodactylus salaris*	Fossoy *et al*., 2020; Rusch *et al*., 2018;
Infection with HPR-deleted or HPRO infectious salmon anaemia virus	Gregory *et al*., 2009
Infection with koi herpesvirus	Haramoto *et al*., 2007; Honjo *et al*., 2010; 2012
Infection with salmonid alphavirus	Bernhardt *et al*., 2020; Weli *et al*., 2021
Crustacean diseases	
Acute hepatopancreatic necrosis disease	Kongrueng *et al*., 2015
Infection with *Aphanomyces astaci* (crayfish plague)	Robinson *et al*., 2018; Rusch *et al*, 2020; Strand *et al*., 2011; 2014; Vralstad *et al*., 2016; Wittwer *et al*., 2018a; 2018b
Infection with white spot syndrome virus	Natividad *et al*., 2008; Quang *et al*., 2009
Mollusc diseases	
Infection with *Bonamia ostreae*	Jorgensen *et al*., 2020
Infection with *Perkinsus marinus*	Audemard *et al*., 2004
Infection with *Xenohaliotis californiensis*	Lafferty & Ben-Horin, 2013

Table 2. Published eDNA studies of pathogenic agents of aquatic animals not listed by the OIE

SUBJECT	PUBLICATION
Ornamental fish parasite detection	Trujillo-Gonzalez *et al*., 2019b; 2019a
Parasitology	Bass *et al*., 2015
Protozoan parasite outbreaks in fish farms	Bastos Gomes *et al*. 2017; 2019
Disease transmission in open water Salmon cages	Salama & Rabe, 2013
Emerging aquatic parasites	Sana *et al*., 2018
Pathogenic microbes in bait	Mahon *et al*., 2018
Waterborne virus detection	Oidtmann *et al*., 2018
Halioticida noduliformans in lobsters	Holt *et al*., 2018
Microcytos mackini	Polinski *et al*., 2017
Trematode parasite *Ribieroia ondatrae*	Huver *et al*., 2015
Schistosoma species	Alzaylaee *et al*., 2020

Return to Agenda

SECTION 2.3.

DISEASES OF FISH

CHAPTER 2.3.0.

GENERAL INFORMATION

. . .

B. MATERIALS AND BIOLOGICAL PRODUCTS REQUIRED FOR THE ISOLATION AND IDENTIFICATION OF FISH PATHOGENS

. . .

2. Techniques

. . .

2.5. Use of molecular techniques for surveillance testing, confirmatory testing and diagnosis (third paragraph)

As with all PCR protocols, optimisation may be necessary depending on the reagents, equipment and the plasticware. PCR is prone to false-positive and false-negative results. False-positive results (negative samples giving a positive reaction), may arise from either product carryover from positive samples or, more commonly, from cross-contamination by PCR products from previous tests. Therefore, each assay and tissue extraction should include a negative control to rule out contamination. False-negative results (positive samples giving a negative result), may occur due to the presence of a new variant that is not recognised by the PCR primer/probe set, which may lead to unwanted transmission of pathogens and biosecurity failure.

[…]

Return to Agenda

CHAPTER 2.3.2.

INFECTION WITH EPIZOOTIC HAEMATOPOIETIC NECROSIS VIRUS

1. Scope

Infection with epizootic haematopoietic necrosis virus means infection with the pathogenic agent *epizootic haematopoietic necrosis virus* (EHNV) of the Genus *Ranavirus* of the Family *Iridoviridae*.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

EHNV is a species of the genus *Ranavirus* in the Family *Iridoviridae* (Chinchar *et al.,* 2005). In addition to fish, ranaviruses have been isolated from healthy or diseased frogs, salamanders and reptiles in America, Europe and Australia (Chinchar*,* 2002; Drury *et al.,* 2002; Fijan *et al.,* 1991; Hyatt *et al.*, 2002; Speare & Smith, 1992; Whittington *et al.*, 2010; Wolf *et al*., 1968; Zupanovic *et al*., 1998). Ranaviruses have large (150–180 nm), icosahedral virions, a double-stranded DNA genome 150–170 kb, and replicate in both the nucleus and cytoplasm with cytoplasmic assembly (Chinchar *et al.,* 2005).

Since the recognition of disease due to EHNV in Australia in 1986, similar systemic necrotising iridovirus syndromes have been reported in farmed fish. These include catfish (*Ictalurus melas*) in France (European catfish virus, ECV) (Pozet *et al*., 1992), sheatfish (*Silurus glanis*) in Germany (European sheatfish virus, ESV) (Ahne *et al.,* 1989; 1990), turbot (*Scophthalmus maximus*) in Denmark (Bloch & Larsen, 1993), and cod (*Gadus morhua*) in Denmark (Cod iridovirus, CodV) (Ariel *et al.,* 2010). EHNV, ECV, ESV, and CodV share >98% nucleotide identity across concatenated sequences across the RNR-α, DNApol, RNR-β, RNAse II and MCP gene regions (Ariel *et al*., 2010).

EHNV and ECV can be differentiated using genomic analysis (Ahne *et al.,* 1998; Holopainen *et al*., 2009; Hyatt *et al.*, 2000; Mao *et al*., 1996; 1997; Marsh *et al*., 2002). This enables epidemiological separation of disease events in finfish in Australia (EHNV) and Europe (ECV), and differentiation of these from ranavirus occurrences in amphibians.

2.1.2. Survival and stability in processed or stored samples

EHNV can persist in frozen fish tissues for more than 2 years (Langdon, 1989) and frozen fish carcases for at least a year (Whittington *et al.*, 1996).

2.1.3. Survival and stability outside the host

EHNV is resistant to drying and remained infective for 97 days at 15°C and 300 days at 4°C in water (Langdon, 1989). For these reasons, it is presumed that EHNV would persist for months to years on a fish farm in water and sediment, as well as on plants and equipment.

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 EHNV according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) are:

Family	Scientific name	Common name
Esocidae	*Esox lucius*	Northern pike
Galaxiidae	*Galaxias olidus*	Mountain galaxias
Ictaluridae	*Ameiurus melas*	Black bullhead
Melanotaeniidae	*Melanotaenia fluviatilis*	Crimson spotted rainbow fish
Percidae	*Perca fluviatilis*	European perch
	Sander lucioperca	Pike-perch
Percichthyidae	*Macquaria australasica*	Macquarie perch
Poeciliidae	*Gambusia holbrooki*	Eastern mosquito fish
	Gambusia affinis	Mosquito fish
Salmonidae	*Oncorhynchus mykiss*	Rainbow trout
Terapontidae	*Bidyanus bidyanus*	Silver perch

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 EHNV according to Chapter 1.5 of the *Aquatic Code* are: none known.

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: Atlantic salmon (*Salmo salar*), freshwater catfish (*Tandanus tandanus*), golden perch (*Macquaria ambigua*), Murray cod (*Maccullochella peelii*) and purple spotted gudgeon (*Mogurnda adspersa*).

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

Natural infections and disease have been limited to European perch (*Perca fluviatilis*) and rainbow trout (*Oncorhynchus mykiss*) in Australia. The disease is more severe in European perch and in juveniles compared with adult fish (Whittington *et al.,* 2010). There are no descriptions of infection of eggs or early life stages of any fish species.

For the purposes of Table 4.1, larvae and fry up to approximately 5 g in weight may be considered to be early life stages, fingerlings and grower fish up to 500 g may be considered to be juveniles, and fish above 500 g may be considered to be adults.

2.2.4. Distribution of the pathogen in the host

Target organs and tissues infected with the virus are kidney, spleen and liver. It is not known if EHNV can be detected in gonadal tissues, ovarian fluid or milt or whether these tissues are suitable for surveillance of broodstock.

2.2.5. Aquatic animal reservoirs of infection

Rainbow trout: The high case fatality rate and low prevalence of infection with EHNV in natural infections in rainbow trout means that the recruitment rate of carriers is likely to be very low (<2%) (Whittington *et al.,* 1994). EHNV has been detected in growout fish but histopathological lesions consistent with infection with EHNV indicated an active infection rather than a carrier state (Whittington *et al.,* 1999). Anti-EHNV serum antibodies were not detected in fingerlings during or after an outbreak but were detected in a low proportion of growout fish, hence, it is uncertain whether these were survivors of the outbreak (Whittington *et al.,* 1994; 1999). There are data for European stocks of rainbow trout in experimental infections where potential carriers were identified (Ariel & Bang Jensen, 2009).

European perch: EHNV was isolated from 2 of 40 apparently healthy adult European perch during epizootics in juveniles in Victoria, Australia (Langdon & Humphrey, 1987), but as the incubation period extends for up to 28 days (Whittington & Reddacliff, 1995), these fish may have been in the preclinical phase.

2.2.6. Vectors

Birds are potential vectors for EHNV, it being carried in the gut, on feathers, feet and the bill (Whittington *et al.,* 1996).

2.3. Disease pattern

2.3.1. Mortality, morbidity and prevalence

Rainbow trout: It appears that under natural farm conditions EHNV is poorly infective but has a high case fatality rate. Infection with EHNV may be present on a farm without causing suspicion because the mortality rate may not rise above the usual background rate. Infection with EHNV has most often been reported in young fingerlings <125 mm fork length with daily mortality of less than 0.2% and total mortality of up to 4%. However, rainbow trout of all ages may be susceptible, although infection has not yet been seen in broodstock (Whittington *et al.*, 1994; 1999). There is a low direct economic impact because of the low mortality rate. Differences in susceptibility between European and Australian stocks of rainbow trout may exist (Ariel & Bang Jensen, 2009).

European perch: There is a very high rate of infection and mortality in natural outbreaks that, over time, leads to loss in wild fish populations (Langdon & Humphrey, 1987; Langdon *et al.,* 1986; Whittington *et al.*, 1996). Experimental bath inoculation with as few as 0.08 $TCID_{50}$ ml^{-1} was lethal, and doses too low to be detected by virus isolation in BF-2 cells were fatal by intraperitoneal inoculation (Whittington & Reddacliff, 1995). Differences in susceptibility between European and Australian stocks of European perch may exist (Ariel & Bang Jensen, 2009).

2.3.2. Clinical signs, including behavioural changes

Moribund fish may have loss of equilibrium, flared opercula and may be dark in colour (Reddacliff & Whittington, 1996). Clinical signs are usually more obvious in fingerlings and juvenile fish than adults of both rainbow trout and European perch. There may be clinical evidence of poor husbandry practices, such as overcrowding and suboptimal water quality manifesting as skin, fin and gill lesions (Reddacliff & Whittington, 1996).

2.3.3 Gross pathology

There may be no gross lesions in affected fish. A small proportion of fish may have enlargement of kidney, liver or spleen. There may be focal white to yellow lesions in the liver corresponding to areas of necrosis (Reddacliff & Whittington, 1996).

2.3.4. Modes of transmission and life cycle

Rainbow trout: EHNV has spread between rainbow trout farms by transfer of infected fingerlings and probably transport water (Langdon *et al.,* 1988; Whittington *et al.*, 1994; 1999). The low prevalence of infection in rainbow trout means that active infection can easily go unrecognised in a population and be spread by trading fish. There are no data on possible vertical transmission of EHNV on or within ova, and disinfection protocols for ova have not been evaluated. EHNV has not yet been isolated from ovarian tissues or from broodstock. Annual recurrence in farmed rainbow trout may be due to reinfection of successive batches of fish from wild European perch present in the same catchment.

European perch: The occurrence of infection with EHNV in European perch in widely separated river systems and impoundments suggested that EHNV was spread by translocation of live fish or bait by recreational fishers (Whittington *et al.,* 2010).

The route of infection is unknown. European perch and rainbow trout are susceptible to immersion exposure. The virus infects a range of cell types including hepatocytes, haematopoietic cells and endothelial cells in many organs (Reddacliff & Whittington, 1996). Virus is shed into water from infected tissues and carcasses as they disintegrate.

2.3.5. Environmental factors

Rainbow trout: Outbreaks appear to be related to poor husbandry, particularly overcrowding, inadequate water flow and fouling of tanks with feed. Damage to skin may provide a route of entry for EHNV. Outbreaks have been seen on farms at water temperatures ranging from 11 to 20°C (Whittington *et al.*, 1994; 1999). The incubation period after intraperitoneal inoculation was 3–10 days at 19–21°C compared with 14–32 days at 8–10°C (Whittington & Reddacliff, 1995).

European perch: Natural epizootics of infection with EHNV affecting juvenile and adult European perch occur mostly in summer (Langdon & Humphrey, 1987; Langdon *et al.,* 1986; Whittington *et al.*, 1994). It has been assumed that the disease in juvenile fish is related to the annual appearance of large numbers of non-immune young fish and their subsequent exposure to the virus while schooling in shallow waters;

adults are uncommonly involved in these outbreaks. It is possible that environmental temperature is the trigger for outbreaks as juvenile fish feed in warm shallow waters on planktonic fauna, whereas adults feed on benthic invertebrates and larger prey in deeper cooler water (Whittington & Reddacliff, 1995). Experimentally, the incubation period ranged from 10 to 28 days at 12–18°C compared with 10–11 days at 19–21°C, and adult perch were refractory to infection at temperatures below 12°C (Whittington & Reddacliff, 1995). European stocks of European perch also displayed temperature-dependent susceptibility (Ariel & Bang Jensen, 2009).

2.3.6. Geographical distribution

Infection with EHNV has been reported from rainbow trout farms within two river catchments in New South Wales, Australia (Whittington *et al*., 2010). Infection with EHNV is endemic in south-eastern Australia, with a discontinuous distribution (Whittington *et al*., 2010).

See WAHIS (https://wahis.oie.int/#/home) for recent information on distribution at the country level.

2.4. Biosecurity and disease control strategies

Not available.

2.4.1. Vaccination

None available.

2.4.2. Chemotherapy including blocking agents

None available.

2.4.3. Immunostimulation

None available.

2.4.4. Breeding resistant strains

There has been no formal breeding programme for resistant strains of susceptible species. However, experimental trials using a bath exposure have shown that European perch from water bodies in New South Wales, Australia with previous EHNV infections showed lower mortality compared with European perch from neighbouring and distant water bodies in Australia that have no previous history of EHNV (Becker *et al.,* 2016).

2.4.5. Inactivation methods

EHNV is susceptible to 70% ethanol, 200 mg litre^{-1} sodium hypochlorite or heating to 60°C for 15 minutes (Langdon, 1989). Data for the inactivation of amphibian ranavirus may also be relevant: 150 mg/litre chlorhexidine and 200 mg/litre potassium peroxymonosulphate were effective after 1 minute contact time (Bryan *et al*., 2009). If it is first dried, EHNV in cell culture supernatant is resistant to heating (Whittington *et al.*, 2010).

2.4.6. Disinfection of eggs and larvae

Not tested.

2.4.7. General husbandry

Disease control in rainbow trout at the farm level relies on reducing the impact of infection by maintaining low stocking rates and adequate water quality. Investigations on one rainbow trout farm indicated that ponds with high stocking rates and low water flow, and thus poorer water quality, may result in higher levels of clinical disease compared with ponds on the same farm with lower stocking rates and higher water flow (Whittington *et al.,* 1994). The mechanism of protection may be through maintenance of healthy integument (Whittington *et al.,* 1994).

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 should be carried out during a period when water temperature is conducive to development of clinical disease (see Section 2.3.5). All production units (ponds, tanks, etc.) should be inspected for the presence of dead, weak or abnormally behaving fish. For the purposes of disease surveillance, fish to be sampled are selected as follows:

i) The most susceptible species (e.g. rainbow trout and European perch) should be sampled preferentially. Other susceptible species listed in Section 2.2.1 should be sampled proportionally.

ii) Risk-based criteria should be employed to preferentially sample lots or populations with a history of abnormal mortality, potential exposure events or where there is evidence of poor water quality or husbandry. If more than one water source is used for fish production, fish from all water sources should be included in the sample.

iii) If weak, abnormally behaving or freshly dead fish are present, such fish should be selected. If such fish are not present, the fish selected should include normal appearing, healthy fish collected in such a way that all parts of the farm as well as all year classes are proportionally represented in the sample.

For disease outbreak investigations, moribund fish or fish exhibiting clinical signs of infection with EHNV should be collected. Ideally fish should be collected while alive, however recently dead fish can also be selected for diagnostic testing. 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.

3.2. Selection of organs or tissues

Liver, anterior kidney and spleen from individual fish are pooled (Jaramillo *et al.,* 2012).

3.3. Samples or tissues not suitable for pathogen detection

Inappropriate tissues include gonads, gonadal fluids, milt and ova, since there is no evidence of reproductive tract infection.

3.4. Non-lethal sampling

No non-lethal samples (blood, fin, gill, integument or mucous) are suitable for testing EHNV.

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

For recommendations on transporting samples for virus isolation to the laboratory, see Section B.2.4 of Chapter 2.3.0 General information (diseases of fish).

3.5.2. Preservation of 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 based on studies in terrestrial animal and human health. The use of lower grade (laboratory or industrial grade) ethanol is not recommended. If material cannot be fixed it may be frozen.

3.5.3. Samples for histopathology, immunohistochemistry or *in-situ* hybridisation

Tissue samples for histopathology should be fixed immediately after collection in 10% neutral buffered formalin. The recommended ratio of fixative to tissue is 10:1.

3.5.4. Samples for other tests

Not recommended for routine diagnostic testing.

3.6. Pooling of samples

Pooling of samples from more than one individual animal for a given purpose should only be recommended where robust supporting data on diagnostic sensitivity and diagnostic specificity have been evaluated and found to be suitable. If the effect of pooling on diagnostic sensitivity has not been thoroughly evaluated, larger fish should be processed and tested individually. Small life stages such as fry or specimens can be pooled to provide the minimum amount of material needed for testing. If pooling is used, it is recommended to pool organ pieces from a maximum of five fish.

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 apparently healthy animals and investigation of clinically affected animals*

Method	A. Surveillance of apparently healthy animals				B. Presumptive diagnosis of clinically affected animals				C. Confirmatory diagnosis[1] of a suspect result from surveillance or presumptive diagnosis			
	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV
Wet mounts												
Histopathology[3]					++	++	++	1				
Cytopathology[3]												
Cell culture	+++	+++	+++	2	+++	+++	+++	2				
Immunohistochemistry					+	+	+	1				
Real-time PCR	+++	+++	+++	2	+++	+++	+++	2				
Conventional PCR	+	+	+	1	++	++	++	1				
Amplicon sequencing[4]									+++	+++	+++	3
***In-situ* hybridisation**												
Bioassy												
LAMP												
Ab-ELISA			+	1								
Ag-ELISA	+	+	+	1	+	+	+	1				
Other antigen detection methods[5]												
Other method[5]												

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; Ab- or Ag-ELISA = antibody or antigen enzyme-linked immunosorbent assay, respectively.
[1]For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). [2]Susceptibility of early and juvenile life stages is described in Section 2.2.3.
[3]Histopathology and cytopathology can be validated if the results from different operators have been statistically compared. [4]Sequencing of the PCR product.
[5]Specify the test used. Shading indicates the test is inappropriate or should not be used for this purpose.

4.1. Wet mounts

Not applicable.

4.2. Histopathology and cytopathology

Light microscopy: routine methods can be used for tissue fixation, such as in 10% buffered neutral formalin, paraffin embedding, preparation of 4–10 µm sections and staining with H&E to demonstrate tissue necrosis and basophilic intracytoplasmic inclusion bodies. These inclusion bodies are indicative but not confirmatory for infection with EHNV. Formalin-fixed paraffin-embedded sections can also be stained using an immunoperoxidase method (see below) to identify EHNV antigen associated with necrotic lesions.

Acute focal, multifocal or locally extensive coagulative or liquefactive necrosis of liver, haematopoietic kidney and spleen are commonly seen in routine haematoxylin and eosin (H&E)-stained sections of formalin-fixed material. A small number of basophilic intracytoplasmic inclusion bodies may be seen, particularly in areas immediately surrounding necrotic areas in the liver and kidney. Necrotic lesions may also be seen in heart, pancreas, gastrointestinal tract, gill and pseudobranch (Reddacliff & Whittington, 1996).

Affected tissues (e.g. kidney liver and spleen) contain cells exhibiting necrosis. Cells contain conspicuous cytoplasmic inclusions that are rarefied areas of the cytoplasm in which the viruses are assembled. Within the cytoplasm, aggregates (paracrystalline arrays) of large (175 nm ± 6 nm) nonenveloped icosahedral viruses are apparent; single viruses are also present. Complete viruses (containing electron-dense cores) bud/egress from the infected cells through the plasma membrane. The nuclei of infected cells are frequently located peripherally and are distorted in shape.

4.3. Cell culture for isolation

4.3.1. Preparation of fish tissues for virus isolation

A simple method for preparation of fish tissues for cell culture and ELISA has been validated (Whittington & Steiner, 1993) (see sampling Section 3).

i) Freeze tubes containing tissues at –80°C until needed.

ii) Add 0.5 ml of homogenising medium (minimal essential medium Eagle, with Earle's salts with glutamine] [MEM] with 200 International Units [IU] ml^{-1} penicillin, 200 µg ml^{-1} streptomycin and 4 µg ml^{-1} amphotericin B) to each tube. Grind tissue to a fine mulch with a sterile fitted pestle.

iii) Add another 0.5 ml of homogenising medium to each tube and mix with a pestle.

iv) Add three sterile glass beads to each tube (3 mm diameter) and close the lid of the tube.

v) Vortex the suspension vigorously for 20–30 seconds and place at 4°C for 2 hours.

vi) Vortex the suspension again as above and centrifuge for 10 minutes at 2500 ***g*** in a benchtop microcentrifuge.

vii) Transfer the supernatant, now called clarified tissue homogenate, to a fresh sterile tube. Homogenates may be frozen at –80°C until required for virus isolation and ELISA.

4.3.2. Cell culture~~/artificial media~~

EHNV grows well in many fish cell lines including BF-2 (bluegill fry ATCC CCL 91), FHM (fathead minnow; ATCC CCL 42), EPC (epithelioma papulosum cyprini [Cinkova *et al.,* 2010]), and CHSE-214 (Chinook salmon embryo cell line; ATCC CRL 1681) at temperatures ranging from 15 to 22°C (Crane *et al.,* 2005). Incubation temperatures of 20°C or 24°C result in higher titres than 15°C; 22°C and BF-2 EPC or CHSE-214 cells are recommended to maximise titres, which might be important for the detection of low numbers of viruses in fish tissues (Ariel *et al.,* 2009). BF-2 cells are preferred by the OIE Reference Laboratory with an incubation temperature of 22°C. The procedure for BF-2 cells is provided below. A procedure for CHSE-214 cells is provided under immunoperoxidase staining below (Section 4.7). The identity of viruses in cell culture is determined by immunostaining, ELISA, immuno-electron microscopy, PCR or other methods.

4.3.3. Cell culture technical procedure

Samples: tissue homogenates.

Cells are cultured (in flasks, tubes or multi-well plates) with growth medium (MEM + 10% fetal calf serum [FCS] with 100 IU ml^{-1} penicillin, 100 µg ml^{-1} streptomycin and 2 µg ml^{-1} amphotericin B). The cells are incubated until almost confluent at 22°C, which can take up to 4 days depending on the seeding rate. Medium is changed to a maintenance medium (MEM with 2% FCS and 100 IU ml^{-1} penicillin, 100 µg ml^{-1} streptomycin and 2 µg ml^{-1} amphotericin B) on the day of inoculation. A 1/10 dilution using homogenising medium is made of single or pooled homogenates. Each culture is inoculated with 100 µl of sample per ml of culture medium. This represents a final 1/100 dilution of a 0.1 mg ml^{-1} tissue homogenate. A further 1/10 dilution is made representing a final 1/1000 dilution, and two cultures are inoculated. No adsorption step is used. As an alternative, two to three cultures can be inoculated directly with 10 µl undiluted homogenate per ml of culture medium. Note that a high rate of cell toxicity or contamination often accompanies the use of a large undiluted inoculum. The cultures are incubated at 22°C in an incubator for 6 days. Cultures are read at day 3 and day 6. Cultures are passed at least once to detect samples with low levels of virus. On day 6, the primary cultures (P1) are frozen overnight at –20°C, thawed, gently mixed and then the culture supernatant is inoculated onto fresh cells as before (P2), i.e. 100 µl P1 supernatant per ml culture medium. Remaining P1 supernatants are transferred to sterile 5 ml tubes and placed at 4°C for testing by ELISA or PCR or another means to confirm the cause of cytopathic effect (CPE) as EHNV. P2 is incubated as above, and a third pass is conducted if necessary.

4.3.4. Interpretation of results

CPE is well developed and consists of focal lysis surrounded by rounded granular cells. This change extends rapidly to involve the entire monolayer, which detaches and disintegrates. Cell cultures can be tested for EHNV DNA using real-time PCR and conventional PCR with sequence analysis as described in Section 4.4. Antigen can be detected using immunocytochemistry in cell cultures with polyclonal antibodies and protocol available from the reference laboratory.

Cell lines should be monitored to ensure that susceptibility to targeted pathogens has not changed.

4.4. Nucleic acid amplification

Although several conventional PCR or quantitative real-time PCR methods have been described (Jaramillo *et al.,* 2012; Pallister *et al,* 2007; Stilwell *et al.,* 2018) none has been validated according to OIE guidelines for primary detection of EHNV. However, identification of ranavirus at genus and species level is possible using several published PCR strategies. Samples can be screened by real-time PCR, but as the assays described are not specific for EHNV, identification of EHNV by conventional PCR and amplicon sequencing must be undertaken on any samples screening positive by real-time PCR. For testing by conventional PCR, two PCR assays using MCP primers are used with amplicon sequencing required to differentiate EHNV from ECV, FV3 and BIV (Marsh *et al*., 2002). Alternatively, PCR of the DNA polymerase gene and neurofilament triplet H1-like protein genes can be used (Holopainen *et al*., 2011) (this method is not described in this chapter).

Samples: virus from cell culture or direct analysis of tissue homogenate.

4.4.1. Real-time PCR

Tissue samples can be homogenised by manual pestle grinding or by bead beating (Rimmer *et al.,* 2012). Commercially available nucleic acid extraction kits (e.g. spin columns, magnetic beads) may be used to extract DNA directly from tissues and from tissue homogenates and cell culture supernatants. Depending on the number of samples to be tested, in the OIE Reference Laboratory, nucleic acids are extracted with either the QIAamp Viral RNA Mini Kit (Qiagen) or MagMAX-96 Viral RNA Isolation Kit (Applied Biosystems) according to the manufacturer's instructions. A negative extraction control, consisting of extraction reagents only, is included when test samples are extracted.

The ranavirus real-time screening protocol in use at the OIE Reference Laboratory, based on Pallister *et al.,* 2007 is as follows; Template (2 µl) is added to 23 µl reaction mixture containing 12.5 µl TaqMan Universal PCR Master Mix (Applied Biosystems), 900 nM for each primer, 250 nM for probe, and molecular grade water. After 1 cycle of 50°C for 2 minutes and 95 °C for 10 minutes, PCR amplification consists of 45 cycles of 95°C for 15 seconds, 60°C for 60 seconds.

Alternative real-time PCR assays can be used according to published protocols for detection of the major capsid protein gene sequence of EHNV and other ranaviruses. The assay described by Jaramillo *et al.* (2012) uses SYBR Green detection chemistry and the assay described by Stilwell *et al.* (2018) was designed to detect multiple ranavirus species using hydrolysis probe detection chemistry.

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

Table 4.4.1.1. *Ranavirus primer and probe sequences*

Primer	Sequence	Reference
RANA CON F	5'-CTC-ATC-GTT-CTG-GCC-ATC-A-3'	Pallister *et al.,* 2007
RANA CON R	5'-TCC-CAT-CGA-GCC-GTT-CA-3'	
Probe		
RANA CON Pr	5'-**6FAM**-CAC-AAC-ATT-ATC-CGC-ATC-**MGB**-3'	
Primer		
C1096	GAC-TGA-CCA-ACG-CCA-GCC-TTA-ACG	Jaramillo *et al.,* 2012
C1097	GCG-GTG-GTG-TAC-CCA-GAG-TTG-TCG	
Primer		
RanaF1	CCA-GCC-TGG-TGT-ACG-AAA-ACA	Stilwell *et al.,* 2018
RanaR1	ACT-GGG-ATG-GAG-GTG-GCA-TA	
Probe		
RanaP1	6FAM-TGG-GAG-TCG-AGT-ACT-AC-MGB	

4.4.2. Conventional PCR

PCR and restriction endonuclease analysis (*REA*): *technical procedure*

Amplified product from PCR assay MCP-1 digested with PflM I enables differentiation of EHNV and BIV from FV3 and ECV. Amplified product from PCR assay MCP-2 digested with Hinc II, Acc I and Fnu4H I (individually) enables differentiation of EHNV and BIV from each other and from FV3 and ECV.

Preparation of reagents

EHNV-purified DNA and BIV-purified DNA PCR control reagents are supplied by the reference laboratory in freeze-dried form. Reconstitute using 0.5 ml of Tris-EDTA (TE) buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0) and allow the vial to stand at RT for 2 minutes. Mix the vial very gently. For routine use, as a PCR control, it is recommended that working stocks be prepared as a 1/10 dilution in TE buffer (pH 8.0). Aliquots of 250 µl should be stored at –20°C. Each aliquot is sufficient for at least 50 reactions (1 to 5 µl added to cocktail) and has a minimum shelf life of 6 months from date of diluting.

Primers M151 and M152 (MCP-1, 321 bp), M153 and M154 (MCP-2, 625 bp) are supplied in working strength (100 ng μl^{-1}) and should be stored at –20°C. Primers can also be ordered from commercial suppliers. For primer sequences, refer to Table 4.4.2.1.

Table 4.4.2.1. *MCP-1 and MCP-2 primer sequences*

PCR assay	Primer	Sequence	Product size	Gene location
MCP-1	M151	AAC-CCG-GCT-TTC-GGG-CAG-CA	321 bp	266–586
	M152	CGG-GGC-GGG-GTT-GAT-GAG-AT		
MCP-2	M153	ATG-ACC-GTC-GCC-CTC-ATC-AC	625 bp	842–1466
	M154	CCA-TCG-AGC-CGT-TCA-TGA-TG		

PCR cocktail

Amplification reactions in a final volume of 50 µl (including 5 µl DNA sample) contain 2.5 µl (250 ng) of each working primer, 200 µM of each of the nucleotides dATP, dTTP, dGTP and dCTP, 5 µl of 10 × PCR buffer (66.6 mM Tris/HCl, 16.6 mM $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, 1.65 mg ml^{-1} BSA, 10 mM beta-mercaptoethanol) and 2 U Taq polymerase. Instructions on preparation of 10 × PCR buffer are included in Table 4.4.2.2.

Table 4.4.2.2. *10 × PCR buffer preparation*

Ingredients	Amount	Final concentration in 50 µl PCR mix
Tris	4.050 g	66.6 mM

Ingredients	Amount	Final concentration in 50 µl PCR mix
Ammonium sulphate	1.100 g	16.6 mM
BSA (albumin bovine fraction V fatty acid free)	0.825 g	1.65 mg ml^{-1}
Magnesium chloride	1.25 ml	2.5 mM
TE buffer (sterile)	50 ml	

NOTE: alternative commercial buffers may also be used.

Two negative controls are included, one comprising PCR cocktail only and the second containing 5 µl TE buffer.

The MCP-1 and MCP-2 reactions have the following profile: 1 cycle of denaturation at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds and extension at 72°C for 1 minute; a final extension of 72°C for 5 minutes, and cooling to 4°C.

NOTE: the annealing temperature may be increased to 60 or 62°C to reduce nonspecific amplification when the assay is used to test fish tissues.

PCR results are assessed by electrophoresis in 2% agarose gels stained with ethidium bromide. EHNV PCR control DNA (1/10 working stock) should give a result similar in intensity to the 10–3 band in both cases.

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

4.4.3. Other nucleic acid amplification methods

Not applicable.

4.5. Amplicon sequencing

Amplicons generated using the MCP-1 and/or MCP-2 primers sets can be sequenced. Amplicons should be gel-purified and sequenced using both the forward and reverse primer. Consensus sequence, generated after analysis of the quality of the sequence chromatograms, can then be compared to reference sequences, for example by BlastN search of the NCBI database.

4.6. *In-situ* hybridisation

Not applicable

4.7. Immunohistochemistry

Immunohistochemistry (*immunoperoxidase stain*)

Samples: formalin-fixed paraffin-embedded tissue sections.

Technical procedure

The following protocol is intended for the qualitative demonstration of EHNV antigens in formalin-fixed paraffin-embedded tissue sections (Reddacliff & Whittington, 1996). It assumes that antigens may have become cross linked and therefore includes a protease digestion step that may be omitted if unfixed samples are examined. A commercial kit (DAKO® LSAB K0679) with peroxidase-labelled streptavidin and a mixture of biotinylated anti-rabbit/anti-mouse/anti-goat immunoglobulins as link antibodies is used for staining. Other commercially supplied reagents are also used. For convenience these are also supplied by DAKO[2]. The primary affinity purified rabbit anti-EHNV antibody (Lot No. M708) is supplied freeze-dried by the OIE Reference Laboratory.

2 Dako Cytomation California Inc., 6392 via Real, Carpinteria, CA 93013, USA, Tel.: (+1-805) 566 6655, Fax: (+1-805) 566

i) Cut 5 µm sections and mount on SuperFrost® Plus G/Edge slides (Menzel-Glaser, HD Scientific Cat. No. HD 041300 72P3). Mark around the section with a diamond pencil to limit the spread of reagents.

ii) Deparaffinise the section:

Preheat slides in a 60°C incubator for 30 minutes.

Place slides in a xylene bath and incubate for 5 minutes. Repeat once. Note that xylene replacements can be used without deleterious effects.

Tap off excess liquid and place slides in absolute ethanol for 3 minutes. Repeat once.

Tap off excess liquid and place slides in 95% ethanol for 3 minutes. Repeat once.

Tap off excess liquid and place slides in distilled or deionised water for 30 seconds.

iii) Expose antigens using a protease treatment. Flood slide with proteinase K (5–7 µg ml^{-1}) and incubate for 20 minutes (ready-to-use solution, DakoCytomation Cat. No. S3020). Rinse slide by immersing three times in water. Place in a PBST bath for 5 minutes (PBS pH 7.2, 0.05% [v/v] Tween 20). Tap off the excess wash solution and carefully wipe around the section.

iv) Perform the immunostaining reaction using the Universal DAKO LSAB®+ Kit, Peroxidase (DakoCytomation Cat No. K0679). Ensuring the tissue section is completely covered, add the following reagents to the slide. Avoid drying out.

v) 3% hydrogen peroxide: cover the section and incubate for 5 minutes. Rinse gently with PBST and place in a fresh wash bath.

vi) Primary antibody (affinity purified rabbit anti-EHNV 1:/1500 Lot No. M708) and negative control reagent (non-immune rabbit serum at a dilution of 1/1500) on a second slide. Cover the section and incubate for 15 minutes. Rinse slides.

vii) Link: cover the section and incubate for 15 minutes. Rinse slides.

viii) Streptavidin peroxidase: cover the section and incubate for 15 minutes. Rinse slides.

ix) Substrate–chromogen solution: cover the section and incubate for 5 minutes. Rinse slides gently with distilled water.

x) Counterstain by placing slides in a bath of DAKO® Mayer's Haematoxylin for 1 minute (Lillie's Modification, Cat. No. S3309). Rinse gently with distilled water. Immerse 10 times into a water bath. Place in distilled or deionised water for 2 minutes.

xi) Mount and cover-slip samples with an aqueous-based mounting medium (DAKO® Faramount Aqueous Mounting Medium Cat. No. S3025).

Interpretation of results

EHNV antigen appears as a brown stain in the areas surrounding degenerate and necrotic areas in parenchymal areas. There should be no staining with negative control rabbit serum on the same section.

Availability of test and reagents: antibody reagents and test protocols are available from the OIE Reference Laboratory.

4.8. Bioassay

Not applicable.

4.9. Antibody- or antigen-based detection methods (ELISA, etc.)

An antigen ELISA for detection of EHNV and an EHNV antibody detection ELISA have been described (Whittington & Steiner, 1993). The same antibodies are suitable for immunohistochemistry on fixed tissues and for detection of ranavirus antigen in cell culture. Reagents and protocols are available from the reference laboratory. It should be noted that polyclonal antibodies used in all related methods (immunoperoxidase, antigen-capture ELISA and immunoelectron microscopy) cross-react with all known ranaviruses except Santee Cooper ranaviruses (Ahne *et al.,* 1998; Cinkova *et al.,* 2010; Hedrick *et al*., 1992; Hyatt *et al*., 2000).

6688; Dako Cytomation Pty Ltd, Unit 4, 13 Lord Street, Botany, NSW 2019, Australia, Fax: (+61-2) 9316 4773; Visit www.dakocytomation.com for links to other countries.

4.10. Other methods

Neutralising antibodies have not been detected in fish or mammals exposed to EHNV. Indirect ELISA for detection of antibodies induced following exposure to EHNV has been described for rainbow trout and European perch (Whittington *et al.*, 1994; 1999; Whittington & Reddacliff, 1995). The sensitivity and specificity of these assays in relation to a standard test are not known and interpretation of results is difficult. Protocols and specific anti-immunoglobulin reagents required to conduct these tests are available from the reference laboratory.

5. Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations

Real-time PCR is the most appropriate method of screening healthy fish populations for EHNV; however, the available methods are not specific for EHNV. Any real-time PCR positive samples should be tested by conventional PCR and sequence analysis to distinguish ranaviruses.

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[3]

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 with EHNV shall be suspected if at least one of the following criteria is met:

i) Positive result for EHNV based on virus isolation in cell cultures;

ii) Positive real-time or conventional PCR result;

iii) Positive EHNV antigen ELISA.

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with EHNV is considered to be confirmed if at least one of the following criteria is met:

i) EHNV-typical CPE in cell culture followed by identification of EHNV by conventional PCR and sequence analysis of the amplicon;

ii) A positive result in tissue samples by real-time PCR and identification of EHNV by conventional PCR and sequence analysis 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.

[3] For example transboundary commodities.

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 with EHNV shall be suspected if at least one of the following criteria is met:

i) Histopathology consistent with EHNV;

ii) EHNV-typical CPE in cell cultures;

iii) Positive real-time or conventional PCR result.

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with EHNV is considered to be confirmed if, in addition to the criteria in Section 6.2.1, at least one of the following criteria is met:

i) EHNV-typical CPE in cell culture followed by identification of EHNV by conventional PCR and sequence analysis of the amplicon;

ii) A positive result in tissue samples by real-time PCR and identification of EHNV by conventional PCR and sequence analysis 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

6.3.1. For presumptive diagnosis of clinically affected animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation
Real-time PCR	Diagnosis	Clinically diseased fish (multiple species) from disease outbreaks and experimental infections	Pool of kidney. Liver and spleen from individual fish	European perch (*Perca fluviatilis*), river blackfish (*Gadopsis marmoratus*), golden perch (*Macquaria ambigua*), trout cod (*Maccullochella macquariensis*), freshwater catfish (*Tandanus tandanus*), Macquarie perch (*Macquaria australasica*) rainbow trout (*Oncorhynchus mykiss*)	94.3% * (n= 105)	100% (n= 441)	Virus isolation in BF-2 cell culture	Jaramillo *et al.*, (2012)
Real-time PCR	Diagnosis	Clinically diseased fish (multiple species) from disease outbreaks and experimental infections	Pool of kidney. Liver and spleen from individual fish	European perch (*Perca fluviatilis*), river blackfish (*Gadopsis marmoratus*), golden perch (*Macquaria ambigua*), trout cod (*Maccullochella macquariensis*), freshwater catfish (*Tandanus tandanus*), Macquarie perch (*Macquaria australasica*) rainbow trout (*Oncorhynchus mykiss*)	95% * (n=106)	100% (n=80)	Virus isolation in BF-2 cell culture	Stilwell *et al.*, 2018

DSe: = diagnostic sensitivity, DSp = diagnostic specificity, *n* = number of samples used in the study;
PCR: = polymerase chain reaction. Note: these assays detect multiple ranaviruses in addition to EHNV that infect amphibian hosts. *A positive result requires characterisation using sequencing to confirm that the result indicates the presence of EHNV.

6.3.2. For surveillance of apparently healthy animals: not available

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation

DSe: = diagnostic sensitivity, DSp = diagnostic specificity, qPCR: = real-time polymerase chain reaction.

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* *

NB: There is an OIE Reference Laboratory for infection with epizootic haematopoietic necrosis virus (EHNV) (see Table at the end of this *Aquatic Manual* or consult the OIE web site for the most up-to-date list: https://www.oie.int/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3).
Please contact the OIE Reference Laboratories for any further information on infection with EHNV.
The OIE Reference Laboratory can supply purified EHNV DNA, heat killed EHNV antigen and polyclonal antibodies against EHNV together with technical methods.
A fee is charged for the reagents to cover the costs of operating the laboratory.

NB: FIRST ADOPTED IN 1995 AS EPIZOOTIC HAEMATOPOIETIC NECROSIS; MOST RECENT UPDATES ADOPTED IN 2018.

Return to Agenda

CHAPTER 2.3.4.

INFECTION WITH HPR-DELETED OR HPR0 INFECTIOUS SALMON ANAEMIA VIRUS

1. Scope

Infection with infectious salmon anaemia virus (ISAV) means infection with the pathogenic agent highly polymorphic region (HPR)-deleted ISAV, or the non-pathogenic HPR0 (non-deleted HPR) ISAV of the Genus *Isavirus* and Family *Orthomyxoviridae*.

HPR-deleted ISAV may cause disease in Atlantic salmon (*Salmo salar*), which ~~is~~ may progress to a generalised and lethal condition characterised by severe anaemia, and variable haemorrhages and necrosis in several organs.

Detection of HPR0 ISAV has ~~never~~ not been associated with clinical signs of disease in Atlantic salmon (Christiansen *et al.,* 2011). A link between non-pathogenic HPR0 ISAV and pathogenic HPR-deleted ISAV has been suggested, with some disease outbreaks potentially occurring as a result of the emergence of HPR-deleted ISAV from HPR0 ISAV (Cardenas *et al.,* 2014; Christiansen *et al.,* 2017; Cunningham *et al*., 2002; Gagne & Leblanc, 2017; Mjaaland, *et al*., 2002).

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

The morphological, physicochemical and genetic properties of ISAV are consistent with those of the *Orthomyxoviridae,* and ISAV has been classified as the type species of the genus *Isavirus* (Kawaoka *et al*., 2005) within this virus family.

ISAV is an enveloped virus, demonstrating a pleomorphic icosahedral shape, 100–130 nm in diameter, with mushroom shaped surface projections approximately 10 nm long (Falk *et al*., 1997). However, there are studies that indicate greater morphological heterogeneity in cells of epithelial origin (Ramirez & Marshall, 2018). ~~ISAV is an enveloped virus, 100–130 nm in diameter, however, there are studies that indicate greater size heterogeneity in cells of epithelial origin (Ramirez & Marshall, 2018).~~ The virus genome consists of eight single-stranded RNA segments with negative polarity (~~Dannevig *et al.,* 1995~~ Mjaaland *et al*., 1997). The virus has haemagglutinating, receptor-destroying and fusion activity (Falk *et al*., 1997; Mjaaland *et al*., 1997~~; Rimstad *et al*., 2011~~).

~~The morphological, physiochemical and genetic properties of ISAV are consistent with those of the *Orthomyxoviridae,* and ISAV has been classified as the type species of the genus *Isavirus* (Kawaoka *et al*., 2005) within this virus family.~~ The nucleotide sequences of all eight genome segments, encoding at least ten proteins, have been described (Clouthier *et al.,* 2002; Rimstad *et al*., 2011), including the 3' and 5' non-coding sequences (Kulshreshtha *et al.,* 2010; Sandvik *et al.,* 2000). Four major structural proteins have been identified, including a 68 kDa nucleoprotein, a 22 kDa matrix protein, a 42 kDa haemagglutinin-esterase (HE) protein responsible for receptor-binding and receptor-destroying activity, and a 50 kDa surface glycoprotein with putative fusion (F) activity, encoded by genome segments 3, 8, 6 and 5, respectively. Segment 1, 2, and 4 encode the viral polymerases PB2, PB1 and PA. The two smallest genomic segments, segments 7 and 8, each contain two open reading frames (ORF). The ORF1 of segment 7 encodes a protein with type I interferon antagonistic properties, while ORF2 has been suggested to encode a nuclear export protein (NEP: Ramly *et al.*, 2013). The smaller ORF1 of segment 8 encodes the matrix protein, while the larger ORF2 encodes an RNA-binding structural protein also with type I interferon antagonistic properties, and also interact with the host RNAi system (Garcia-Rosado *et al.,* 2008; Thukral *et al.,* 2018).

~~Sequence analysis of various gene segments has revealed differences between isolates both within and between defined geographical areas. According to sequence differences in a partial sequence of segment 6, two groups have been defined: one designated as a European clade and one designated as a North American clade (Gagne & LeBlanc, 2017).~~ In the HE gene, a small HPR near the transmembrane

domain has been identified. This region is characterised by the presence of gaps rather than single-nucleotide substitutions (Cunningham *et al*., 2002; Mjaaland *et al*., 2002). A full-length gene (HPR0) has been suggested to represent a precursor from which all ISAV HPR-deleted (pathogenic) variants of ISAV originate. The presence of non-pathogenic HPR0 ISAV genome has been reported in both apparently healthy wild and farmed Atlantic salmon~~., but has not been detected in~~ Fish with clinical disease and pathological signs consistent with ISA are infected ~~infection~~ with HPR-deleted ISAV (Christiansen *et al*., 2011; Cunningham *et al*., 2002; Markussen *et al*., 2008~~; McBeath *et al*., 2009~~). A mixed infection with HPR-deleted and HPR0 ISAV variants has been reported in the same fish (Cardenas *et al.,* 2014; Kibenge *et al.,* 2009). Recent studies show that HPR0 ISAV variants occur frequently in sea-reared Atlantic salmon (Christiansen *et al.,* 2017). HPR0 ISAV is seasonal and transient in nature and displays a tissue tropism with high prevalence in gills (Christiansen *et al.*, 2011; Lyngstad *et al*., 2011). To date there has been no direct evidence linking the presence of HPR0 ISAV to a clinical disease outbreak. The risk of emergence of pathogenic HPR-deleted ISAV variants from a reservoir of HPR0 ISAV is considered to be low but not negligible (Cardenas *et al.,* 2014; Christiansen *et al.,* 2011; 2017; EFSA, 2012).

Sequence analysis of various gene segments has revealed differences between isolates both within and between defined geographical areas. According to sequence differences in a partial sequence of segment 6, two groups have been defined: one designated as a European clade and one designated as a North American clade (Gagne & LeBlanc, 2017).

In addition to the variations seen in the HPR of the HE gene, other gene segments ~~may also be~~ are of importance for development of clinical disease. A putative virulence marker has been identified in the fusion (F) protein. Here, a single amino acid substitution, or different sequence insertion, near the protein's putative cleavage site has been found to be a prerequisite for virulence (Kibenge *et al.,* 2007; Markussen *et al*., 2008). Aside from insertion/recombination, ISAV also uses gene segment reassortment in its evolution, with potential links to virulence (Cardenas *et al.,* 2014; Devold *et al*., 2006; Gagne & Leblanc, 2017; Markussen *et al*., 2008; Mjaaland *et al*., 2005).

2.1.2. Survival and stability in processed or stored samples

A scientific study concluded that ISAV retains infectivity for at least 6 months at –80°C in tissue homogenates (Smail & Grant, 2012). Isolation in cell culture has been successful even from fish kept frozen whole at –20°C for several years. The experience of diagnostic laboratories has indicated the suitability of general procedures for sample handling (see Chapter 2.3.0) for ISAV.

2.1.3. Survival and stability outside the host

ISAV RNA has been detected by reverse-transcription polymerase chain reaction (RT-PCR) in seawater sampled at from net-pens ~~at farm sites~~ with ISAV-positive Atlantic salmon but not from a sample collected 80–100 metres downstream of the farm (Lovdal & Enger, 2002 ~~Kibenge *et al*., 2004~~). ~~It is difficult to estimate exactly how long the virus may remain infectious in the natural environment because of a number of factors, such as the presence of particles or substances that may bind or inactivate the virus. Exposing cell culture-propagated ISAV suspended in cell culture supernatant to 15°C for 10 days or to 4°C for 14 days had no effect on virus infectivity (Falk *et al*., 1997).~~ A study using natural seawater held at 10°C, whether exposed to UVA and UVB or not, demonstrated that the starting titre of ISA diminished substantially over a period of 72 hours with some indication that infectiousness in an IP challenge model was lost between 3 and 6 hours (Vike *et al*., 2014).

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 ISAV according to Chapter 1.5 of *Aquatic Animal Health Code* (*Aquatic Code*) are: Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*).

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 ISAV according to Chapter 1.5 of the *Aquatic Code* are: Atlantic herring (*Clupea harengus*) and amago trout (*Oncorhynchus masou*).

In addition, pathogen-specific positive RT-PCR results have been reported in the following species, but an active infection has not been demonstrated *in vivo*: Coho salmon (*Oncorhynchus kisutch*).

2.2.3. Non-susceptible species

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

Family	Scientific name	Common name	Reference
Caligidae	*Caligus rogercresseyi*	sea lice	Ito *et al*., 2015
Cyclopteridae	*Cyclopterus lumpus*	lumpfish	Ito *et al*., 2015
Cyprinidae	*Cyprinus carpio*	common carp	Ito *et al*., 2015
Gadidae	*Gadus morhua*	Atlantic cod	MacLean *et al*., 2003; Snow & Raynard, 2005
	Pollachius virens	saithe	Snow *et al*., 2002
	Pollachius virens	pollack	Ito *et al*., 2015
Mytilidae	*Mytilus edulis*	blue mussel	Molloy *et al*., 2014; Skar & Mortensen, 2007
Pleuronectidae	*Hippoglossus hippoglossus*	Atlantic halibut	Ito *et al*., 2015
Salmonidae	*Onchorhynchus tshawytscha*	Chinook salmon	Rolland & Winton, 2003
	Carassius auratus	goldfish	Ito *et al*., 2015

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

In Atlantic salmon, life stages from yolk sac fry to adults are known to be susceptible. Disease outbreaks are mainly reported in seawater cages, and only a few cases have been reported in the freshwater stage, including one case in yolk sac fry (Rimstad *et al*., 2011). Infection with HPR-deleted ISAV has been experimentally induced in both Atlantic salmon fry and parr kept in freshwater.

2.2.5 4. Distribution of the pathogen in the host

There is evidence of the presence of the virus in practically all organs of the fish, as well as in ovarian fluids and ova (Marshall *et al*., 2014), however, the HPR0 variant has a predilection for gills.

HPR-deleted ISAV: Endothelial cells lining blood vessels seem to be the primary target cells for ISAV replication as demonstrated by electron microscopy, immunohistochemistry and *in-situ* hybridisation. Virus replication has also been demonstrated in leukocytes, and sinusoidal macrophages in kidney tissue stain positive for ISAV using immunohistochemistry (IHC). Furthermore, red blood cells may have virus aggregates on the outer cell membrane as indicated by indirect fluorescent antibody test (IFAT) with a monoclonal antibody (MAb) against the HE protein. As endothelial cells support replication and virus may be carried on red blood cells, virus may occur in any organ. Repeated sampling over the course of a chronic infection point to kidney and heart as the organs most likely to become test-positive. Clinical disease and macroscopic organ lesions appear foremost in severely anaemic Atlantic salmon (Aamelfot *et al.,* 2012; McBeath *et al.,* 2015; Rimstad *et al*., 2011).

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

HPR0 ISAV: As HPR0 ISAV has not been isolated in cell culture, controlled, experimental studies on virus distribution within the host are generally lacking. Observed tissue tropism was foremost in the gills when PCR testing was carried out on various organs of Atlantic salmon (Christiansen *et al*., 2011). *In-situ* immunostaining of HPR0 ISAV PCR-positive gills show staining limited to the epithelium indicating replication and shedding to water, rather than invasive infection. Immunostaining was unable to demonstrate HPR0 ISAV infection of internal organs.

2.2.6 5. Aquatic animal reservoirs of infection

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

2.2.~~7~~ 6. Vectors

Transmission of ISAV by salmon lice and sea lice (*Lepeophtheirus salmonis* and *Caligus rogercresseyi;* Oelckers *et al.,* 2014) has been demonstrated under experimental conditions.

2.3. Disease pattern

2.3.1. Mortality, morbidity and prevalence

The disease pattern with HPR-deleted ISAV depends on many factors, including the strain of the virus. During outbreaks of infection with HPR-deleted ISAV, morbidity and mortality may vary greatly between net pens in a seawater fish farm, and between farms (Hammell & Dohoo, 2005). Morbidity and mortality within a net pen may start at very low levels, with typical daily mortality between 0.5 and 1% in affected cages. Without intervention, mortality increases and often peaks in early summer and winter. The range of cumulative mortality during an outbreak is generally insignificant to moderate, but in severe cases, lasting several months, cumulative mortality may exceed 90%. Initially, a clinical disease outbreak may be limited to one or two net pens. In such cases, if affected fish are slaughtered immediately, further development of clinical infection with HPR-deleted ISAV at the site may be prevented. ~~In outbreaks where smolts have been infected in well boats, simultaneous outbreaks on several farms may occur.~~

HPR0 ISAV has not been associated with clinical disease in Atlantic salmon.

2.3.2. Clinical signs, including behavioural changes

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

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

Affected fish are generally in good condition, but diseased fish have no feed in the digestive tract.

2.3.3. Gross pathology

Fish infected with HPR-deleted ISAV may show a range of pathological changes, from none to severe, depending on factors such as infective dose, virus strain, temperature, age and immune status of the fish. No lesions are pathognomonic to infection with HPR-deleted ISAV, but anaemia and circulatory disturbances are always present. The following findings have been described to be consistent with infection with HPR-deleted ISAV, though all changes are seldom observed in a single fish: i) yellowish or blood-tinged fluid in peritoneal and pericardial cavities; ii) oedema of the swim bladder; iii) small haemorrhages of the visceral and parietal peritoneum; iv) focal or diffusely dark red liver (a thin fibrin layer may be present on the surface); v) swollen, dark red spleen with rounded margins; vi) dark redness of the intestinal wall mucosa in the blind sacs, mid- and hind-gut, without blood in the gut lumen of fresh specimens; vii) swollen, dark red kidney with blood and liquid effusing from cut surfaces; and viii) pinpoint haemorrhages of the skeletal muscle.

2.3.4. Modes of transmission and life cycle

The main route of infection is most likely horizontally through the gills for both HPR0 and HPR-deleted ISAV, but infection via the intestine or skin cannot be excluded. Vertical transmission cannot be excluded (Marshall *et al.,* 2014).

ISAV may be shed in skin, mucous, urine, faeces (Totland *et al.,* 1996), ovarian fluid and ova (Marshall *et al*., 2014) ~~but shedding from localised gill infection may be most important~~.

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

2.3.5. Environmental factors

Generally, outbreaks of infection with HPR-deleted ISAV tend to be seasonal, occurring in early summer and winter; however, outbreaks can occur at any time of the year.

2.3.6. Geographical distribution

ISAV was initially reported in Norway in the mid-1980s (Thorud & Djupvik, 1988). It has since been reported in other countries in Europe, North America and South America. ~~The presence of the HPR0 ISAV variant has been reported in all countries where infection with HPR-deleted ISAV has occurred.~~ See WAHIS (https://wahis.oie.int/#/home) for recent information on distribution at the country level.

2.4. Biosecurity and disease control strategies

2.4.1. Vaccination

Vaccination against infection with ISAV has been carried out in North America since 1999 and the Faroe Islands since 2005. In Norway, vaccination is ~~not~~ normally ~~done, but was~~ carried out ~~for the first time in 2009~~ in ~~a~~ regions ~~where~~ with high prevalence of outbreaks ~~were associated with a high rate of infection with HPR-deleted ISAV~~. Chile started vaccinating against infection with ISAV in 2010. However, vaccine efficacy seems insufficient given all cases of both HPR0 and HPR-deleted ISAV that occurred in the Faroe Islands have occurred in vaccinated fish. The same lack of efficacy has been observed in Norway after vaccination around outbreak areas.

2.4.2. Chemotherapy including blocking agents

Chemotherapy is currently not available. However, the broad-spectrum antiviral drug Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) is effective in inhibiting ISAV replication both *in vitro* and *in vivo* (Rivas-Aravena *et al.,* 2011). It should also be noted that interfering peptides have recently been shown to have a non-toxic antiviral effect against ISAV (Cardenas *et al*., 2020).

2.4.3. Immunostimulation

Not applicable.

2.4.4. Breeding resistant strains

Differences in susceptibility among different family groups of Atlantic salmon in freshwater have been observed in challenge experiments and in field tests (Gjoen *et al*., 1997). Breeding companies are using infection trials, family selection and genomic selection to improve ISA resistance, but scientific information on the effect of this on disease incidence or prevalence of subclinical infection is lacking.

2.4.5. Inactivation methods

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

2.4.6. Disinfection of eggs and larvae

Disinfection of eggs according to standard procedures is suggested as an important control measure (see chapter 4.4 of the *Aquatic Code*).

2.4.7. General husbandry

The incidence of infection with ISAV may be greatly reduced by implementation of legislative measures or husbandry practices regarding the movement of fish, mandatory health control, transport and slaughterhouse regulations. Specific measures including restrictions on affected, suspected and neighbouring farms, enforced sanitary slaughtering, generation segregation ('all in/all out') as well as disinfection of offal and wastewater from fish slaughterhouses and fish processing plants may also contribute to reducing the incidence of the disease.

Handling of fish (e.g. sorting or treatment, splitting or moving of cages) may initiate disease outbreaks on infected farms, especially if long-term undiagnosed problems have been experienced (Lyngstad *et al*., 2008).

The experience from the Faroe Islands, where the prevalence of HPR0 ISAV is high, demostrates that the combination of good biosecurity and husbandry substantially reduces the risk of outbreaks of infection with HPR-deleted ISAV (Christiansen *et al.,* 2017).

3. Specimen selection, sample collection, transportation and handling

3.1. Selection of populations and individual specimens

For detection of HPR-deleted ISAV, clinical inspections should be carried out during a period when water temperature is conducive to development of clinical disease (see Section 2.3.5). All production units (ponds, tanks, net-cages etc.) should be inspected and fish displaying clinical signs~~,~~ and gross pathology and anaemia consistent with those described in Sections 2.3.2 and 2.3.3 should be sampled.

For detection of HPR0 ISAV, gills from randomly selected individuals should be sampled at different time points throughout the production cycle.

For the purposes of disease surveillance, fish to be sampled are selected as follows:

i) The most susceptible species should be sampled preferentially (see Section 2.2.3). Other susceptible species listed in Section 2.2.1 should be sampled proportionally.

ii) Risk-based criteria should be employed to preferentially sample lots or populations with a history of abnormal mortality, potential exposure events or where there is evidence of poor water quality or husbandry. If more than one water source is used for fish production, fish from all water sources should be included in the sample.

iii) If weak, abnormally behaving or freshly dead fish are present, such fish should be selected. If such fish are not present (e.g. during surveillance of apparently healthy populations), the fish selected should include normal appearing, healthy fish collected in such a way that all parts of the farm as well as all year classes are proportionally represented in the sample.

For disease outbreak investigations, moribund fish or fish exhibiting clinical signs of infection with ISAV should be collected. Ideally fish should be collected while alive, however recently dead fish can also be selected for diagnostic testing. 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.

3.2. Selection of organs or tissues

3.2.1. Detection of HPR-deleted ISAV

Only internal organs that have not been exposed to the environment should be used for diagnostic testing.

The organs or tissue material to be sampled ~~and examined must be~~ can include: i) for histology: mid-kidney, liver, heart, pancreas, intestine, spleen and gill; ii) for immunohistochemistry: mid-kidney and heart including valves and bulbus arteriosus; iii) for RT-PCR (conventional and real-time) analysis: mid-kidney and heart; and iv) for virus culture: mid-kidney, heart, liver and spleen.

3.2.2. Detection of HPR0 ISAV

Gill tissue is recommended~~, however, HPR0 ISAV has also been detected in the mid-kidney and heart. It is, therefore, suggested to use pools of the three organs for detection purposes~~.

3.3. Samples or tissues not suitable for pathogen detection

Information on samples or tissues not suitable for pathogen detection is lacking; follow recommendations in Section 3.2 for virus detection.

3.4. Non-lethal sampling

Blood is preferred for non-lethal sampling for HPR-deleted ISAV based on a study by Giray *et al*. (2005) in which blood and mucus was compared with kidney samples derived from both infected fish with or without clinical signs ~~clinical and non-clinical fish~~ and tested by RT-PCR and virus isolation in cell culture.

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~~ depends 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. To avoid degradation of samples, use alternative storage methods only after consultation with the receiving laboratory.

3.5.2. Preservation of samples for molecular detection

Tissue samples for real-time RT-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 based on studies in terrestrial animal and human health. The use of lower grade (laboratory or industrial grade) ethanol is not recommended. If material cannot be fixed it may be frozen. Commercial RNA preservatives are available, ~~such as RNAlater,~~ which have better efficacy than ethanol at room temperature. Commercial fixatives validated to be at least as effective as the fixatives described above may be used.

3.5.3. Samples for histopathology, immunohistochemistry or *in-situ* hybridisation

Tissue samples for histopathology should be fixed immediately after collection. Gills need to be fixed immediately after euthanasia. Thickness of tissues for fixation must not exceed 4–5 mm. The recommended ratio of fixative to tissue is 10:1, and neutral, phosphate-buffered, 10% formalin is recommended as this fixative is compatible with the immunohistochemistry procedure for ISAV.

3.5.4. Samples for electron microscopy

ISAV has been characterised by transmission electron microscopy (TEM) using general procedures (Falk *et al*., 1997).

3.5.5. Samples for other tests

At present, other tests, for example serology tests, are not used for diagnostic purposes.

3.6. Pooling of samples

Pooling of samples from more than one individual animal for a given purpose should only be recommended where robust supporting data on diagnostic sensitivity and diagnostic specificity have been evaluated and found to be suitable. If the effect of pooling on diagnostic sensitivity has not been thoroughly evaluated, larger fish should be processed and tested individually. ~~Data are available regarding the effect of pooling samples on the detection of ISAV that indicate the effects are related to the prevalence of the disease in the fish population (Hall *et al*., 2013; 2014).~~ Small life stages such as fry ~~or specimens up to 0.5 g~~ can be pooled to provide the minimum amount of material needed for testing. If pooling is used, it is recommended to pool organ pieces from a maximum of five fish.

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 apparently healthy animals and investigation of clinically affected animals*

Method	A. Surveillance of apparently healthy animals				B. Presumptive diagnosis of clinically affected animals				C. Confirmatory diagnosis[1] of a suspect result from surveillance or presumptive diagnosis			
	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV
Gross signs					+	+	+	1				
Histopathology[3]					++	++	++	1				
Cell ~~or artificial media~~ culture					++	++	++	1	+++	+++	+++	NA
Real-time RT-PCR	+++	+++	+++	1	+++	+++	+++	3				
~~Conventional~~ RT-PCR	+	+	+	1	++	++	++	1	+	+	+	NA
Amplicon sequencing[4]									+++	+++	+++	NA
***In-situ* hybridisation**												
Immunohistochemistry					++	++	++	1	++	++	++	NA
IFAT on kidney imprints or blood smears					++	++	++	1	+++	+++	+++	NA
Bioassay												
LAMP												
Ab-ELISA												
Ag-ELISA												
Other antigen detection methods[5]												

LV = level of validation, refers to the stage of validation in the OIE Pathway (chapter 1.1.2); NA = not applicable; RT-PCR = reverse-transcription polymerase chain reaction; LAMP = loop-mediated isothermal amplification; Ab- or Ag-ELISA = antibody or antigen enzyme-linked immunosorbent assay, respectively.

[1]For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). [2]Susceptibility of early and juvenile life stages ~~have been defined~~ is described in Section 2.2.3.

[3]Histopathology and cytopathology can be validated if the results from different operators have been statistically compared. [4]Sequencing of the PCR product.

Shading indicates the test is inappropriate or should not be used for this purpose.

4.1. Wet mounts

Not applicable.

4.2. Histopathology and cytopathology

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

i) Numerous erythrocytes in the central venous sinus and lamellar capillaries where erythrocyte thrombi also form in the gills.

ii) Multifocal to confluent haemorrhages and/or hepatocyte necrosis at some distance from larger vessels in the liver. Focal accumulations of erythrocytes in dilated hepatic sinusoids.

iii) Accumulation of erythrocytes in blood vessels of the intestinal lamina propria and eventually haemorrhage into the lamina propria.

iv) Spleen stroma distended by erythrocyte accumulation.

v) Slight multifocal to extensive diffuse interstitial haemorrhage with tubular necrosis in the haemorrhagic areas, erythrocyte accumulation in the glomeruli in the kidney.

vi) Erythrophagocytosis in the spleen and secondary haemorrhages in liver and kidney.

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

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

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

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

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

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

For virus isolation, cells grown in 25 cm^2 tissue culture flasks or multi-well cell culture plates, which may be sealed with parafilm or a plate sealer to stabilise the pH of the medium, may be used. Cells grown in 24-well plates may not grow very well into monolayers, but this trait may vary between laboratories and according to the type of cell culture plates used. Serially diluted HPR-deleted ISAV-positive controls should be inoculated in parallel with the tissue samples as a test for cell susceptibility to HPR-deleted ISAV (this should be performed in a separate location from that of the test samples). See Chapter 2.3.0 for the methods used for inoculation of cell monolayers, monitoring the cultures and sub-cultivation.

Inoculated cell cultures are incubated for at least 14 days and examined as described in Chapter 2.3.0. At the end of the incubation period, or earlier if obvious CPE appears, the medium is collected for virus identification by immunofluorescence (IFAT) (see Section 4.9), real-time PCR or conventional PCR (see Sections 4.4.1 .and 4.4.2) as virus replication may occur without apparent CPE.

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

Cell lines should be monitored to ensure that their susceptibility to targeted pathogens has not changed.

4.4. Nucleic acid amplification

4.4.1. Real-time RT-PCR

The primers and probes shown in Table 4.4.1.1 for real-time RT-PCR will detect both European and North-American HPR-deleted ISAV and HPR0 ISAV. Real-time RT-PCR may be used for detection of ISAV from total RNA (or total nucleic acid) extracted from recommended organs/tissues (see Section 3.2) and is recommended over RT-PCR (see Section 4.4.2.) as it has increased specificity and, probably, also sensitivity. The primer sets derived from genomic segment 8 and segment 7 have been used by several laboratories and have been found suitable for detection of ISAV during disease outbreaks and in apparently healthy carrier fish.

With the widespread occurrence of HPR0 ISAV variants, it is essential to follow up any positive RT-PCR results based on segment 7 or 8 primer sets by ~~sequencing~~ sequence analysis of the HPR ~~of~~ in segment 6 in order to determine if the ~~isolate~~ virus is either HPR-deleted or HPR0 ISAV or both. Primers, designed and validated by the OIE Reference Laboratory, are given in Table 4.4.2.1. Validation of the HPR primer set for the North American HPR0 isolates is restricted by the limited sequence data available in the Genbank for the 3' end of ISAV segment 6 (Marshall *et al.,* 2014).

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

Table 4.4.1.1. *Primer and probes sequences and cycling conditions for ISAV real-time RT-PCR*

Primer and probe sequences (5'–>3') (concentration)	Cycling conditions	Genomic segment	Amplicon size (bp)	Reference
For: CAG-GGT-TGT-ATC-CAT-GGT-TGA-AAT-G (900nM) Rev: GTC-CAG-CCC-TAA-GCT-CAA-CTC- (900nM) Probe: *6FAM*-CTC-TCT-CAT-TGT-GAT-CCC-*MGBNFQ* (250nM)	1 × 2 minutes @ 50°C 1 × 10 minutes @ 95°C 45 × 15 seconds @ 95°C and 1 minute @ 60°C	7	155	Snow *et al*., 2006
For: CTA-CAC-AGC-AGG-ATG-CAG-ATG-T (900 nM) Rev: CAG-GAT-GCC-GGA-AGT-CGA-T (900 nM) Probe: *6FAM*-CAT-CGT-CGC-TGC-AGT-TC-*MGBNFQ* (250 nM)		8	104	Snow *et al.,* 2006

The following controls should be run with each assay: negative extraction control; positive control; no template control; internal RT-PCR control. The positive control should be distinguishable from viral genomic sequence, thus allowing detection of any cross-contamination leading to false positive results.

4.4.2. ~~Conventional~~ RT-PCR

The primers described in Table 4.4.2 for RT-PCR will detect both European and North-American HPR-deleted ISAV and HPR0 ISAV. RT-PCR may be used for detection of ISAV from total RNA (or total nucleic acid) extracted from recommended organs/tissues (see Section 3.2). However, the real-time RT-PCR (see Section 4.4.1.) for the detection of ISAV is recommended as it has increased specificity and, probably, also sensitivity.

Table 4.4.2.1. *Primer sequences and cycling conditions for ISAV Segment 6 RT-PCR*

Primer sequences (5'–>3') (concentration)	Cycling conditions	Amplicon size (bp)	Reference
For: GAC-CAG-ACA-AGC-TTA-GGT-AAC-ACA-GA (200 nM) Rev: GAT-GGT-GGA-ATT-CTA-CCT-CTA-GAC-TTG-TA (200 nM)	1 × 30 minutes @ 50°C 1 × 2 minutes @ 94°C 40 × 1 minute @ 94°C, 1 minute @ 50°C, 1 minute @ 68°C 1 × 7 minutes @ 68°C	304 if HPR0	Designed by OIE Ref. Lab.

~~With the widespread occurrence of HPR0 ISAV variants, it is essential to follow up any positive PCR results based on segment 7 or 8 primer sets by sequencing s the HPR of in segment 6 in order to determine if the isolate is either HPR-deleted or HPR0 ISAV or both. Primers, designed and validated by the OIE Reference Laboratory, are given in Table 4.4.2. Validation of the HPR primer set for the North-American HPR0 isolates is restricted by the limited sequence data available in the Genbank for the 3' end of ISAV segment 6.~~

~~The primers for segment 7 and 8 may also be used for conventional RT-PCR if necessary.~~

~~The following controls should be run with each assay: negative extraction control; positive control; no template control; internal PCR control. The positive control should be distinguishable from viral genomic sequence, thus allowing detection of any cross-contamination leading to false positive results.~~

4.5. Amplicon sequencing

There is evidence of the generation of complete amplicons for the eight segments of the viral genome that include the 5' and 3' ends of each one (Toro-Ascuy *et al.,* 2015).

The segment 6 assay primers given in Section 4.4.2 are used for RT-PCR and amplicon sequencing.

4.6. *In-situ* hybridisation

Published methods are available but not recommended due to lack of validation.

4.7. Immunohistochemistry (IHC)

4.7.1. IHC on paraffin sections from formalin-fixed tissue

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

i) Preparation of tissue sections

The tissues are fixed in neutral phosphate-buffered 10% formalin for at least 1 day, dehydrated in graded ethanol, cleared in xylene or isopropanol and embedded in paraffin, according to standard protocols. Approximately 3 µm thick sections (for IHC sampled on poly-L-lysine-coated slides) are heated at 56–58°C (maximum 60°C) for at least 20 minutes, dewaxed in xylene, rehydrated through graded ethanol, and stained with haematoxylin and eosin for pathomorphology and IHC as described below.

ii) Staining procedure for IHC

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

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

b) Sections are then incubated overnight at 4°C with primary antibody (~~monospecific rabbit~~ e.g. an antibody against ISAV nucleoprotein) diluted in TBS with 1% non-fat dry milk, followed by three washes in TBS, the last wash with 0.1% Tween 20.

c) For detection of bound antibodies, sections are incubated with biotinylated ~~goat anti-rabbit~~ species specific IgG (diluted ~~1/200~~ in 2.5% BSA in Tris buffer) for 60 minutes, followed by ABC-AP (diluted ~~1/100~~ in Tris buffer) for 45 minutes. Following a final wash, Fast Red (1 mg ml–1) and Naphthol AS-MX phosphate (0.2 mg ml^{-1}) with 1 mM Levamisole in 0.1 M TBS (pH 8.2) are added to develop for 20 minutes. Sections are then washed in tap water before counterstaining with Harris haematoxylin and mounted in aqueous mounting medium. ISAV positive and ISAV negative tissue sections are included as controls in every setup.

iii) Interpretation

Negative control sections should not have any significant colour reactions. Positive control sections should have clearly visible red-coloured cytoplasmic and intranuclear staining of endothelial cells in blood vessels or heart endocardium. A test sample section should only be regarded as positive if clear, intranuclear red staining of endothelial cells is found. The intranuclear localisation is particular

to the orthomyxovirus nucleoprotein during a stage of virus replication. Concurrent cytoplasmic staining is often dominant. Cytoplasmic and other staining patterns without intranuclear localisation must be considered as nonspecific or inconclusive.

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

4.7.1 ~~2~~. ~~Indirect fluorescent antibody test~~ IFAT on tissue imprints and blood smears

An indirect fluorescent antibody test (IFAT) using validated MAbs against ISAV haemagglutinin-esterase (HE) on kidney ~~smears (~~imprints~~)~~, on blood smears or on frozen tissue sections of kidney, heart and liver has given positive reactions in both experimentally and naturally infected Atlantic salmon. Suspect cases (see Section 6.1) may be confirmed with a positive IFAT.

i) Preparations of tissue ~~smears (~~imprints~~)~~

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

ii) Staining procedure

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

iii) Preparation of blood smear ~~(imprint)~~

Blood fraction is obtain using a discontinuous Percoll gradient. A small fraction is smeared on poly-L-lysine-coated microscope slide. The ~~imprint~~ smear is air-dried, fixed in chilled 100% acetone for 10 minutes and stored either at 4°C for a few days or at –80°C until use.

iv) Staining procedure

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

4.8. Bioassay

Not available.

4.9. Antibody- or antigen-based detection methods

4.9.1. Virus identification by IFAT

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

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

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

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

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

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

4.10. Other methods

None published or validated.

5. Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations

Real-time RT-PCR is validated for surveillance to demonstrate freedom in apparently healthy populations.

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[4]

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.

6.1.1. Definition of suspect case in apparently healthy animals

The presence of infection with HPR0 or HPR-deleted ISAV shall be suspected if at least one of the following criteria is met:

~~i) ISAV-typical CPE in cell cultures (HPR-deleted only)~~

ii) Positive result by ~~conventional~~ RT-PCR

iii) Positive result by real-time RT-PCR

6.1.2. Definition of confirmed case in apparently healthy animals

[4] For example transboundary commodities.

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.

Definition of confirmed case of infection with HPR-deleted ISAV

The presence of infection with HPR-deleted ISAV is considered to be confirmed if, in addition to the criteria in Section 6.1.1, at least one ~~or more~~ of the following ~~criteria~~ points ~~are~~ is met:

~~i) ISAV-typical CPE in ASK cell culture and virus identification by by conventional RT-PCR and sequencing of the HE-gene to verify HPR-deletion~~

~~ii~~) Detection of ISAV in tissue ~~preparations~~ samples by ~~conventional~~ RT-PCR (conventional or real-time) and detection of ISAV in histological sections by immunoassay using specific anti-ISAV antibodies (IFAT or immunohistochemistry)

~~iii~~) Detection of ISAV in tissue ~~preparations~~ samples by ~~real-time~~ RT-PCR (conventional or real-time) and ~~detection of ISAV in tissue preparations by~~ conventional PCR of segment 6 ~~followed by~~ and sequencing of the ~~HE-gene~~ amplicon to verify HPR-deletion

iii ~~iv~~) Detection of ISAV in tissue samples by real-time RT-PCR and detection of ISAV in histological sections by immunoassay using specific anti-ISAV antibodies (IFAT or immunohistochemistry)

~~v) Detection of ISAV in tissue preparations by real-time RT-PCR and ISAV-typical CPE in cell culture followed by virus identification by conventional RT-PCR and sequencing of the amplicon~~

~~vi) Detection of ISAV in tissue preparations by conventional PCR followed by sequencing of the amplicon~~

Definition of confirmed case of infection with HPR0 ISAV

The presence of infection with HPR0 ISAV is considered to be confirmed if the following criterion is met:

i) Detection of ISAV in tissue samples by real-time RT-PCR and detection of ISAV by conventional RT-PCR of segment 6 ~~followed by amplification~~ and sequencing of the ~~HE-gene of segment 6~~ amplicon to verify HPR0-deletion

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 with HPR-deleted ISAV 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) Histo- or cytopathological changes consistent with the presence of the pathogen or the disease

iii) ISAV-typical CPE in ~~ASK~~ cell culture

iv) Positive result by a real-time RT-PCR

v) Positive result of a conventional RT-PCR

vi) Positive result by immunohistochemistry

vii) Positive result by IFAT on tissue imprints

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with HPR-deleted ISAV is considered to be confirmed if at least one ~~or more~~ of the following criteria is met:

~~i) ISAV-typical CPE in ASK cell culture and virus identification by by conventional RT-PCR and sequencing of the HE-gene to verify HPR-deletion~~

i) Virus isolation with ISAV-typical CPE in cell culture and virus identification by RT-PCR (conventional or real-time) followed by sequencing of the amplicon

ii) Detection of ISAV in tissue ~~preparations~~ samples by ~~conventional~~ RT-PCR (conventional or real-time) and detection of ISAV in histological sections by immunoassay using specific anti-ISAV antibodies (IFAT or immunohistochemistry)

iii) Detection of ISAV in tissue ~~preparations~~ samples by ~~real-time~~ RT-PCR (conventional or real-time) and ~~followed by~~ conventional RT-PCR of segment 6 and sequencing of the ~~HE-gene~~ amplicon to verify HPR-deletion

~~iv) Detection of ISAV in tissue preparations by real-time RT-PCR and detection of ISAV in tissue preparations by means of specific antibodies against ISAV (IFAT or immunohistochemistry)~~

~~v) Detection of ISAV in tissue preparations by real-time RT-PCR and ISAV-typical CPE in cell culture followed by virus identification by conventional RT-PCR and sequencing of the amplicon~~

~~vi) Detection of ISAV in tissue preparations by conventional 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

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with ISAV are provided in Table 6.3. This information can be used for the design of surveys for infection with ISAV, however, it should be noted that diagnostic performance is specific to the circumstances of each diagnostic accuracy study (including the test purpose, source population, tissue sample types and host species) and diagnostic performance may vary under different conditions. Data are only presented where tests are validated to at least level two of the validation pathway described in Chapter 1.1.2. and the information is available within published diagnostic accuracy studies.

6.3.1. For presumptive diagnosis of clinically affected animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation
Cell Culture	Diagnosis	Clinically diseased Atlantic salmon from farm	Gills, Kidney, and heart	*Salmo salar*	Non-available	Non-available	Real-time RT-PCR	Dannevig *et al.,* 1995

DSe: = diagnostic sensitivity, DSp = diagnostic specificity, *n* = number of samples used in the study,
PCR: = polymerase chain reaction; NA = not available.

6.3.2. For surveillance of apparently healthy animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation
Real-time PCR	Surveillance	Salmonids	Gills, Kidney, and heart	*Salmo salar* and other salmonids	Non available	Non available	Cell culture	Snow *et al.,* 2006

DSe: = diagnostic sensitivity, DSp = diagnostic specificity, *n* = number of samples used in the study,
PCR: = polymerase chain reaction.

7. References

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

NB: FIRST ADOPTED IN 1995 AS INFECTIOUS SALMON ANAEMIA; MOST RECENT UPDATES ADOPTED IN 2018.

Return to Agenda

CHAPTER 2.3.6.

INFECTION WITH KOI HERPESVIRUS

1. Scope

Infection with koi herpesvirus (KHV) means infection with all genotypes of the pathogenic agent cyprinid herpesvirus-3 (CyHV-3), of the Genus *Cyprinivirus* in the Family *Alloherpesviridae* (Engelsma *et al.,* 2013; Haramoto *et al*., 2007; Waltzek *et al.,* 2009). However, for familiarity, the abbreviation KHV will be used in this chapter.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

KHV, also known as carp interstitial nephritis and gill necrosis virus (CNGV) (Ilouze *et al.,* 2010), has been classified as cyprinid herpesvirus-3 (CyHV-3) following the nomenclature of other cyprinid herpesviruses: CyHV-1 (carp pox virus, fish papilloma virus) and CyHV-2 (goldfish haematopoietic necrosis virus). Analysis of the complete genome has shown that CyHV-3 is closely related to CyHV-1, CyHV-2, anguillid herpesvirus-1 (AngHV-1) and distantly related to channel catfish virus (Ictalurid herpesvirus: IcHV-1) and Ranid (frog) herpesvirus (RaHV-1) (Waltzek *et al.,* 2005). CyHV-3 was designated the type species of the new *Cyprinivirus* genus within the *Alloherpesviridae* family, that also contains CyHV-1 and CyHV-2. However, the designation KHV has been retained in the *Aquatic Code* and *Aquatic Manual* for reasons of continuity and is used here synonymously with CyHV-3.

Early estimates of the genome size of KHV varied from at least 150 kbp to 277 kbp; the size is now confirmed as 295 kbp. Virus nucleocapsids have been measured at 100–110 nm in diameter and are surrounded by an envelope (review: Ilouze *et al.,* 2010). The enveloped virions range in size from 170 to 230 nm in the different infected cell types (Hedrick *et al.,* 2000; Miwa *et al.,* 2007;Miyazaki *et al,* 2008). Aoki *et al.* (2007) initially described the complete genome sequence of three isolates of ~~CyHV-3~~ KHV and the genome includes 164 open reading frames (ORFs) as well as 156 unique protein-coding genes. They suggested that the finding that 15 KHV genes are homologous with genes in IcHV-1 confirms the proposed place of KHV in the family Herpesviridae. Forty viral proteins and 18 cellular proteins are incorporated into mature virions.

2.1.2. Survival and stability in processed or stored samples

No information available.

2.1.3. Survival and stability outside the host

Studies in Israel have shown that KHV remains viable in water for at least 4 hours, but less than 21 hours, at water temperatures of 23–25°C (Perelberg *et al.,* 2003). Studies in Japan have shown a significant reduction in the infectious titre of KHV within 3 days in river or pond water or sediment samples at 15°C. However, KHV remained infective for >7 days when kept in environmental water samples that had been sterilised by autoclaving or filtration (Shimizu *et al.,* 2006).

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with KHV according to Chapter 1.5 of *Aquatic Animal Health Code* (*Aquatic Code*) are: all varieties and subspecies of common carp (*Cyprinus carpio*), and common carp~~/goldfish~~ hybrids (e.g. *Cyprinus carpio* × *Carassius auratus, Cyprinus carpio* × *Carassius carassius*).

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is insufficient evidence to fulfil the criteria for listing as susceptible to infection with KHV according to Chapter 1.5 of the *Aquatic Code* are: Goldfish (*Carassius auratus*), grass carp (*Ctenopharyngodon idella*) and Crucian carp (*Carassius carassius*).

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

Family	Scientific name	Common name
Acipenseridae	*Acipenser gueldenstaedtii*	Atlantic sturgeon
	Acipenser ruthenus × *Huso huso*	hybrid sterlet × beluga
	Acipencer oxyrinchus	Russian sturgeon
Cyprinidae	*Leuciscus idus*	blue back ide
	Rutilus rutilus	common roach
	Tinca tinca	tench
	Hypophthalmichthys molitrix	silver carp
Gammaridae	*Gammarus pulex*	scud (crustacean)
Nemacheilidae	*Barbatula barbatula*	stone loach
Percidae	*Gymnocephalus cernuus*	Eurasieans ruffe
	Perca fluviatilis	European perch
~~Salmonidae~~	~~*Oncorhynchus mykiss*~~	~~rainbow trout~~
Unionidae	*Anodonta cygnea*	swan mussel

2.2.3. ~~Non-susceptible species~~

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

~~Family~~	~~Scientific name~~	~~Common name~~
~~Agamidae~~	~~*Intellagama lesueurii*~~	~~Eastern water dragon~~
~~Ambassidae~~	~~*Ambassis agassizii*~~	~~olive perchlet~~
~~Anguillidae~~	~~*Anguilla australis*~~	~~short-finned eel~~
~~Ariidae~~	~~*Neoarius graeffei*~~	~~salmon catfish~~
~~Chelidae~~	~~*Emydura macquarii*~~	~~Macquarie short-necked turtle~~
~~Clupeidae~~	~~*Nematalosa erebi*~~	~~bony bream~~
~~Eleotridae~~	~~*Hypseleotris* sp.~~	~~carp gudgeon~~
~~Galaxiidae~~	~~*Galaxias maculatus*~~	~~common galaxias~~
~~Limnodynastidae~~	~~*Limnodynastes tasmaniensis*~~	~~spotted marsh frogs~~
~~Melanotaeniidae~~	~~*Melanotaenia duboulayi*~~	~~crimson-spotted rainbowfish~~
~~Mordaciidae~~	~~*Mordacia mordax*~~	~~short-headed lamprey ammocoetes~~
~~Mugilidae~~	~~*Mugil cephalus*~~	~~sea mullet~~
~~Parastacidae~~	~~*Cherax destructor*~~	~~common yabby~~
~~Pelodryadidae~~	~~*Litoria peronii*~~	~~Peron's tree frog~~
~~Percichthyidae~~	~~*Maccullochella peelii*~~	~~Murray cod~~
	~~*Macquaria ambigua*~~	~~golden perch~~
~~Plotosidae~~	~~*Tandanus tandanus*~~	~~eel-tailed catfish~~
~~Retropinna~~	~~*Retropinna semoni*~~	~~Australian smelt~~
~~Terapontidae~~	~~*Bidyanus bidyanus*~~	~~silver perch~~

2.2.4 ~~3~~. Likelihood of infection by species, host life stage, population or sub-populations

For the purposes of Table 4.1, larvae and fry up to approximately 1 g in weight may be considered to be early life stages, fingerlings and grower fish up to 250 g may be considered to be juveniles, and fish above 250 g may be considered to be adults.

All age groups of fish, from juveniles upwards, appear to be susceptible to infection with KHV but, under experimental conditions, 2.5–6 g fish were more susceptible than 230 g fish (Perelberg *et al.,* 2003). Carp larvae appear to be tolerant to infection with KHV.

Common carp or varieties, such as koi or ghost (koi × common) carp, are most susceptible and should be preferentially selected for virus detection, followed by any common carp hybrids, such as goldfish × common carp or crucian carp × common carp. Experimental challenges studies by Ito *et al.,* 2014a; 2014b, demonstrated that mortality due to infection with KHV was higher in indigenous Japanese carp (95–100%) compare with domesticated common carp and koi carp, where mortality varied from 30% to 95% and from 35% to 100%, respectively.

2.2.5 ~~4~~. Distribution of the pathogen in the host

Gill, kidney, gut and spleen are the organs in which KHV is most abundant during the course of clinical disease (Gilad *et al.,* 2004). In fish surviving experiment challenge by immersion, KHV DNA was more likely to be detected from the caudal fin and brain compared with gill and kidney (Ito *et al*., 2014b).

2.2.~~6~~ 5. Aquatic animal reservoirs of infection

There is evidence to indicate that survivors of infection with KHV may become persistently infected with virus and may retain the virus for long periods without expression of clinical signs of infection. The virus has been shown to persist in common carp experimentally infected at a permissive temperature and subsequently maintained at a lower than permissive temperature (St-Hilaire *et al.,* 2005). Researchers in Japan conducted a PCR and serological survey of ~~CyHV-3~~ KHV in Lake Biwa in 2006, where episodic outbreaks of infection with KHV had been reported in the 2 years following a major outbreak in 2004. Further analysis of the surviving population showed that 54% of the older carp were seropositive and 31% PCR positive. The maintenance of high levels of antibody to the virus suggests that latent virus may be reactivating periodically in some animals, leading to excretion and a low level of virus circulation in the population, which boosts herd immunity.

2.2.~~7~~ 6. Vectors

No species of vector have been demonstrated to transmit KHV to susceptible species. Studies in Japan have however, reported the detection of ~~CyHV-3~~ KHV DNA in plankton samples and, in particular, Rotifera species ~~Plankton samples were collected in 2008 from Iba-naiko, a shallow lagoon connected to Lake Biwa, a favoured carp spawning area~~ (Minamoto *et al*., 2011). ~~Statistical analysis revealed a significant positive correlation between CyHV-3 in plankton and the numbers of Rotifera and the authors suggested that CyHV-3 binds to or is concentrated by the filter feeding behaviour of Rotifera species. In an earlier report of a study in Poland, CyHV-3~~ KHV ~~was~~ has also been detected by PCR in swan mussels (*Anodonta cygnea*) and freshwater shrimp (*Gammarus pulex*) (Kielpinski *et al*., 2010). ~~The invertebrates were collected from ponds in Southern Poland where outbreaks had occurred in common carp populations over 5 to 6 years. More work is needed to determine how long the infectious virus persists and remains viable in the invertebrates in the absence of the host species.~~

2.3. Disease pattern

2.3.1. Mortality, morbidity and prevalence

The clinical signs of infection may become apparent 3–21 days after naïve fish have been introduced to a pond containing infected fish (Bretzinger *et al.,* 1999; Hedrick *et al*., 2000). Morbidity of affected populations can be 100%, and mortality 70–100% (Bretzinger *et al.,* 1999; Haenen *et al.,* 2004). However, in several experiments, differential resistance to infection with KHV among common carp strains was reported (Dixon *et al.*, 2009; Ito *et al*., 2014a; Shapira *et al*., 2005). In these reports, the cumulative mortalities of the most resistant strains were approximately 40%. Secondary and concomitant bacterial or parasitic infections are commonly seen in diseased carp and may affect both the mortality rate and clinical signs of infection (Haenen *et al.,* 2004).

2.3.2. Clinical signs, including behavioural changes

During an outbreak of infection with KHV there will be a noticeable increase in mortality in the population. All age groups of fish, except larvae, appear to be susceptible to infection with KHV, although, under experimental infection, younger fish (up to 1 year of age) are more susceptible to infection. Changes to the skin are also commonly observed and include: focal or total loss of epidermis, irregular patches of pale colouration or reddening, excessive or reduced mucous secretion (on skin or gills) and sandpaper-like skin texture. Other clinical signs include enophthalmia (sunken eyes) and haemorrhages on the skin and base of the fins, and fin erosion.

Fish become lethargic, separate from the shoal and gather at the water inlet or sides of a pond and gasp at the surface of the water. Some fish may experience loss of equilibrium and disorientation, but others may show signs of hyperactivity.

2.3.3. Gross pathology

There are no pathognomic gross lesions. However, the most consistent gross pathology is seen in the gills, which can vary in extent from pale necrotic patches to extensive discolouration, severe necrosis and inflammation. Internal lesions are variable in occurrence and often absent in cases of sudden mortality. Other gross pathologies that have been reported include adhesions in the abdominal cavity, with or without abnormal colouration of internal organs (lighter or darker). The kidney or liver may be enlarged, and they may also exhibit petechial haemorrhages. Co-infections, for example with ectoparasites such as gill monogeneans, may alter the observed gross pathology.

2.3.4. Modes of transmission and life cycle

Virus is shed via faeces, urine, gills and skin and the main mode of transmission of KHV is horizontal. Early reports suggested that the gills are the major portal of virus entry in carp (Dishon *et al.,* 2005; Gilad *et al.,* 2004; Pikarsky *et al*., 2004).

However, a more recent experimental study has demonstrated that the skin covering the fins and body of the carp is the major portal of entry for KHV (Costes *et al.,* 2009). Another study has shown that KHV DNA was detected in two of three fish from the caudal fin and gill, and caudal fin and spleen one day after exposure to sub-clinically infected fish (Ito *et al*., 2014a; 2014b). The virus spreads systemically from main points of entry to the internal organs; high levels of KHV DNA have been detected in kidney, spleen, liver and gut tissue (Dishon *et al.,* 2005; Pikarsky *et al*., 2004). The assembly and morphogenesis of KHV in infected cells is the same as other herpesviruses (Miwa *et al.*, 2007). An ultrastructural examination of experimentally infected carp has provided evidence for immature capsids and mature nucleocapsid assembly in the nucleus and further maturation of the virion in the cytoplasm of infected cells. Hyper-secretion of mucous is very evident in the early stages of infection with KHV and KHV DNA has been detected at high levels in mucous sampled from experimentally infected carp (Gilad *et al.,* 2004). This is further evidence for active involvement of the skin in viral pathogenesis and an important site of virus shedding. Excretion of virus via urine and faeces may also be an important mechanism for virus shedding; infectious virus has been detected in faeces sampled from infected carp (Dishon *et al.,* 2005; Gilad *et al*., 2004).

2.3.5. Environmental factors

Disease patterns are influenced by water temperature, virulence of the virus, age, population genetics and condition of the fish, population density and stress factors (e.g. transportation, spawning, poor water quality). The disease is temperature dependent, occurring mainly between 16 and 29°C (Haenen *et al.,* 2004; Hedrick *et al*., 2000; Perelberg *et al.,* 2003; Sano *et al.,* 2004). Under experimental conditions, infectious virus was continually shed for a longer period from infected common carp at 16°C than those kept at 23°C or 28°C (Yuasa *et al.,* 2008). However, experimental challenge resulted in high mortality at 28°C but not at 29°C or 30°C, nor at 13°C (Gilad *et al.,* 2004; Ilouze *et al.,* 2010) (optimal temperature range for viral replication may vary with the virus strain).

2.3.6. Geographical distribution

Following the first reports of infection with KHV in Israel and Germany in 1998 and detection of KHV DNA in tissue samples taken during a mass mortality of carp in the UK in 1996, the geographical range of the disease has become extensive and includes most continents, including Europe, Asia, the Middle East, Southern Africa, and North America.

See WAHIS (https://wahis.oie.int/#/home) for recent information on distribution at the country level.

2.4. Biosecurity and disease control strategies

2.4.1. Vaccination

A safe and effective commercial vaccine is not currently widely available. However, live attenuated virus has been used to vaccinate carp. The vaccine preparation induced antibody against the virus and the duration of the protection was at least 8 months (Ilouze *et al.,* 2010). The vaccine was licensed for emergency use in Israel and has been widely used in carp farms across the country. Various vaccine candidates against KHV have been developed. Results of studies in Japan have shown that oral administration of a liposome-based vaccine containing inactivated KHV was also effective in protecting carp against clinical disease (reviewed by Ilouze *et al.,* 2010). A vaccine candidate based on the double deletion of ORF56 and ORF57 was produced using BAC cloning technology, and the effectiveness of attenuated recombinant vaccines has been demonstrated in experimental challenge experiments (Boutier *et al.*, 2015). The DNA vaccines consisting of plasmids encoding ORF25, ORF81 and ORF 149 showed efficient results under lab conditions (Hu *et al.,* 2020; Zhou *et al.,* 2014a; 2014b;).

2.4.2. Chemotherapy including blocking agents

Chemotherapy is not currently available, however, the antiviral activity of exopolysaccharides against KHV *in vitro* has been reported (Reichert *et al.*, 2017).

2.4.3. Immunostimulation

There is currently no published information on the use of immunostimulants to control infection with KHV in carp. However, it is known to be an area of research interest (Reichert *et al.,* 2017).

2.4.4. Breeding resistant strains

Differential resistance to infection with KHV, but not to virus entry, has been shown among different carp strains (Dixon *et al.,* 2009; Ito *et al*., 2014a; 2014b; Shapira *et al.,* 2005). The progeny of crosses of two strains of domesticated carp and one strain of wild carp were challenged by experimental or natural infection. The lowest survival rate was approximately 8% but the survival rate of the most resistant strain was 60.7% for experimental exposure and 63.5% for natural exposure in ponds (Shapira *et al.,* 2005). In a more recent resistance study, 96 families derived from di-allele crossing of four European/Asian strains of common carp were experimentally challenged with KHV. Survival rates of the five most resistant crosses in the final virus challenge trial ranged from 42.9 to 53.4% (Dixon *et al.,* 2009).

2.4.5. Inactivation methods

The virus is inactivated by UV radiation at a dose of 4.0×10^3 µ Ws/cm^2, temperatures above 50°C for 1 minute and by iodophor (200 mg litre^{-1}) treatment for 30 seconds at 15°C ~~(Kasai *et al*., 2005)~~. The following disinfectants are also effective for inactivation: iodophor at 200 mg litre^{-1} for 20 minutes, benzalkonium chloride at 60 mg litre^{-1} for 20 minutes, ethyl alcohol at 30% for 20 minutes and sodium hypochlorite at 200 mg litre^{-1} for 30 seconds, all at 15°C (Kasai *et al.,* 2005).

2.4.6. Disinfection of eggs and larvae

Disinfection of the surface of the eggs can be achieved by iodophor treatment (Kasai *et al.,* 2005). There are no publications on the disinfection of larvae.

2.4.7. General husbandry

Biosecurity measures should include ensuring that new introductions of fish are from disease-free sources and installation of a quarantine system where new fish can be held with sentinel fish at permissive temperatures for infection with KHV. The fish should be quarantined for a minimum of 4 weeks to 2 months before transfer to the main site and mixing with naïve fish. Hygiene measures on site should include disinfection of eggs, regular disinfection of ponds, chemical disinfection of farm equipment, careful handling of fish to avoid stress and safe disposal of dead fish.

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 conducive to development of clinical disease, i.e. above 16°C (see Section 2.3.5). All production units (ponds, tanks, net-cages, etc.) should be inspected for the presence of dead, weak or abnormally behaving fish. If moribund fish or fish showing clinical signs are sampled, the probability of detecting KHV is higher than if randomly selected, apparently healthy fish are sampled.

~~Fish to be sampled are selected as follows:~~ For the purposes of disease surveillance, fish to be sampled are selected as follows:

i) ~~Susceptible species should be sampled proportionally or following~~ The most susceptible species should be sampled preferentially (see Section 2.2.3). Other susceptible species listed in Section 2.2.1 should be sampled proportionally.

ii) Risk-based criteria ~~for targeted selection of~~ should be employed to preferentially sample lots or 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 disease status) or where there is evidence of poor water quality or husbandry. Younger fish up to 1 year are more susceptible to clinical disease and are recommended for sampling. If more than one water source is used for fish production, fish from all water sources should be included in the sample.

~~ii) If more than one water source is used for fish production, fish from all water sources should be included in the sample.~~

iii) If weak, abnormally behaving or freshly dead (not decomposed) fish are present, such fish should be selected. If such fish are not present, the fish selected should include normal appearing, healthy fish collected in such a way that all parts of the farm as well as all year classes are proportionally represented in the sample.

For disease outbreak investigations, moribund fish or fish exhibiting clinical signs of infection with KHV should be collected. Ideally fish should be collected while alive, however recently dead fish can also be selected for diagnostic testing. 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.

3.2. Selection of organs or tissues

When testing clinically affected fish by PCR methods, and particularly if virus isolation is to be attempted, it is recommended to sample gill, kidney, and spleen tissues. The virus is most abundant in these tissues during the course of overt infection and high levels of virus have also been detected in encephalon (brain) and intestine (gut) tissue (Dishon *et al.,* 2005; Gilad *et al.,* 2004). Moreover, KHV DNA was detected with high probability from the encephalon of the surviving fish at 120 days post-infection (Ito *et al.,* 2014a). When testing subclinical, apparently healthy, fish by PCR methods, it is recommended to also include intestine (gut) and encephalon in a separate sample. In addition, KHV DNA was detected in the caudal and pectoral fin of all sampled dead fish from the field. As fins can be easily collected using tweezers and scissors, the fins are a suitable organ for PCR detection of KHV in clinically affected fish (Ito *et al*., 2014a; 2014b).

3.3. Samples or tissues not suitable for pathogen detection

Fish carcasses showing very advanced signs of tissue decomposition are not suitable for testing by any method.

3.4. Non-lethal sampling

While some research has been carried out on the use of non-lethal sampling during the first few days after experimental challenge (Monaghan *et al*., 2015), due to the lack of formal validation non-lethal sampling is currently not recommended for the detection of KHV.

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 depends strongly on the quality of samples (which is influenced by 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. To avoid degradation of samples, use alternative storage methods only after consultation with the receiving laboratory.

3.5.2. Preservation of samples for molecular detection

Tissue samples for PCR testing should be preserved in 80–100% (v/v) analytical/reagent-grade (undenatured) ethanol. The recommended ratio of ethanol to tissue is 10:1 based on studies in terrestrial animal and human health and will ensure that the ethanol does not fall to below 70% The use of lower grade (laboratory or industrial grade) ethanol is not recommended. If material cannot be fixed it may be frozen, but repeated freezing and thawing should be avoided.

3.5.3. Samples for histopathology, immunohistochemistry or *in-situ* hybridisation

~~Tissue samples for histopathology should be fixed in neutral buffered formalin immediately after collection. To ensure adequate penetration of the fixative the recommended ratio of fixative to tissue is 10:1.~~ Standard sample collection, preservation and processing methods for histological techniques can be found in Section 2.2. of Chapter 2.3.0. General information (diseases of fish).

3.5.4. Samples for electron microscopy

Samples for electron microscopy are not routinely required and are collected only when it is considered beneficial to facilitate further diagnostic investigation. A 2 mm cubed 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

~~Blood samples extracted from the caudal vessel into a vacuum blood collection tube should be centrifuged for the collection of serum or plasma as soon as possible after sampling to avoid lysis of the red blood cells. Serum or plasma samples should be shipped on ice to the laboratory to ensure maintenance of virus infectivity.~~ Not applicable.

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.~~ Pooling of samples from more than one individual animal for a given purpose should only be recommended where robust supporting data on diagnostic sensitivity and diagnostic specificity have been evaluated and found to be suitable. If the effect of pooling on diagnostic sensitivity has not been thoroughly evaluated, larger fish should be processed and tested individually. Small life stages such as fry ~~or specimens up to 0.5 g,~~ can be pooled to obtain the minimum amount of 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 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 apparently healthy animals and investigation of clinically affected animals*

Method	A. Surveillance of apparently healthy animals				B. Presumptive diagnosis of clinically affected animals				C. Confirmatory diagnosis[1] of a suspect result from surveillance or presumptive diagnosis			
	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV
Wet mounts												
Histopathology[3]						++	++	1				
Cell ~~or artificial media~~ culture						++	++	1				
Real-time PCR	++±	++±	++±	~~1~~ 3	++±	++±	++±	~~1~~ 3				
Conventional PCR					++	+++	+++	1	++	++	++	1
Conventional nested PCR	++	++	++	1	+++	+++	+++	1	++	++	++	1
Amplicon sequencing[4]									+++	+++	+++	1
***In-situ* hybridisation**												
Bioassay												
LAMP						+++	+++	1				
IFAT						+	+	1				
ELISA												
Other antigen detection methods[5]												
Other method[5]												

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; ELISA = enzyme-linked immunosorbent assay, respectively.

[1]For confirmatory diagnoses, methods need to be carried out in combination (see Section 6).

[2]Susceptibility of early and juvenile life stages ~~have been defined~~ is described in Section 2.2.3.

[3]Histopathology and cytopathology can be validated if the results from different operators have been statistically compared.

[4]Sequencing of the PCR product.[5]Specify the test used. 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

Examination of the gills by low-power light microscopy can reveal erosion of primary lamellae, fusion of secondary lamellae, and swelling at the tips of the primary and secondary lamella. The histopathology of the disease is variable and not pathognomonic, but inflammation and necrosis of gill tissues is a consistent feature. Gills also exhibit hyperplasia and hypertrophy of branchial epithelium, and fusion of secondary lamellae and adhesion of gill filaments can be seen. Gill necrosis, ranging from small areas of necrotic epithelial cells of secondary lamellae to complete loss of the lamellae is observed. Branchial epithelial cells and leucocytes may have prominent nuclear swelling, margination of chromatin to give a 'signet ring' appearance, and pale diffuse eosinophilic intranuclear inclusions can be observed. Inflammation, necrosis and nuclear inclusions have also been observed (individually or together) in other organs, particularly the kidney, but also in the spleen, pancreas, liver, brain, gut and oral epithelium.

4.3. Cell ~~or artificial media~~ culture for virus isolation

The recommended cell lines for KHV detection are: CCB and KF-1. Cell lines should be monitored to ensure that susceptibility to targeted pathogens has not changed.

Diagnosis of infection with KHV in clinically affected fish can be achieved by virus isolation in cell culture. However, the virus is isolated in only a limited number of cell lines which can be difficult to handle. Also, cell culture isolation is not as sensitive as the published PCR-based methods to detect KHV DNA and is not considered to be a reliable diagnostic method for KHV (Haenen *et al.,* 2004).

~~*Cell line to be used:* KF-1, KFC or CCB.~~

Use the procedure described in Chapter 2.3.0 *General information* (on diseases of fish), Section A.2.2.2.

Confirmatory identification

The most reliable method for confirmatory identification of a CPE is by PCR, followed by sequence analysis of the PCR product. The PCR methods recommended for identification of KHV are the same methods recommended for direct detection in fish tissues ~~(Section 4.3.1.2.3 below)~~. For final confirmation, PCR products of the correct size should be identified as KHV in origin by sequence analysis ~~(Section 4.4.5 below).~~

i) Using a suitable DNA extraction kit or reagent, extract DNA from a sample of the virus culture that includes both cellular and supernatant material.

ii) Extracted DNA is then amplified using the PCR protocols described below (Section 4.4.3). Amplified PCR products may then be ~~excised from the gel and~~ sequenced as described in Section ~~4.3.1.2.3~~ 4.4.5

4.4. Nucleic acid amplification

The following controls should be run with each stage of the assay: negative extraction control; positive extraction control; no template PCR control; internal PCR control or positive PCR control. Ideally, the positive extraction control should be distinguishable from viral genomic sequence, thus allowing detection of any cross-contamination leading to false positive results.

4.4.1. Sample preparation and extraction of DNA

DNA from infected cells and/or tissues is extracted using a phase-separation method or by use of a commercially available DNA isolation kit used according to the manufacturer's instructions.

4.4.2. Real-time PCR

Real-time PCR assays, such as TaqMan real-time PCR, are favoured by many diagnostic laboratories over conventional PCR, and real-time Taqman PCR is now a common diagnostic procedure that has been shown to detect and quantitatively assess very low copy numbers of target nucleic acid sequences. The most commonly used quantitative assay for detection of KHV is the Gilad Taqman real-time PCR assay (Gilad *et al.,* 2004). ~~However, it should be noted that real-time PCR positive results are presumptive only and should be confirmed by convention PCR and sequence analysis.~~

Furthermore, it should be noted that there is evidence that the published conventional PCR and real-time PCR methods, developed for the detection of KHV DNA in fresh tissue samples from clinically diseased carp, fail to detect some KHV ~~variants~~ genotypes in clinically affected fish (Engelsma *et al.,* 2013). Until this is resolved, in geographic locations where these variants may be present it is highly recommended that the assay described by Engelsma *et al*. (2013) is used ~~in place of the current assays;~~ i.e. it is recommended to use ~~using~~ the nested or one-tube semi-nested PCR assay or increas~~e~~ing the cycle number of the single-round assay to detect the virus in apparently healthy carriers.

~~The following controls should be run with each assay: negative extraction control; control; no template control; internal PCR control. Ideally, the positive control should be distinguishable from viral genomic sequence, thus allowing detection of any cross-contamination leading to false positive results.~~ The primer and probe sequences and cycling conditions for the Gilad *et al*. (2004) KHV and ~~koi glucokinase~~ an internal housekeeping gene (used as the internal PCR control) real-time PCRs are shown in Table 4.4.2.1.

Table 4.4.2.1*. Primer and probe sequences and cycling conditions for the KHV real-time PCR (Gilad* et al.*, 2004).*

Target	Primer/probe sequence (5'->3') (concentration)	Cycling conditions	Amplicon size (bp)	Reference
KHV	KHV-86f: GAC-GCC-GGA-GAC-CTT-GTG (400 nM)	1 × 2 minutes @ 50°C 1 × 10 minutes @ 95°C 40 × 15 seconds @ 95°C and 60 seconds @60°C	78	Gilad *et al*. (2004)[1]
	KHV-163r: CGG-GTT-CTT-ATT-TTT-GTC-CTT-GTT (400 nM)			
	KHV-109p: 6FAM-CTT-CCT-CTG-CTC-GGC-GAG-CAC-G-TAMRA (80 nM)			
Glucokinase	CgGluc-162f: ACT-GCG-AGT-GGA-GAC-ACA-TGA-T (400 nM)		69	
	CgGluc-230r: TCA-GGT-GTG-GAG-CGG-ACA-T (400 nM)			
	CgGluc-185p: 6FAM-AAG-CCA-GTG-TCA-AAA-TGC-TGC-CCA-CT-TAMRA (80 nM)			

[1]The Gilad *et al.* (2014) assay was modified slightly by increasing the probe quantity to 100 nM by Clouthier *et al.* (2017).

4.4.3. Conventional PCR

Engelsma *et al.* (2013) reported that the published single-round PCR methods traditionally thought to be the most sensitive for detection of KHV DNA in fresh tissue samples fail to detect some KHV genotypes in clinically affected fish. Therefore, the assay described by Engelsma *et al*. (2013) is highly recommended when detecting KHV ~~variants~~ genotypes. By extending the number of cycles ~~to 50~~ or using the nested second round of amplification the assay may also be suitable to detect virus in sub-clinical carriers. This method and other commonly used PCR protocols are shown in Table 4.4.3.1.

Table 4.4.3.1. *Primer sequences and cycling conditions for KHV conventional PCR methods*

Primer sequence (5'->3') (concentration)	Cycling conditions	Amplicon size (bp)	References
Primary step: CyHVpolfor: CCA-GCA-ACA-TGT-GCG-ACG-G (200 nM) CyHVpolrev: CCG-TAR-TGA-GAG-TTG-GCG-CA (200 nM) Nested PCR:	1 × 2 minutes @ 95°C 40 × 30 seconds @ 95°C, 30 seconds @ 55°C and 45 seconds @ 72°C 1 × 10 minutes @ 72°C	361	Engelsma *et al*. (2013)

Primer sequence (5'->3') (concentration)	Cycling conditions	Amplicon size (bp)	References
CyHVpolforint: CGA-CGG-VGG-YAT-CAG-CCC (200 nM) CyHVpolrevint: GAG-TTG-GCG-CAY-ACY-TTC-ATC (200 nM)		339	
For: GGG-TTA-CCT-GTA-CGA-G (200 nM) Rev: CAC-CCA-GTA-GAT-TAT-GC (200 nM)	1 × ~~15~~ 5 minutes @ ~~94~~ 95°C 40 × ~~45~~ 60 seconds @ 95°C, ~~45~~ 60 seconds @ 55°C and 60 seconds @ 72°C 1 × ~~7~~ 10 minutes @ 72°C	409	Bercovier *et al*. (2005)[1] Clouthier *et al.* (2017)
For: GAC-ACC-ACA-TCT-GCA-AGG-AG (1000 nM) Rev: GAC-ACA-TGT-TAC-AAT-GGT-CGC (1000 nM)	1 × 30 seconds @ 94°C 40 × 30 seconds @ 94°C, 30 seconds @ 63°C and 30 seconds @ 72°C 1 ×7 minutes @ 72°C.	292	Gray *et al*. (2002) Yuasa *et al*. (2005)
For: GAC-GAC-GCC-GGA-GAC-CTT-GTG (300 nM) Rev: CAC-AAG-TTC-AGT-CTG-TTC-CTC-AAC (300 nM)	1 × 5 minutes @95°C 39 ×1 minute @ 94°C, 1 minute @ 68°C and 30 seconds @ 72°C 1 × 7 minutes @ 72°C	484	Gilad *et al.,* (2004)

[1]The annealing temperature and cycling programme described by Bercovier *et al.* (2005) were slightly modified to improve detection limits and the specificity of the assay. See Clouthier *et al*. (2017) for the details.

4.4.4. Other nucleic acid amplification methods

A loop-mediated isothermal amplification (LAMP) targeting TK gene has been developed for detection of KHV and shown to be more or equally sensitive as the single-round conventional PCR assays. An assay incorporating DNA hybridisation technology and antigen–antibody reactions in combination with LAMP has also been developed and reported to have improved sensitivity and specificity (Soliman & El-Matbouli, 2010).

4.5. Amplicon sequencing

PCR products are excised from the gel and purified using a commercial kit for gel purification. Single, intense (bright) PCR products, after purification, are sequenced directly in both directions with the primers used in the initial amplification. Alternatively, less intense (faint) PCR products are cloned using a TA cloning vector and both DNA strands are sequenced. The amplification, cloning and sequencing are performed in duplicate to eliminate potential errors introduced by the Taq polymerase. Sequence reactions are then analysed on a Genetic Analyser and the alignments and consensus sequences generated using appropriate computer software. Testing laboratories that have no sequencing facilities are recommended to use commercial companies that offer a sequencing service. Testing laboratories should follow the instructions supplied by the chosen sequencing service for submission of samples.

4.6. *In-situ* hybridisation

In-situ hybridisation (ISH) and immunofluorescence (IF) methods performed on separated fish leucocytes, have been used in research applications for detection, confirmation, or identification of KHV. Although these methods have not been thoroughly compared with other techniques, they are non-destructive (non-lethal) techniques and some laboratories may find them useful in a diagnostic setting and for confirmation of PCR results. Details of the methods are not given here but detailed protocols for separation of leucocytes from blood and for IF and ISH can be found in published reports by Bergmann *et al.* (2009; 2010).

4.7. Indirect fluorescent antibody test (IFAT)

KHV can be detected in touch imprints of liver, kidney and brain of infected fish by immunofluorescence (IF). Highest levels of positive IF were seen in the kidney and the virus could be detected by IF on a kidney imprint 1 day post-infection (Pikarsky *et al.,* 2004; Shapira *et al.,* 2005). The detection of KHV by immunostaining must be interpreted with care, as positive-staining cells could result from cross-reaction with serologically related virus (e.g. CyHV-1) or a non-viral protein (Pikarsky *et al.,* 2004).

A method for direct detection of KHV from kidney imprints by indirect fluorescent antibody test (IFAT) is detailed below.

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.

iii) Allow the imprint to air-dry for 20 minutes.

iv) 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 glass slides or a mixture of 30% acetone/70% ethanol, also stored at –20°C, for plastic wells.

v) Let the fixative act for 15 minutes. A volume of 0.5 ml/2 cm^2 well is adequate for imprints in cell culture plates.

vi) Allow the fixed imprints to air-dry for at least 30 minutes and process immediately or freeze at –20°C.

vii) Rehydrate the dried imprints by four rinses with 0.01 M PBS solution, pH 7.2, containing 0.05% Tween 20 (PBST), and remove this buffer completely after the last rinse.

viii) Prepare a solution of purified antibody or antiserum to ~~CyHV-3~~ KHV in 0.01 M PBS, pH 7.2, containing 0.05% Tween 20 (PBST), at the appropriate dilution (which has been established previously or is given by the reagent supplier).

ix) Block with a solution of 5% skim milk or 1% bovine serum albumin, in PBST for 30 minutes at 37°C.

x) Rinse four times with PBST.

xi) Treat the imprints with the antibody solution (prepared at step viii) for 1 hour at 37°C in a humid chamber and do not allow evaporation to occur. A volume of 0.25 ml/2 cm^2 well is adequate for imprints in cell culture plates.

xii) Rinse four times with PBST.

xiii) Treat the imprints for 1 hour at 37°C 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.

xiv) Rinse four times with PBST.

xv) Add PBS (0.5 ml/2 cm^2 well) to the treated imprints in cell culture plates and examine immediately or mount the glass slides with cover-slips using glycerol saline at pH 8.5 prior to microscopic observation.

xvi) Examine under incident UV light using a microscope. Positive and negative controls must be found to give the expected results prior to any other observation.

Paraffin wax tissue sections fixed in 10% neutral buffered formalin (NBF) are also suitable for detection of KHV antigen by IFAT. However, the deparaffinised sections, rehydrated in PBS, may need to be further treated to reveal antigen that may be masked by over fixation of the tissue. A common treatment is incubation of the sections with 0.1% trypsin in PBS at 37°C for 30 minutes. The sections are then washed in cold PBS before proceeding with steps viii–xvi above. Tissues collected for direct detect by IFAT (or other immunohistochemical staining, e.g. immunoperoxidase) should be fixed for 24–48 hours in 10% NBF and then the fixative should be replaced with 70% ethanol for prolonged storage.

4.8. Bioassay

Bioassay is not recommended as a diagnostic procedure.

4.9. Antibody- or antigen-based detection methods

Enzyme-linked immunosorbent assay (ELISA)-based methods for direct detection of KHV antigen in infected tissues are under development in a number of laboratories ~~and these methods may also be suitable for confirmatory identification of KHV~~. Currently, two published ELISA methods are available and ~~was~~ were developed in Israel to detect KHV in fish faeces (Dishon *et al.,* 2005) but also after isolation in cell culture using different KHV isolates at different temperatures (Bergmann *et al.* 2017~~b~~). The ELISA methods

developed will have low sensitivity that may be suitable for detection of the high levels of KHV found in clinically diseased fish tissue but not suitable for KHV surveillance in healthy populations.

4.10. Other methods

~~Infected carp produce antibodies against the virus, and ELISA-based tests that reliably detect these antibodies at high serum dilution have been published (Adkison *et al.*, 2005; Bergman *et al.*, 2017a; Ilouze *et al.*, 2010; St-Hilaire *et al.*, 2005). Antibody has been detected in the serum at 3 weeks after experimental infection and in survivors after 1 year following a natural infection (Adkison *et al.*, 2005; Ilouze *et al.*, 2010; St-Hilaire *et al.*, 2005; Taylor *et al.*, 2010).~~

~~Serum from koi containing antibodies to KHV has been shown to cross-react, in low dilutions, with CyHV-1. a further indication that these viruses are closely related. Evidence of cross-reacting antibodies was demonstrated in ELISA and western blot analyses of serum from koi infected with CyHV-1 or KHV (Adkison *et al.*, 2005). Diagnostic virologists should also be aware that fish recently vaccinated against KHV may test positive in antibody detection ELISAs.~~

None published or validated.

5. Test(s) recommended for surveillance to demonstrate disease freedom in apparently healthy populations

~~There are no well validated methods that are currently recommended for testing healthy populations of susceptible fish for declaration of freedom from infection with KHV; there is increasing evidence that the published real-time PCR assays may fail to detect all genotypes of KHV. Therefore, conventional nested PCR assays described by Engelsma *et al.* (2013) which will detect all known KHV genotypes is currently recommended for surveillance to demonstrate freedom in apparently health populations.~~ Real-time PCR is the recommended test for surveillance in apparently healthy animals to declare freedom from infection with KHV. However, there have been unpublished observations that the method may not detect the KHV variants that were described by Englesma *et al.* (2013). In geographic locations where these variants may be present, the conventional nested PCR published by Englesma *et al*. (2013) should also be considered.

6. Corroborative diagnostic criteria

This section only addresses the diagnostic test results for detection of infection in the ~~presence~~ absence (6.1) or ~~absence~~ presence of clinical signs (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[5]

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.

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:

i) A positive result from a real-time PCR assay

ii) A positive result from a conventional nested PCR assay.

5 For example transboundary commodities.

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with KHV is considered to be confirmed if at least one of the following criteria is met:

i) Detection of KHV in tissue samples by real-time PCR followed by ~~and~~ conventional PCR or conventional nested PCR ~~followed by~~ and sequencing of the amplicon

ii) Detection of KHV in tissue samples by conventional nested PCR and sequencing of the amplicon

6.2. Clinically affected animals

No clinical signs are pathognomonic for infection with KHV 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~~ is met:

i) Gross pathology or clinical signs associated with infection with KHV as described in this chapter, with or without elevated mortality

ii) Histopathological changes consistent with infection with KHV as described in this chapter

iii) KHV typical CPE in cell culture

iv) A positive result by a real-time PCR

v) A positive result by a conventional (single round or nested) PCR

vi) A positive result by LAMP assay

vii) A positive result by IFAT

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection shall be confirmed if at least one of the following criteria is met:

i) KHV isolation in cell culture followed by virus identification by conventional PCR or conventional nested PCR and sequencing of the amplicon

ii) Detection of KHV in tissue samples by real-time PCR ~~and by conventional PCR~~ followed by conventional PCR or conventional nested PCR and sequencing of the amplicon

iii) A positive result by LAMP assay ~~and~~ followed by conventional PCR or conventional nested PCR and ~~followed by~~ sequencing of the amplicon

iv) A positive result by IFAT ~~and~~ followed by conventional PCR or conventional nested PCR and ~~followed by~~ sequencing of the amplicon

iv) Detection of KHV in tissue samples by conventional PCR or conventional nested PCR and ~~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

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with KHV are provided in Tables 6.3.1. and 6.3.2. This information can be used for the design of surveys for infection with KHV, however, it should be noted that diagnostic performance is specific to the circumstances of each diagnostic accuracy study (including the test purpose, source population, tissue sample types and host species) and diagnostic performance may vary under different conditions. Data are only presented where tests are validated to at least level two of the validation pathway described in Chapter 1.1.2. and the information is available within published diagnostic accuracy studies.

The diagnostic sensitivity (DSe) and specificity (DSp) of the real-time and conventional PCR assays, based on an analysis of field collections and experimentally infected carp and koi, are 94–100% (DSe) and 93–100% (DSp) (Amita *et al*., 2002, Clouthier *et al.,* 2017; Ito *et al*., 2014a; 2014b) ~~demonstrated 94–100% DSe and 100% DSp~~.

6.3.1. For presumptive diagnosis of clinically affected animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation
Real-time PCR	Diagnosis	Experimentally infected koi and apparently healthy wild common carp	kidney	Common carp & koi (*Cyprinus carpio* L.)	99	93	None; Bayesian latent class modelling	Clouthier *et al.*, 2017
PCR	Diagnosis	Experimentally infected koi and apparently healthy wild common carp	kidney	Common carp & koi (*Cyprinus carpio* L.)	99	93	None; Bayesian latent class modelling	Clouthier *et al.*, 2017

DSe: = diagnostic sensitivity, DSp = diagnostic specificity.

6.3.2. For surveillance of apparently healthy animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation
Real-time PCR	Diagnosis	Experimentally infected koi and apparently healthy wild common carp	kidney	Common carp & koi (*Cyprinus carpio* L.)	99	93	None; Bayesian latent class modelling	Clouthier *et al.*, 2017[1]
PCR	Diagnosis	Experimentally infected koi and apparently healthy wild common carp	kidney	Common carp & koi (*Cyprinus carpio* L.)	99	93	None; Bayesian latent class modelling	Clouthier *et al.*, 2017[1]

DSe: = diagnostic sensitivity, DSp = diagnostic specificity.

[1]The diagnostic accuracy study did not include samples that were known to be positive for all KHV genotypes.

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* *

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

NB: FIRST ADOPTED IN 2006; MOST RECENT UPDATES ADOPTED IN 2019.

Return to Agenda

CHAPTER 2.3.7.

INFECTION WITH RED SEA BREAM IRIDOVIRUS

1. Scope

Infection with red sea bream iridovirus is considered to be infection with the pathogenic agent red sea bream iridovirus (RSIV) of the genus *Megalocytivirus*, Family *Iridoviridae*.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

The pathogen is an icosahedral virion 140−200 nm in diameter consisting of a central electron-dense core (120 nm) and an electron translucent zone (Inouye *et al.,* 1992) with a double-stranded DNA genome approx. 110 kbp in length (Kawato *et al.,* 2017a). The viral genome has a G+V content of 53–55%, containing about 120 potential open reading frames (ORFs).

Phylogenetic analyses using major capsid protein (MCP) and ATPase genes shows that the viruses causing the similar clinical signs can be divided into three different genotypes: RISV, infectious spleen and kidney necrosis virus (ISKNV) (He *et al.,* 2000; 2001), and turbot reddish body iridovirus (TRBIV) genotype (Shi *et al.,* 2004; 2010).

The aetiological agent of infection with RSIV is RSIV (Inouye *et al.,* 1992; Jeong *et al.,* 2003) and other strains belonging in the RSIV genotype (Go *et al.,* 2016; Koda *et al.,* 2018; Kurita & Nakajima, 2012). Similar diseases with the characteristic, enlarged basophilic cells within infected organs, typical of infections with megagalocytiviruses, classified into ISKNV genotype and TRBIV genotypes are excluded in this chapter. RSIV genotypes are differentiated from ISKNV and TRBIV genotypes based on nucleotide sequence analysis which is required for confirmatory diagnosis. Scale drop disease virus is another virus in the genus *Megalocytivirus* causing different clinical signs in Asian seabass, *Lates calcarifer* (Groof *et al.,* 2015).

RSIV was first found in red sea bream, *Pagrus major,* from which the virus name (RSIV) is derived (Inouye *et al.,* 1992). As RSIV has a broad host range as shown in Section 2.2.1. *Susceptible host species*, many viruses that can be classified into RSIV genotype are synonyms of RSIV and defined to be the aetiological agents in this chapter, e.g. rock bream iridovirus (RBIV) (Do *et al.,* 2004; Jung & Oh 2000), Taiwan grouper iridovirus (TGIV) (Chou *et al.,* 1998), large yellow croaker iridovirus (LYCIV) (Chen *et al.,* 2003), orange-spotted grouper iridovirus (OSGIV) (Lu *et al.,* 2005), spotted knifejaw iridovirus (SKIV) (Dong *et al.,* 2010) and giant seaperch iridovirus (GSIV) (Wen & Hong, 2016).

2.1.2. Survival and stability inside the host tissues

Unknown

2.1.3. Survival and stability outside the host

Unknown

For inactivation methods, see Section 2.4.6.

2.2. Host factors

2.2.1. Susceptible host species

In the case of RSIV infection:

Family	Scientific name	Common name
Carangidae	*Pseudocaranx dentex*	striped jack
	Seriola dumerili	greater amberjack

	Seriola lalandi	yellowtail amberjack
	Seriola lalandi × *Seriola quinqueradiata*	hybrid of yellowtail amberjack and Japanese amberjack
	Seriola quinqueradiata	Japanese amberjack
	Trachinotus blochii	snubnose pompano
Centrarchidae	*Micropterus salmoides*	largemouth bass
Centropomidae	*Lates calcarifer*	barramundi or sea bass
Haemulidae	*Parapristipoma trilineatum*	chicken grunt
	Plectorhinchus cinctus	crescent sweetlips
	Trachurus japonicus	Japanese jack mackerel
Kyphosidae	*Girella punctata*	largescale blackfish
Lateolabracidae	*Lateolabrax japonicas*	Japanese sea perch
	Lateolabrax sp.,	
Lethrinidae	*Lethrinus haematopterus*	Chinese emperor
	Lethrinus nebulosus	spangled emperor
Moronidae	*Morone saxatilis* × *M. chrysops*	hybrid of striped sea bass and white bass
Oplegnathidae	*Oplegnathus fasciatus*	Japanese parrotfish
Paralichthyidae	*Paralichthys olivaceus*	bastard halibut
Pleuronectidae	*Verasper variegatus*	spotted halibut
Rachycentridae	*Rachycentron canadum*	cobia
Sciaenidae	*Pseudosciaena crocea*	croceine croaker
Scombridae	*Scomber japonicus*	chub mackerel
	Scomberomorus niphonius	Japanese Spanish mackerel
	Thunnus thynnus	northern bluefin tuna
Sebastidae	*Sebastes schlegeli*	rockfish
Serranidae	*Epinephelus akaara*	Hong Kong grouper
	Epinephelus awoara	yellow grouper
	Epinephelus bruneus	longtooth grouper
	Epinephelus coioides	orange-spotted grouper
	Epinephelus fuscoguttatus	brown-marbled grouper
	Epinephelus lanceolatus	giant grouper
	Epinephelus malabaricus	Malabar grouper
	Epinephelus septemfasciatus	convict grouper
	Epinephelus tauvina	greasy grouper
	Oplegnathus punctatus	spotted knifejaw
Sparidae	*Acanthopagrus latus*	yellowfin sea bream
	Acanthopagrus schlegeli	black porgy
	Evynnis japonica	crimson sea bream
	Pagrus major	red sea bream
Tetraodontidae	*Takifugu rubripes*	torafugu

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 (RSIV) according to Chapter 1.5 of the *Aquatic Code* are: Under study.

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

Juvenile through to adult stages are susceptible; however, the susceptibility of juveniles is generally higher than adults. Fish belonging to the genus *Oplegnathus* may be more susceptible than others.

2.2.4. Distribution of the pathogen in the host

Infected cells are observed in the spleen, kidney, heart, liver, intestine and gill.

2.2.5. Aquatic animal reservoirs of infection

Unknown

2.2.6. Vectors

Unknown

2.3. Disease pattern

2.3.1. Mortality, morbidity and prevalence

Depending on host fish species, fish size, fish age, water temperature, and other culture conditions, mortality rates range between 0% and 100%. Morbidity is unknown.

2.3.2. Clinical signs, including behavioural changes

Affected fish become lethargic and show abnormal and conspicuous respiratory movements.

2.3.3. Gross pathology

Fish exhibit severe anaemia, petechiae in the gills, and enlargement of the spleen and kidney.

2.3.4. Modes of transmission and life cycle

The principal mode of transmission of RSIV is horizontal via the water. Vertical transmission of RSIV has not yet been investigated.

2.3.5. Environmental factors

Outbreaks have been seen mostly in the summer season at water temperatures of 25°C and above.

2.3.6. Geographical distribution

The first outbreak was recorded in marine cultured red sea bream in Japan in 1990. From then on, further outbreaks and infections have been reported in many marine fish and freshwater fish. The international trade of ornamental fish has contributed significantly to the spread of megalocytiviruses (Johan & Zainathan, 2020).

See WAHIS (https://wahis.oie.int/#/home) for recent information on distribution at the country level.

2.4. Biosecurity and disease control strategies

2.4.1. Vaccination

Effectiveness of a vaccine consisting of formalin-inactivated supernatant from RSIV-infected GF cell culture has been confirmed experimentally and in field trials (Nakajima *et al.,* 1997; 1999). Currently, the formalin-inactivated vaccine for infection with RSIV is commercially available for red sea bream (*Pagrus major*), striped jack (*Pseudocaranx dentex*), Malabar grouper (*Epinephelus malabaricus*), orange-spotted grouper (*Epinephelus coioides*) and other fish species belonging to the genus *Seriola* in Japan. Protection of fish belonging to the genus *Oplegnathus* by vaccination is difficult.

2.4.2. Chemotherapy including blocking agents

Not available.

2.4.3. Immunostimulation

Not applicable.

2.4.4. Breeding resistant strains

An RSIV-resistant strain of red sea bream (*Pagrus major*) has been developed using marker-assisted selection combined with DNA-based family selection (Sawayama *et al.*, 2019).

2.4.5. Inactivation methods

RSIV is inactivated at 56°C for 30 minutes and by treatment with either ether, chloroform or formalin (0.1%), and by exposure to pH 3.0. The virus is stable in tissue at –80°C and at pH 7.0 and pH 11.0 (Nakajima & Sorimachi, 1994).

2.4.6. Disinfection of eggs and larvae

Unknown

2.4.7. General husbandry

Not available.

3. Specimen selection, sample collection, transportation and handling

This section draws on information in 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 should be carried out during a period when water temperature is conducive to development of clinical disease (see Section 2.3.5). All production units (ponds, tanks, etc.) should be inspected for the presence of dead, weak or abnormally behaving fish. For the purposes of disease surveillance, fish to be sampled are selected as follows:

i) The most susceptible species should be sampled preferentially (see Section 2.2.3). Other susceptible species listed in Section 2.2.1 should be sampled proportionally.

ii) Risk-based criteria should be employed to preferentially sample lots or populations with a history of abnormal mortality, potential exposure events or where there is evidence of poor water quality or husbandry. If more than one water source is used for fish production, fish from all water sources should be included in the sample.

iii) If weak, abnormally behaving or freshly dead fish are present, such fish should be selected. If such fish are not present (e.g. during surveillance of apparently healthy populations), the fish selected should include normal appearing, healthy fish collected in such a way that all parts of the farm as well as all year classes are proportionally represented in the sample. Smaller fish may be more appropriate because infection with RSIV can cause higher mortality in juvenile or yearling fish. However, adult fish are also susceptible to RSIV infection as the viral genome has been detected from apparently healthy broodstock. Infection with RSIV has not been reported in hatchery fish.

For disease outbreak investigations, moribund fish or fish exhibiting clinical signs of infection with RSIV should be collected. Ideally fish should be collected while alive, however recently dead fish can also be selected for diagnostic testing. 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.

3.2. Selection of organs or tissues

Although gill and visceral organs such as spleen, heart, kidney, liver and intestine can be used, it is recommended to sample spleen or kidney tissues; spleen is the most appropriate organ for the preparation of imprints for use in the IFAT. For surveillance of apparently healthy populations, spleen or kidney should be sampled.

3.3. Samples or tissues not suitable for pathogen detection

Fish carcasses showing advanced signs of tissue decomposition are not suitable for testing by any method.

Use of inappropriate fixatives (where required), poor sample quality, inappropriate tissues and lack of information provided with the submission may render samples unsuitable for testing.

3.4. Non-lethal sampling

Not available.

3.5. Preservation of samples for submission

Store fish at 4°C for use within 24 hours (or at –80°C for longer periods [up to a few years]).

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 depends strongly on the quality of samples (which will be affected by 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. To avoid degradation of samples, use alternate storage methods only after consultation with the receiving laboratory.

3.5.2. Preservation of 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 based on studies in terrestrial animal and human health. The use of lower grade (laboratory or industrial grade) ethanol is not recommended. If material cannot be fixed it may be frozen.

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

Tissue samples for histopathology should be fixed immediately after collection. The recommended ratio of fixative to tissue is 10:1.

3.5.4. Samples for other tests

Not applicable.

3.6. Pooling of samples

Pooling of samples from more than one individual animal for a given purpose should only be recommended where robust supporting data on diagnostic sensitivity and diagnostic specificity have been evaluated and found to be suitable. If the effect of pooling on diagnostic sensitivity has not been thoroughly evaluated, larger fish should be processed and tested individually. Small life stages such as fry or specimens can be pooled to provide the minimum amount of material needed for testing. If pooling is used, it is recommended to pool organ pieces from a maximum of five fish.

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 apparently healthy animals and investigation of clinically affected animals*

Method	A. Surveillance of apparently healthy animals				B. Presumptive diagnosis of clinically affected animals				C. Confirmatory diagnosis[1] of a suspect result from surveillance or presumptive diagnosis			
	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV
Tissue imprints	+	+	+	1	+	+	+	1				
Histopathology[3]	+	+	+	1	++	++	++	1				
IFAT or ICC	+	+	+	1	++	++	++	1				
Cytopathology[3]												
Cell culture	+	+	+	1	++	++	++	1				
Real-time PCR	++	++	++	2	++	++	++	2				
Conventional PCR	++	++	++	2	++	++	++	2	+++	+++	+++	
Amplicon sequencing[4]									+++	+++	+++	
***In-situ* hybridisation**												
Bioassay												
LAMP												
Ab-ELISA												
Ag-ELISA												
Other antigen detection methods[5]												
Other serological method[5]												

LV = level of validation, refers to the stage of validation in the OIE Pathway (chapter 1.1.2); IFAT = Indirect fluorescent antibody test. ICC = Immunocytochemistry PCR = polymerase chain reaction; LAMP = loop-mediated isothermal amplification; Ab- or Ag-ELISA = antibody or antigen enzyme-linked immunosorbent assay, respectively;
[1]For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). [2]Susceptibility of early and juvenile life stages is described in Section 2.2.3.
[3]Histopathology and cytopathology can be validated if the results from different operators have been statistically compared. [4]Sequencing of the PCR product.
[5]Specify the test used. Shading indicates the test is inappropriate or should not be used for this purpose.

4.1. Tissue imprints

Samples to be tested include tissue imprints of spleen from affected fish. Use a tissue imprint of spleen from known uninfected fish as a negative control and if possible, use a tissue imprint of spleen from confirmed RSIV-infected fish as a positive control.

i) Bleed the fish thoroughly.

ii) Make spleen imprints on cleaned glass microscope slides.

iii) Store the spleen pieces at 4°C together with the other organs that may be required for virus isolation or PCR tests later.

iv) Allow the imprints to air-dry for 20 minutes.

v) Fix the imprints with cold acetone.

vi) Stain with Giemsa or Diff-Quik.

vii) Mount the microscope slides with cover-slips using a drop of mounting fluid.

viii) Examine under light microscopy using ×40–100 magnification.

A presumptive positive result is indicated by the presence of abnormally enlarged cells. Negative control slides should not exhibit any abnormally enlarged cells. If enlarged cells are observed in the test samples, identification procedures PCR followed by amplicon sequencing must be undertaken immediately.

4.2. Histopathology and cytopathology

Examination of histological sections from diseased fish may reveal abnormally enlarged cells from the spleen, heart, kidney, liver, intestine or gill. These enlarged cells react to anti-RSIV MAb M10 (4.9.1.) using an immunohistochemistry test (Bermudez *et al.,* 2018). However, this method is not yet fully validated.

4.3. Cell culture for isolation

Cell lines should be monitored to ensure that susceptibility to targeted pathogens has not changed.

Isolation of RSIV (and ISKNV) is undertaken using the Grunt fin (GF) cell line[6] or SKF-9 cell line (Kawato *et al.,* 2017b); isolation of the viruses from freshwater fish such as gourami is difficult. Spleen and/or kidney tissues from diseased fish are suitable samples. Cells should be grown in Eagle's basal medium (BME) for GF cell line and Hank's minimum essential medium (HMEM) for SKF-9 cell line, supplemented with 10% fetal bovine serum (FBS) at 25°C in a temperature-controlled incubator. A virus isolate to be used as positive control can be obtained from the OIE Reference Laboratory for RSIV. Use uninfected cells as negative control. Following development of viral cytopathic effect (CPE), virus identification would be undertaken using conventional PCR and sequencing. SKF-9 cell line can be obtained from the OIE Reference Laboratory for RSIV.

4.3.1. Virus isolation in cell cultures

4.3.1.1. Inoculation of cell monolayers

i) Cell cultures (GF or SKF-9) maintained at 25°C and passaged 7-14 day intervals should be used for virus isolation to ensure virus susceptibility. Prepare cell monolayers in 25 cm^2 flask, 6-well, 24-well, or 96-well plates according to the purpose and sample size on the day before sample inoculation.

ii) Following the virus isolation procedure described in Chapter 2.3.0 *General information* (on diseases of fish), Section A.2.3.2, make an additional tenfold dilution of the 1/10 spleen homogenate supernatants and transfer an appropriate volume of each of the two dilutions onto the cell monolayers. To avoid cytotoxic effect (CTE), final concentration of the organ in the cultured medium should be less than 1% w/v.

[6] European Collection of Authenticated Cell Cultures (ECACC) Catalogue No. 88010601; www.phe-culturecollections.org.uk/products/celllines/generalcell/detail.jsp?refId=88010601&collection=ecacc_gc

iii) Without withdrawing the inoculum, incubate at 25°C.

4.3.1.2. Monitoring incubation

i) Follow the course of infection in positive controls and other inoculated cell cultures by daily microscopic examination at ×40–100 magnification for 10 days. The use of a phase-contrast microscope is recommended.

ii) If CPE appears in those cell cultures inoculated with dilutions of the test homogenates, identification procedures by PCR followed by amplicon sequence analysis must be undertaken immediately.

iii) If no CPE develops in the inoculated cultures (despite normal progression of CPE in the positive controls cultures) after 10 days incubation, the inoculated cultures should be subcultured and incubated for a further 7 days. Should the virus control fail to develop CPE, the process should be repeated with fresh susceptible cells and new batches of samples.

4.3.1.3. Subcultivation procedure

i) Collect aliquots of cell culture medium from all monolayers inoculated with dilutions of each supernatant of the test homogenates.

ii) Inoculate cell monolayers as described above (Section 4.3.1.1 Inoculation of cell monolayers, steps i and ii).

iii) Incubate and monitor as described above (Section 4.3.1.1 Inoculation of cell monolayers, steps ii and iii and Section 4.3.1.2 Monitoring incubation steps i and ii).

If no CPE occurs, the test may be declared negative.

4.4. Nucleic acid amplification

See Chapter 2.3.0 *General information* (on diseases of fish), Section B.2.5 for information on the use of molecular techniques for virus identification. Both real-time PCR and conventional PCR tests are available for RSIV identification. Samples to be tested include spleen from affected fish or supernatants from cell cultures that have developed CPE. The following controls should be run with each assay: negative extraction control; positive control; no template control; internal PCR control. Use extracted DNA from the spleen and kidney of uninfected fish or extracted DNA from the supernatant of an uninfected cell culture as the negative control. Use extracted DNA from the spleen of confirmed RSIV-infected fish or extracted DNA from the supernatant of an infected cell culture or Viral DNA or plasmid in which target sequence is inserted as the positive control. Select controls depending on the kinds of samples to be tested.

Tissue samples can be homogenised by manual pestle grinding or by bead-beating. Commercially available nucleic acid extraction kits may be used to extract DNA directly from tissues, from tissue homogenates and cell culture supernatants according to the manufacturer's instructions. A negative extraction control, consisting of extraction reagents only, is included when test samples are extracted. Use a pre-confirmed RSIV-affected organ or supernatant from RSIV-infected cell cultures as positive controls. Use organs from healthy fish or supernatants from non-infected cell cultures as negative controls

N.B. Viral DNA or a plasmid in which the PCR target sequence is inserted that can be used as the positive control can be obtained from the OIE Reference Laboratory for RSIV.

4.4.1. Real-time PCR

Several real-time PCR assays available for detection of RSIV have been evaluated (Kawato *et al*., 2021). Two probe-based real-time PCR assays, designated as the Mohr et al. assay (Mohr *et al*., 2015) and the Cummins assay, were deemed equivalent to each other and superior to the other tests evaluated in this study. The primer sets and probes of each of these two assays are designed to detect a major capsid protein (MCP) gene sequence and are as follows:

i) Mohr real-time PCR

RSIV RT F: 5'-TGA-CCA-GCG-AGT-TCC-TTG-ACT-T-3'

RSIV RT R: 5'-CAT-AGT-CTG-ACC-GTT-GGT-GAT-ACC-3'

RSIV Probe: 5'-FAM-AAC-GCC-TGC-ATG-ATG-CCT-GGC-TAMRA-3'

ii) Cummins real-time PCR

AFDL Megalo F: 5'-GGC-GAC-TAC-CTC-ATT-AAT-GTG-3'

AFDL Megalo R: 5'-CAC-CAG-GTC-GTT-AAA-TGA-CA-3'

AFDL Megalo Pr: 5'-FAM-CTG-CGT-GTT-AAG-ATC-CCC-TCC-A-TAMRA-3'

The protocol in use at the OIE Reference Laboratory for RSIV is as follows: Template (2 µl) is added to 23 µl reaction mixture containing 12.5 µl TaqMan Universal PCR Master Mix (Applied Biosystems), 900 nM for each primer, 250 nM for probe, and molecular grade water. After 1 cycle of 50°C for 2 minutes and 95°C for 10 minutes, amplification consists of 45 cycles of 95°C for 15 seconds, 60°C for 60 seconds.

The detection sensitivity limits of both real-time PCRs are approximately 1-10 copies/µl template DNA which is higher than that of conventional PCR. However, since the real-time PCRs have cross-reactivity to the ISKNV and TRBIV genotypes, conventional PCR followed by amplicon sequence analysis (see Section 4.5.) is required for confirmatory diagnosis.

4.4.2. Conventional PCR

The conventional PCR primer set consisting of the forward primer 1-F (5'-CTC-AAA-CAC-TCT-GGC-TCA-TC-3') and reverse primer 1-R (5'-GCA-CCA-ACA-CAT-CTC-CTA-TC-3') is used for amplification of the genome sequence across two ORFs (Kurita *et al.,* 1998).

The protocol in use at the OIE Reference Laboratory for RSIV, based on Kurita *et al.*, (1998), is as follows: Template (1 µl) is added to 19 µl reaction mixture containing 2 µl 10× reaction buffer, 1.6 µl dNTP mixture (2.5 mM each), 0.2 µl *TaKaRa ExTaq* HS (5 U/µl) (TaKaRa), 1 µM each primer, and molecular grade water. After 1 cycle of 94°C for 2 minutes, PCR amplification consists of 40 cycles of 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds, followed by final extension at 72°C for 2 minutes. Amplified DNA (567 or 570 bp) is analysed by agarose gel electrophoresis using a 1.5% agarose/TAE (Tris-acetate-EDTA) gel containing SYBR™ Safe (Thermo Fisher Scientific) or equivalent.

The detection sensitivity limit of the PCR is approximately 10-100 copies/µl template DNA. However, the primer set 1-F and 1-R is confirmed to amplify both RSIV and ISKNV DNA, and hence, amplicon sequencing is required for confirmatory diagnosis. The cross reactivity of these primer sets against TRBIV has not yet been validated.

4.4.3. Other nucleic acid amplification methods

Not applicable

4.5. Amplicon sequencing of the amplicon

The primer set 1-F and 1-R can amplify both RSIV and ISKNV DNA and sequencing of the amplicon is required for virus identification. Amplicons should be gel-purified and sequenced using both the forward and reverse primer. Consensus sequence, generated after analysis of the quality of the sequence chromatograms, can then be compared to reference sequences, for example by BlastN search of the NCBI database.

4.6. *In-situ* hybridisation

Not applicable

4.7. Histoimmunochemistry

Not applicable

4.8. Bioassay

Not applicable

4.9. Antibody-based or antigen detection methods

4.9.1. Antibody-based antigen detection methods: indirect fluorescent antibody test (IFAT) or immunocytochemistry (ICC)

Reagent and protocols for detecting RSIV proteins with a monoclonal antibody (MAb) M10 have been published (Kawato *et al.*, 2017b; 2020; Nakajima & Sorimachi, 1995). The MAb M10-reactive epitope has been demonstrated to be a 7 amino acid sequence (EYDCPEY) of a non-structural protein encoded by the laminin-type epidermal growth factor-like domain gene in RSIV and ISKNV (Takano *et al.,* 2019). MAb M10 detects both RSIV- and ISKNV-infected cells (Kawato *et al.*, 2020) but it does not detect ranaviruses (Nakajima & Sorimachi, 1995). The reactivity of MAb against TRBIV has not yet been confirmed. MAb M10 can be obtained from the OIE Reference Laboratory for RSIV.

Samples to be tested include tissue imprints of spleen from affected fish. Use a tissue imprint of spleen from uninfected fish as a negative control and if possible, use a tissue imprint of spleen from confirmed RSIV-infected fish as a positive control. Similarly, IFAT can be conducted directly after virus isolation in cell culture. Samples to be taken for testing include acetone-fixed infected cell monolayers that have developed CPE. Use an uninfected cell monolayer as a negative control and if possible, use a confirmed RSIV-infected cell monolayer as a positive control. The protocol for tissue imprints is as follows and can be adapted for IFAT on cell cultures.

i) Bleed the fish thoroughly.

ii) Make spleen imprints on cleaned glass microscope slides.

iii) Store the spleen pieces at 4°C together with the other organs that may be required for virus isolation.

iv) Allow the imprints to air-dry for 20 minutes.

v) Fix the imprints with cold acetone.

vi) Prepare a diluted solution of MAb M10 in PBS (1/100)

vii) Treat the imprints with the MAb M10 solution for 30 minutes at 37°C in a humid chamber.

viii) Rinse three times with PBS.

ix) Incubate the imprints for 30 minutes at 37°C in a humid chamber with a solution of a specific anti-mouse FITC-conjugated antibody prepared according to the supplier's instructions.

x) Rinse three times with PBS.

xi) Mount the microscope slides with cover-slips using glycerol saline prior to microscopic observation.

xii) Examine using a fluorescence microscope. Positive and negative controls must be found to give the expected results prior to any other observation.

A positive result is indicated by the presence of abnormally enlarged cells with strong fluorescence. Negative control slides should not exhibit any strong fluorescence.

If the test is positive, the fish from which the samples were obtained is considered infected with RSIV or ISKNV.

Alternatively a peroxide-conjugated second antibody could be used rather than fluorescence conjugate.

4.10. Other methods

RSIV cannot be identified by neutralisation tests as the antisera generated by the immunisation of rabbits have few neutralising antibodies.

5. Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations

As indicated in Table 4.1, real-time PCR is the most appropriate method of screening healthy fish populations for RSIV; however, the available methods are not specific for RSIV. Any real-time positive samples should be tested by

conventional PCR followed by amplicon sequence analysis to distinguish megalocytiviruses.

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[7]

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.

6.1.1. Definition of suspect case in apparently healthy animals

The presence of infection with RSIV shall be suspected if at least one of the following criteria is met:

i) Positive result by real-time or conventional PCR;

ii) Cyto- or histopathological changes consistent with RSIV infection or disease;

iii) Cytopathic effect in cell culture;

iv) Positive result from IFAT or ICC.

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with RSIV is considered to be confirmed if at least one of the following criteria is met:

i) Positive result by a recommended molecular or antigen detection test with confirmation by conventional PCR and sequence analysis, with sequence consistent with RSIV;

ii) Cyto- or histopathological changes consistent with the presence of the pathogen or the disease with confirmation by conventional PCR and sequence analysis, with sequence consistent with RSIV;

iii) Cytopathic effect in cell culture with confirmation by conventional PCR and sequence analysis, with sequence consistent with RSIV.

Reference Laboratories should be contacted for specimen referral when testing laboratories cannot undertake any of the recommended test methods. Positive test results will result in notification to the OIE.

6.2 Clinically affected animals

Clinical signs (see Section 2.3.2) 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 with RSIV shall be suspected if at least one of the following criteria is met:

[7] For example transboundary commodities.

i) Presence of gross pathology or clinical signs associated with RSIV disease as described in this chapter, with or without elevated mortality;

ii) Cyto- or histopathological changes consistent with the presence of the pathogen or the disease;

iii) Positive result from IFAT or ICC;

iv) Cytopathic effect typical for RSIV infectin in cell culture;

v) Positive result by real-time PCR or conventional PCR.

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with RSIV is considered to be confirmed if at least one of the following criteria is met:

i) Positive result by a recommended antigen detection test with confirmation by conventional PCR and sequence analysis, with sequence consistent with RSIV;

ii) Cyto- or histopathological changes consistent with the presence of the pathogen or the disease with confirmation by conventional PCR and sequence analysis, with sequence consistent with RSIV;

iii) Cytopathic effect in cell culture with confirmation by conventional PCR and sequence analysis, with sequence consistent with RSIV;

iv) Positive real-time PCR test with confirmation by conventional PCR and sequence analysis, with sequence consistent with RSIV.

Reference Laboratories should be contacted for specimen referral when testing laboratories cannot undertake any of the recommended test methods. Positive test results will result in notification to the OIE.

6.3. Diagnostic sensitivity and specificity for diagnostic tests [under study]

6.3.1. For presumptive diagnosis of clinically affected animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation

DSe: = diagnostic sensitivity, DSp = diagnostic specificity, qPCR: = real-time polymerase chain reaction.

6.3.2. For surveillance of apparently healthy animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation

DSe: = diagnostic sensitivity, DSp = diagnostic specificity, qPCR: = real-time polymerase chain reaction.

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* *

NB: There is an OIE Reference Laboratory for red sea bream iridoviral disease
(see Table at the end of this *Aquatic Manual* or consult the OIE web site for the most up-to-date list:
https://www.oie.int/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3).
Please contact the OIE Reference Laboratories for any further information on
infection red sea bream iridoviral disease.

NB: FIRST ADOPTED IN 2000); MOST RECENT UPDATES ADOPTED IN 20XX.

Return to Agenda

CHAPTER 2.4.1.

INFECTION WITH ABALONE HERPESVIRUS

[…]

2.2. Host factors

~~Currently, species known to be susceptible to AVG in Australia are the greenlip abalone (*Haliotis laevigata*), blacklip abalone (*H. rubra*) and hybrids of these two species. Clinical signs consistent with AVG have not been reported in other molluscan species in areas where AVG is suspected to be enzootic. In Chinese Taipei, ganglioneuritis associated with a herpes viral infection and high mortalities in the *H. diversicolor supertexta* abalone species have been reported. The disease was reported only in *H. diversicolor supertexta*, while cohabitating Japanese black abalone *H. discus* remained normal (Chang et al., 2005).~~

2.2.1. Susceptible host species

~~Greenlip abalone – Haliotis laevigata~~
~~Blacklip abalone – H. rubra~~
~~Hybrid (greenlip × blacklip) – H. laevigata × H. rubra~~
~~Diversicolor abalone or jiukong abalone - H. diversicolor~~

Species that fulfil the criteria for listing as susceptible to infection with abalone herpesvirus according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) are: Blacklip abalone (*Haliotis rubra),* greenlip abalone (*Haliotis laevigata),* hybrids of greenlip x blacklip abalone (*Haliotis laevigata* x *Haliotis rubra) and* small abalone (*Haliotis diversicolor).*

2.2.2. ~~Susceptible stages of the host~~ Species with incomplete evidence for susceptibility

~~All ages.~~

Species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to infection with abalone herpesvirus according to Chapter 1.5 of the *Aquatic Code* are: none known.

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following species, but no active infection has been demonstrated: Japanese abalone (*Haliotis discus*) and rainbow abalone (*Haliotis iris*).

[…]

Return to Agenda

CHAPTER 2.4.2.

INFECTION WITH *BONAMIA EXITIOSA*

[…]

2.2. Host factors

2.2.1. Susceptible host species

~~Oyster species *Ostrea chilensis* (= *Tiostrea chilensis* = *T. lutaria*) (Dinamani et al., 1987), *O. angasi* (Corbeil et al., 2006b; Hine, 1996; Hine & Jones, 1994), *O. edulis* (Abollo et al., 2008; Narcisi et al., 2010) and *O. stentina* (Hill et al., 2010).~~

Species that fulfil the criteria for listing as susceptible to infection with *Bonamia exitiosa* according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) are: Argentinean flat oyster (*Ostrea puelchana*), australian mud oyster *(Ostrea angasi),* chilean flat oyster *(Ostrea chilensis),* dwarf oyster *(Ostrea stentina),* eastern oyster *(Crassostrea virginica),* european flat oyster *(Ostrea edulis),* olympia oyster *(Ostrea lurida)* and suminoe oyster *(Crassostrea ariakensis)*

2.2.2. ~~Susceptible stages of the host~~ Species with incomplete evidence for susceptibility

~~In *O. chilensis*, recruit-sized oysters (oysters greater than or equal to 58 mm in length) are known to be susceptible (Dinamani et al., 1987). In *O. edulis*, the parasite was detected in market-sized (>60 mm) oysters (Abollo et al., 2008). There are no data concerning the other oyster stages, including spat.~~

~~DNA of *B. exitiosa* has recently been detected in larvae of flat oysters *Ostrea edulis* (Arzul et al., 2011).~~

Species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to infection with *B. exitiosa* according to Chapter 1.5 of the *Aquatic Code* are: none known

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following species, but no active infection has been demonstrated: Pacific cupped oyster (*Crassostrea gigas*) and sydney rock oyster (*Saccostrea glomerata*).

[…]

Return to Agenda

***Aquatic Manual* disease chapters Table 4.1. *OIE recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals:* current Key for Table 4.1 with suggested edits tracked)**

4.Diagnostic methods

The methods currently available for identifying infection that can be used in i) surveillance of apparently healthy ~~populations~~animals, ii) presumptive diagnosis in clinically affected animals and iii) confirmatory diagnostic purposes are listed in Table 4.1. by life stage.

~~The designations used in the Table indicate:~~

Ratings against purposes of use. For each recommended assay a qualitative rating against the purpose of use is provided. The ratings are determined based on multiple performance and operational factors relevant to application of an assay for a defined purpose. These factors include appropriate diagnostic performance characteristics, level of assay validation, successful application by diagnostic laboratories, cost, timeliness, and sample throughput. For a specific purpose of use, assays are rated as:

~~Key:~~

+++ =	Most suitable methods – desirable performance and operational characteristics.
++ =	Suitable method(s) acceptable performance and operational characteristics under most circumstances.
+ =	Less suitable methods – performance or operational characteristics may significantly limit 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.~~

Validation stage. The validation stage corresponds to the assay development and validation pathway in chapter 1.1.2. The validation stage is specific to each purpose of use. Where available, information on the diagnostic performance of recommended assays is provided in Section 6.3.

OIE Reference Laboratories welcome feedback on diagnostic performance of recommended assays, in particular PCR methods. Of particular interest are any factors affecting expected assay sensitivity (e.g. tissue components inhibiting amplification) or expected specificity (e.g. failure to detect particular genotypes). These issues should be communicated to the OIE Reference Laboratories so that advice can be provided to diagnostic laboratories and the standards amended if necessary.

[…]

Return to Agenda