

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

**Virtual meeting, 24 and 27 January, and from 16–23 February 2022**

**PART A – Texts to be proposed for adoption at the OIE 89th General Session in May 2022**

The OIE Aquatic Animal Health Standards Commission (the Aquatic Animals Commission) held its meeting electronically on the 24 and 27 January and from 16 to 23 February 2022. The list of participants is attached as [Annex 1](#).

Considering the ongoing COVID-19 pandemic, the 89th Annual General Session will be held in a semi-hybrid format from Monday 23 to Thursday 26 May 2022. During the 89th General Session new and revised chapters of the OIE International standards (the *Aquatic Animal Health Code*, the *Terrestrial Animal Health Code*, the *Manual of Diagnostic Tests for Aquatic Animals* and the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*) will be proposed for adoption.

To facilitate this process, the February 2022 meeting report of the Aquatic Animals Commission will be distributed in two parts: Part A (herewith) provides information about the new and revised texts for the *Aquatic Code* and the *Aquatic Manual* that will be proposed for adoption at the 89th General Session; and Part B (to be published in April 2022) will provide information about other topics discussed at the Commission's February 2022 meeting including texts circulated for comment and information.

In preparation for the 89th General Session, the OIE will once again organise pre-General Session information webinars to ensure that Members are aware of the background and key aspects of the standards being presented for adoption. Attendance to these webinars will be by invitation only. Please note that Delegates will soon receive detailed information about the 89th General Session, and in particular the process for the adoption of standards.

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 the Commission's September 2021 report: Australia, Canada, Chile, China (People's Rep. of), Chinese Taipei, Colombia, Japan, Korea (Rep. of), New Caledonia, New Zealand, Norway, Switzerland, Thailand, United Kingdom (the UK), United States of America (the USA), the Member States of the European Union (the EU) and the African Union Inter-African Bureau for Animal Resources (AU-IBAR) on behalf of the African Members of the OIE. 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 prior to the deadline and were supported by a rationale. The Commission made amendments to draft texts, where relevant, in the usual manner by 'double underline' and '~~strike through~~'. In relevant annexes, amendments proposed at this meeting are highlighted with a coloured background to distinguish them from those made previously. Due to the large number of comments, the Commission was not able

to provide a detailed explanation of 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 reminded Members that *ad hoc* Group reports can be found on the [OIE Website: https://www.oie.int/en/what-we-do/standards/standards-setting-process/ad-hoc-groups/](https://www.oie.int/en/what-we-do/standards/standards-setting-process/ad-hoc-groups/). 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 below includes the agenda items addressed by the Aquatic Animals Commission at this meeting and includes links to relevant items within this report. Members should note that the texts in **Annexes 2, 3, 4, 7, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21 and 22** will be proposed for adoption at the 89th General Session in May 2022. **Annexes 5, 6, 8 and 17** are provided for Members information.

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## **1. WELCOME FROM THE DEPUTY DIRECTOR GENERAL INTERNATIONAL STANDARDS AND SCIENCE**

Dr Montserrat Arroyo, the OIE Deputy Director General, International Standards and Science, welcomed members of the Aquatic Animals Commission and thanked them for their ongoing contributions to this work, noting the significant challenges posed by the ongoing COVID-19 pandemic, such as virtual meetings. Dr Arroyo commended the Commission for its ambitious agenda and on consistently providing high quality information in its reports. She extended her appreciation to the members' employing institutions and national governments.

Dr Arroyo briefed the members on preparations for the semi-hybrid 2022 OIE General Session, including pre-General Session webinars that will be conducted by each of the OIE Specialist Commissions to inform Members about the revised and new standards that will be proposed for adoption. She also informed the Commission that the Technical Item would be on the OIE and Veterinary Services engagement in global, regional and national Emergency Management Systems. Dr Arroyo provided a summary of ongoing work on the OIE standards development and review system, including the development and planning for digital tools. Finally, she informed the Commission of an 'after-action review' conducted by the OIE in response to the COVID-19 pandemic.

Dr Arroyo and the members of the Aquatic Animals Commission discussed the importance of ensuring Member's involvement in the OIE Standards setting process, and how to best support them to engage in this process. Dr Arroyo informed the Aquatic Animals Commission of the launch of a survey by the OIE Observatory to investigate the barriers to the implementation of aquatic animal health and welfare standards as part of the implementation of the Aquatic Animal Health Strategy. She also thanked the members of the Commission for participating in a pilot phase to test an online commenting system.

The members of the Aquatic Animals Commission thanked Dr Arroyo for the excellent support provided by the OIE Secretariat.

## **2. MEETING WITH THE DIRECTOR GENERAL**

Dr Monique Eloit, the OIE Director General, met the Aquatic Animals Commission on 23 February 2022 and thanked its members for their support and commitment to achieving OIE objectives. She recognised the Commission's efforts and adaptability to develop new ways of working despite the challenges imposed by the COVID-19 pandemic. Dr Eloit provided an update on the 89th OIE General Session preparation and informed the Commission of a new initiative to review the OIE Science system.

Dr Eloit informed the Commission of the budgetary situation of the Organisation and noted that due to the continued increase of activities, the current regular budget would not be sufficient to ensure the sustainable delivery of some core OIE activities. Dr Eloit highlighted that this situation might impact how the Commission and its Secretariat undertake some of their work. Dr Eloit acknowledged the work already being done by the Commission and the OIE Secretariat in prioritisation of its work and ensuring alignment with the priorities of the OIE Aquatic Animal Health Strategy.

The Commission welcomed the initiative to review the OIE Science system and noted that this work should also take into consideration how this system interacts with the OIE Standard setting process.

The Aquatic Animals Commission thanked Dr Eloit for making time to meet with its members and commended the excellent work of the Secretariat for meeting preparations and its work during the meeting especially given the challenges of virtual meetings.

## **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 4.1.2.2.).

#### 4. THE OIE AQUATIC ANIMAL HEALTH CODE

##### 4.1. Texts to be proposed for adoption in May 2022

The Aquatic Animals Commission thanked Members for highlighting translation issues in some of the Annexes circulated for comments in the French and Spanish versions of the September 2021 Aquatic Animals Commission report, and noted that these have been reviewed and corrected.

###### 4.1.1. User's Guide

Comments were received from Colombia, New Caledonia, Switzerland and the EU.

###### *Background*

At its September 2021 meeting, the Aquatic Animals Commission proposed amendments to the User's Guide to improve readability and ensure that it reflected key amendments made in the 2021 edition of the *Aquatic Code*.

###### **Previous Commission reports where this item was discussed:**

September 2021 report (Item 5.1.1., page 6).

###### **February 2022 meeting**

In point 1 of Section A. Introduction, the Commission acknowledged a general comment requesting that an emphasis be placed on the importance of welfare for aquatic animals in general rather than focusing only on farmed aquatic animals. The Commission did not agree and reminded Members that the *Aquatic Code* currently only addresses welfare standards related to farmed fish.

In the second sentence of point 6 in Section B. *Aquatic Code* content, the Commission agreed with a comment that 'disposal of aquatic animal waste' would be complemented by the addition of 'handling, and treatment' to align with the title of Chapter 4.8. Handling, disposal and treatment of aquatic animal waste, and amended the text accordingly.

The revised User's Guide is presented as [Annex 2](#) and will be proposed for adoption at the 89th General Session in May 2022.

###### 4.1.2. Glossary definitions

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

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

###### *Background*

At its February 2021 meeting, the Aquatic Animals Commission proposed amendments to the Glossary definitions for 'Basic biosecurity conditions', 'Early detection system' and proposed a new Glossary definition for 'Passive surveillance'. These amendments were to ensure alignment with the proposed amendments to Chapter 1.4. Aquatic Animal Health Surveillance. The revised definitions were circulated for comment in the Commission's February 2021 report.

At its September 2021 meeting, the Commission considered comments received and amended the definitions as appropriate. The Commission also proposed to amend the definition of ‘Biosecurity plan’, which had not previously been circulated for comment, to include a reference to Chapter 4.1. Biosecurity for aquaculture establishments. The revised definitions were circulated for comment in the Commission’s September 2021 report.

**Previous Commission reports where this item was discussed:**

February 2021 (Part B: Item 1.1., page 3); September 2021 (Item 5.1.2.1., page 6).

**February 2022 meeting**

**Basic biosecurity conditions**

The Commission noted Member’s support for the proposed definition.

**Biosecurity plan**

The Commission did not agree with a comment to delete ‘to zone and compartment’ as Article 5.3.7. Sequence of steps to be taken in establishing a zone or compartment and having it recognised for international trade purposes, indicates the requirement of a ‘biosecurity plan’ for aquaculture establishments and for recognition of a zone or compartment for the purposes of international trade.

The Commission also did not agree with a comment that the measures applied to mitigate the identified risk in the biosecurity plan should only be in accordance with the recommendations in Article 4.1.7. as it considered that the recommendations for a biosecurity plan are broader than the recommendations in Article 4.1.7. However, the Commission agreed that the context for the use of the term ‘biosecurity plan’ is broader than Chapter 4.1. and agreed to delete the reference to ‘Chapter 4.1.’ and revert to the current text ‘*Aquatic Code*’. Consequently, there are no proposed amendments to the Glossary definition of ‘biosecurity plan’.

**Early detection system**

The Commission did not agree to add ‘including an attempt for disease diagnosis’ after ‘investigation’ as it considered that this was clear as written and that this point is addressed in the proposed new Article 1.4.18. Diagnostic confirmation of a listed disease or an emerging disease.

The Commission did not agree with a comment to harmonise this definition with that in the *Terrestrial Code*. Members were reminded that the definition of ‘Early detection system’, like all Glossary definitions, is for the purposes of the *Aquatic Code* and that it had been modified in conjunction with the amendments made to Chapter 1.4. The *Terrestrial Code* definition of an ‘Early detection system’ does not align with the proposed amendments to Chapter 1.4.

The Commission did not agree to add ‘control or eradication’ after ‘investigation’ as it considered that the definition should not include all steps within a disease response, but rather that an ‘early detection system’ would contribute to an initial disease investigation.

**Passive surveillance**

The Commission noted some divergent views on the definition but reminded Members that the definitions in the Glossary are for the purposes of the *Aquatic Code*, as indicated at the top of the Glossary.

The Commission agreed with a comment to provide more guidance and clarity on the types and sources of information that would be part of a passive surveillance system and amended the definition accordingly.

The revised Glossary definitions for ‘Basic biosecurity conditions’, ‘Early detection system’ and ‘Passive surveillance’ are presented as [Annex 3](#) and will be proposed for adoption at the 89th General Session in May 2022.

#### *4.1.2.2. ‘Competent Authority’, ‘Veterinary Authority’ and ‘Aquatic Animal Health Services’*

Comments were received from Australia, Canada, China (People’s Rep. of), Colombia, New Caledonia, Switzerland and the EU.

##### *Background*

At its September 2018 meeting, 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. The revised definitions were circulated for comment in the Code Commission’s September 2018 report. The *ad hoc* Group on Veterinary Services considered the comments submitted and proposed revised definitions.

At their respective September 2020 meetings, the Code Commission and the Aquatic Animals Commission discussed the importance of ensuring alignment of these definitions in the two Codes except where differences could be justified and agreed to circulate 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* for comment in the September 2020 report of the Code Commission and the Aquatic Animals Commission, respectively. Neither Commission addressed comments received during their respective February 2021 meetings due to time constraints.

In preparation for the September 2021 meetings, the Presidents of the two Commissions met to review all comments previously received. 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 respective September 2021 Commission reports, as well as some more detailed information on the use of these terms in each Code.

At its September 2021 meeting, the Aquatic Animals Commission considered the comments received on its September 2020 report, as well as the feedback from the Presidents discussions, and the outcome of the Code Commission’s discussions at its September 2021 meeting. The Aquatic Animals Commission made one additional amendment to the definition for ‘Veterinary Authority’ that was not included in the Code Commission proposal but otherwise the definitions were aligned. The revised definitions were circulated for comment in the Aquatic Animals Commission September 2021 report.

##### **Previous Commission reports where this item was discussed:**

September 2020 (Item 4.5.3., page 9); September 2021 (Item 5.1.2.2., page 7).

##### **February 2022 meeting**

The Commission noted that most comments submitted were in support of the proposed definitions.

In response to a general comment requesting clarification regarding the responsibilities and interactions between the different organisations who fulfil the roles of ‘Aquatic Animal Health



Services’, ‘Competent Authority’ and ‘Veterinary Authority’ the Commission reminded Members that a detailed explanation was provided in the Commission’s September 2021 report. The Commission reiterated that 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 Aquatic Animal Health Services.

In response to a comment to clarify the meaning of the term ‘standards’, the Commission agreed with the suggestion to revise the paragraph on the SPS agreement in the foreword of the *Aquatic Code* to clarify that ‘standards’ refers to all chapters and articles of the *Aquatic Code*. The Commission will also consider amendments elsewhere in the *Aquatic Code* where appropriate. The Commission informed Members that when undertaking this work that it would ensure that any changes are aligned in the *Terrestrial Code*, where relevant.

The revised Glossary definitions for ‘Competent Authority’, ‘Veterinary Authority’ and ‘Aquatic Animal Health Services’, are presented as [Annex 3](#) and will be proposed for adoption at the 89th General Session in May 2022.

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

Comments were received from Australia, Chinese Taipei, Colombia, New Caledonia, Switzerland, Thailand and the EU.

##### *Background*

At its September 2017 meeting, 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.

The *ad hoc* Group on Infection with tilapia lake virus conducted its work electronically between November 2017 and September 2021.

At its September 2021 meeting, the Commission considered the *ad hoc* Group’s final report and noted its conclusion that there are reliable diagnostic methods for TiLV. The Commission reviewed its previous assessment of infection with TiLV against the criteria in Article 1.2.2. It agreed that Criteria 1, 2, 3, 4b and 4c were met and therefore infection with TiLV should be proposed for listing in Article 1.3.1. of Chapter 1.3. Diseases listed by the OIE. The Commission circulated the revised Article 1.3.1. for comment in its September 2021 report.

The Assessment for listing infection with tilapia lake virus was provided for Member information in the September 2021 Report of the Commission (<https://www.oie.int/en/what-we-do/standards/standards-setting-process/aquatic-animals-commission/#ui-id-3>).

##### **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 (Item 5.1.3., page 11).

## February 2022 meeting

The Commission noted the general support of Members for the listing of infection with tilapia lake virus in Chapter 1.3. Diseases listed by the OIE, and updated the assessment against the listing criteria to reflect recently published scientific information.

The Commission agreed with a comment requesting the OIE to apply the same approach for future emerging disease events as was applied to infection with TiLV. The Commission informed Members that this approach would be formalised through future work of the Commission.

The Commission did not agree with a comment that infection with TiLV does not meet Criteria No. 4b and No. 4c of Article 1.2.2. (i.e. affect the health of cultured and wild animals respectively) and therefore should not be proposed for listing. The Commission noted that different strains of TiLV have shown different virulence between susceptible species and that those strains that are highly virulent pose a threat to farmed and wild tilapia populations. The Commission agreed that the study (Piamsomboon *et al.*, 2021), which was provided as support for infection with TiLV not meeting all the criteria for listing, did not provide any evidence on the absence of pathogenicity. Of most significance within the study was the detection of PCR positives in Asian sea bass (*Lates calcarifer*). The Commission reiterated that a finding of subclinical infection in one circumstance cannot be extrapolated to absence of pathogenicity in all circumstances.

### Reference:

PIAMSOMBOON, P.& WONGTAVATCHAI, J. (2021). Detection of Tilapia Lake Virus (TiLV) in healthy fish from the pre-existing disease environment using different RT-PCR methods. *Turkish Journal of Fisheries and Aquatic Sciences*, **21**, 205-209. [http://doi.org/10.4194/1303-2712-v21\\_4\\_05](http://doi.org/10.4194/1303-2712-v21_4_05)

The Commission agreed with a comment that if listing of infection with tilapia lake virus in Chapter 1.3. Diseases listed by the OIE, is adopted in May 2022, an OIE Reference Laboratory for infection with tilapia lake virus will need to be designated.

The revised and updated 'Assessment of infection with tilapia lake virus (TiLV) for listing in Chapter 1.3. of the *Aquatic Code*', is presented as [Annex 5](#) for Member information.

The revised Article 1.3.1. of Chapter 1.3. Diseases listed by the OIE is presented as [Annex 4](#) and will be proposed for adoption at the 89th General Session in May 2022.

## 4.1.4. Approaches to demonstrate disease freedom

### Background

At its September 2018 meeting, the Aquatic Animals Commission developed a discussion paper on approaches for determining periods required to demonstrate disease freedom, which was circulated for comments. At its September 2019 meeting, the Commission considered comments received and circulated a revised paper for comments. At its February 2020 meeting, the Commission developed model Articles X.X.4.–X.X.8. to replace the existing articles in the disease-specific chapters of the *Aquatic Code*. The model articles were circulated for comments in the Commission's February 2020 report.

At its September 2020 meeting, the Commission considered all comments received and agreed that Chapter 1.4. Aquatic animal health surveillance, needed to be revised to better complement the proposed model articles. The revised Chapter 1.4. and the model Articles X.X.4. to X.X.8. for disease-specific chapters to address declaration of freedom from [Pathogen X], were circulated for comment in the Commission's February 2021 report. At its September 2021 meeting, the

Commission considered all comments received and amended the texts as appropriate, and circulated the revised Chapter 1.4. and the model Articles X.X.4. to X.X.8. for disease-specific chapters to address declaration of freedom from [Pathogen X] for another round of comments.

**Previous Commission reports where this item was discussed:**

September 2018 (Item 2.10., page 11); September 2019 (Item 6.6., page 9); February 2020 (Item 7.2.2., page 15); September 2020 (Item 6.2., page 16); February 2021 (Part B: Item 1.2., page 4); September 2021 (Item 5.1.4., page 12).

*4.1.4.1. Chapter 1.4. Aquatic Animal Health Surveillance*

Comments were received from Australia, Canada, Chile, China (People's Rep. of), Colombia, New Caledonia, Norway, Switzerland, the UK, the USA and the EU.

**February 2022 meeting**

**General Comments**

The Commission thanked Members for their comprehensive comments and noted the general support for the proposed chapter.

The Commission agreed with a comment to further develop the application procedures for OIE publication of self-declarations of freedom and for possible mechanisms to encourage Members to submit applications. The Commission agreed that it would discuss this issue further and also investigate proposal to require annual updates to confirm that the requirements for maintenance of freedom in Chapter 1.4 are being met.

The Commission agreed that 'Competent Authorities', 'the Competent Authority' and 'a Competent Authority' had been applied inconsistently throughout the proposed chapter. It noted that there may be more than one Competent Authority involved in a self-declaration of freedom and so replaced 'the Competent Authority' with 'a Competent Authority' or 'Competent Authorities', where relevant.

In response to a comment requesting the publication of scientific assessments for the default minimum periods for basic biosecurity conditions included within the disease-specific chapters, the Commission informed Members that detailed information on the proposed default minimum periods has been provided in previous reports of the Commission. The Commission encouraged Members to refer to its previous reports.

The Commission did not agree with a comment requesting a delay in proposing the amended Chapter 1.4. for adoption. It noted that the development of this chapter has been thoroughly consulted since 2018 and Members' have expressed overall support for the proposed amended chapter, which the Commission considered to be a vast improvement on the current chapter. The Commission emphasised that it is important to provide guidance to Members on the requirements for surveillance to support the proposed changes in the disease-specific chapters regarding self-declaration of freedom, and agreed to propose the revised chapter for adoption. The Commission reminded Members that it will be possible to continue to improve the chapter, if necessary, after adoption.

**Article 1.4.1.**

The Commission did not agree with a comment to add 'a specific' after 'self-declaration of freedom from' as 'self-declaration of freedom from disease' is a defined term in the Glossary that includes a reference to a specific disease.

## **Article 1.4.2.**

Minor editorial changes were made to this article as described in the general comments above.

## **Article 1.4.3.**

In response to a comment to add a point on shared water bodies in Article 1.4.12., the Commission agreed that this was an important point that had been captured in the disease-specific chapters of the *Aquatic Code*, but not in Chapter 1.4. The Commission agreed that it would be more appropriate to add it to the first paragraph of Article 1.4.3., to emphasise that the evidence used to support a claim of freedom must account for shared water bodies.

In point 1, the Commission did not agree to add a new pathway for shared water bodies as the proposed pathways consider the situations at the country and zone levels, both of which may include shared water bodies. The Commission also added wording in the introductory text to clarify that all pathways must account for shared water bodies.

In point 1, the Commission did not agree to add ‘(excluding species with incomplete or no evidence of susceptibility)’ as it considered the glossary definition of ‘susceptible species’ to provide sufficient clarity.

The Commission did not agree with a comment to change ‘passive surveillance information’ to ‘passive surveillance data’ as passive surveillance may provide more qualitative information than just data, which implied empirical evidence. Similar replacements had previously been made throughout the chapter at the September 2021 meeting.

In point 2, the Commission agreed with a comment to add ‘at the country or zone level’ to align with Table 1.1. The Commission also agreed to add the levels of application for the other pathways within the respective points for consistency.

The Commission did not agree with a comment to add ‘when historical freedom can not be demonstrated’ to the title of pathway 3 as the capacity to claim freedom using any pathway would not be restricted based on the inability to demonstrate freedom through any other pathway. The choice of which pathway to use for a self-declaration of freedom depends on the specific circumstances of the situation. To remove any ambiguity, the Commission deleted part of the first sentence.

In the final sentence of pathway 3, the Commission did not agree with a comment that supplementary passive surveillance information must be quantitative and agreed it would need to be judged on its merits, not on whether it is qualitative or quantitative.

In the final sentence of pathway 3, the Commission did not agree to replace ‘information may also be used in this pathway’ with ‘may also contribute evidence to this pathway’ as it was not considered an improvement.

The Commission agreed with a comment that a flowchart providing a visual representation of the pathways of freedom, in theory, may be of assistance to Members but did not want to delay proposing the new chapter for adoption in order to incorporate such a flowchart.

In pathway 1 of Table 1.1., the Commission did not agree with a comment to add compartment as a level of application for pathway 1, as it considered that targeted surveillance is always required to demonstrate that biosecurity measures are effective to establish a free status for a compartment. However, the Commission agreed that additional guidance on compartmentalisation is required and noted that this would be a logical progression of work following adoption of Chapter 4.1 on Biosecurity for Aquaculture Establishments and the new guidance on declaration of freedom. The commission agreed to prioritise revision of Chapter 4.2. Zoning and Compartmentalisation, within

it's work plan to ensure additional guidance and clarity on compartmentalisation is provided to Members.

In pathway 3 of Table 1.1., the Commission did not agree to replace 'population' with the defined term 'study population' under 'Secondary evidence to claim freedom' as it considered that the use of 'population' within this context is more appropriate than the Glossary definition of 'study population'.

In Table 1.1., the Commission did not agree that there was no difference between pathways 3 and 4. The Commission agreed that while the two pathways were similar, the context of applying them is different and that the proposed chapter provides guidance when declaring freedom using those pathways.

#### **Article 1.4.4.**

In point 2, the Commission agreed with a comment to replace 'confirm' with 'verify' as this was a more suitable word.

In the first sentence of the final paragraph, the Commission deleted 'Except when otherwise provided for in the disease-specific chapter' to remove inconsistencies with Article 1.4.16. which indicates that 'Apparent disease at any level in a target population automatically invalidates any freedom from disease claim.'. The Commission also noted that the *Aquatic Code* disease-specific chapters do not allow for Members to maintain a claim of freedom when an outbreak has occurred.

#### **Article 1.4.5.**

The Commission did not agree with a comment to reconcile Article 1.4.5. with Chapter 4.1. Biosecurity for aquaculture establishments, as Article 1.4.5. provides guidance on the biosecurity and surveillance that is applied at the national level while Chapter 4.1. provides guidance on biosecurity applied at the establishment level.

The Commission agreed with a comment to remove point 3 as an early detection system is included within the requirements of Article 1.4.6. Basic biosecurity conditions. However, to ensure that the requirement for an early detection system was emphasised, the Commission added '(which include an early detection system)' to point 2 after 'basic biosecurity conditions'.

#### **Article 1.4.6.**

In point 1, the Commission did not agree with a comment to revert to the previous text and include a requirement for 'compulsory requirement for notification of a specific disease, or suspicion of the disease to a Competent Authority'. The Commission considered that it was unnecessary as point 1 in Article 1.4.6. refers to Article 1.4.7. which includes a legal obligation to report listed diseases. However, the Commission added 'emerging diseases' to point 2 of Article 1.4.7. as it considered that recognition and reporting of emerging diseases is of significance to the performance of the early detection system.

In point 2, the Commission did not agree with a comment to add 'anthropogenic' after 'measures to prevent the' as the scope of basic biosecurity conditions is for a country, zone or compartment. While some pathways relating to the movement of wild animals may not be manageable at some levels, at others they can be; for example, compartments may erect barriers to prevent the entry of wild aquatic animals (as recommended in Article 4.1.7. point 1 j).

#### **Article 1.4.7.**

In the first paragraph, the Commission agreed with a comment to clarify that the objective of an early detection system extended beyond the collection of information for a declaration of freedom. As such

the Commission amended the wording to ‘The early detection system of the Competent Authority is important to generate evidence for claims of disease freedom and to provide assurance that a change in disease status would be rapidly discovered’.

In point 3, the Commission did not agree with a comment to add ‘Veterinary Authority or designated Competent Authority’ and delete ‘Aquatic Animal Health Services’ as Aquatic Animal Health Services is the appropriate term to use because a disease investigation may not always be completed by a government authority.

In point 3, the Commission agreed with a comment to add ‘led by a Competent Authority’ at the end of the sentence as a Competent Authority should lead emergency aquatic animal disease response activities.

In point 4, the Commission did not agree with a comment to delete ‘Aquatic Animal Health Services’ and add ‘Competent Authority’ as laboratory services are not always within the Competent Authority of Member Countries and could be contracted by the private sector or other countries.

In point 5, the Commission did not agree with a comment to delete ‘with an occupational role with aquatic animals’ as aquatic animal health professionals are authorised by the Competent Authority and ‘others’ indicates a broad public responsibility. The Commission noted that Members had previously requested other possible occupational roles be included within point 5 and proposed adding ‘with an occupational role with aquatic animals’ to broaden the scope of the point to address those comments.

The Commission did not agree to add a point 6 requiring listing of specific notifiable diseases within Member Country legislation as it considered that the inclusion of ‘listed diseases and emerging diseases’ within point 5 already addressed this concern. However in order to emphasise this, the Commission amended point 5 to ‘..suspicion of the occurrence of listed diseases or emerging diseases...’.

The Commission did not agree to add a point 6 requiring ‘enhanced awareness of the status of susceptible species populations through time’ as the proposed point is an outcome measure while the list within Article 1.4.7. includes input measures.

In the first sentence of the eighth paragraph, the Commission did not agree to add ‘in a timely manner’ after ‘detected’ as it could be confusing considering that sensitivity for passive surveillance is estimated as a default annual basis of 30% which accumulates over a 10-year period to 95%. In the second sentence, the Commission also did not agree to add ‘or investigation’ as investigation is covered in the definitions of passive surveillance and an early detection system.

In the second sentence of the last paragraph, the Commission did not agree to delete ‘diagnostic’ before ‘assays’ as it was considered that the use of ‘diagnostic assays’ assists with understanding and is consistent with the Glossary definitions for disease and diagnosis.

In the last paragraph, the Commission did not agree with a comment to delete ‘can be quantified, for example, by use of a scenario tree model, however, in most circumstances a qualitative assessment will be sufficient’ and to add text on reporting that is repeated elsewhere, as the proposed changes would remove unique guidance on measuring sensitivity and did not improve the existing text.

#### **Article 1.4.8.**

In point 1 a), the Commission agreed that there was repetitive information to that provided in point 4. The Commission agreed to delete point 4 as it did not provide any additional information.

In point 1 a), the Commission did not agree with a comment to add ‘in that species’ after ‘disease’ as it was not considered an improvement.

In point 1 b), the Commission agreed that there was some repetition between point 1 and 5 in Article 1.4.7. and point 1b) of Article 1.4.8. Therefore, the Commission deleted ‘there should be sufficient awareness by potential observers of the study population, such that’ to remove any repetition while maintaining the unique information regarding investigation.

In point 1 d), the Commission did not agree to add ‘(or proxy or sentinel populations)’ after ‘they’ as it considered the current text more explicit.

In point 1 d ii), the Commission did not agree to:

- add ‘the Competent Authority can demonstrate that’ as the Commission considered that may be difficult to obtain evidence that can demonstrate an epidemiological link;
- remove the point as it was considered necessary guidance for Members and that disease occurrence in an adjacent farmed population would be part of the early detection system and passive surveillance;
- add ‘epidemiologically linked’ before ‘farmed populations’ as the concept is already embedded at the beginning of the sentence.

In point 2, the Commission did not agree to delete the point as it was considered necessary guidance for Members.

In the second sentence of point 2, the Commission agreed to amend the reference to ‘points 1a), b and d) may not be’ to clarify that for wild populations, some aspects stated under point d ii) should be met for passive surveillance.

In the second sentence of point 3, the Commission did not agree to delete ‘and surveys (e.g. of wild populations)’ but agreed that to clarify the purpose of the surveys a different example should be provided ‘(e.g. fisheries and aquatic fauna surveys)’.

#### **Article 1.4.9.**

In point 1 the Commission agreed with a comment to add ‘or’ after point 1a) and replace the ‘and’ after point 1b) with an ‘or’ as the different pathways would not all apply in one situation.

In the second sentence of point 2 and in point 2b), the Commission did not agree to add an option and guidance for a shorter default minimum periods as the consensus from the consultation process with Members was that the 10 year default minimum should be retained. 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. However, the Commission does not intend to develop such standards and if a faster pathway for a self- declaration of freedom was desired, pathway 3- Targeted surveillance, could be used.

At the end of point 2 b), the Commission agreed to add ‘recommended in the disease-specific chapters’ as it is important to clarify that these are criteria for disease-specific chapter determinations and not intended for country-specific evaluation.

In point 2 b) iv), the Commission agreed to delete ‘and therefore the likelihood of detection’ as all listed factors (i-vi) are intended to inform the likelihood of annual detection and is a repetition from 2 b) above.

In point 2 b v), the Commission agreed to amend the wording to ‘(i.e., periods of the year when prevalence and intensity of infection is highest and most conducive to detection)’ to ensure consistency with point 2 c) and Article 1.4.10.

In point 2 c), the Commission did not agree to add the possibility of having a shorter requirement for basic biosecurity conditions as it was considered that basic biosecurity conditions are controls implemented at a national level and not related to production cycle within a premises.

Within sentences 2 and 3 in point 2d), the Commission agreed to add wording to ensure that it is explicit that the introduction route for the disease occurrence must be identified and mitigated before pathway 4 can be completed.

#### **Article 1.4.10.**

In the fourth sentence of the sixth paragraph, the Commission agreed with a comment that wild populations should be considered for sampling as there may be different species in the wild that may be more likely to show signs of disease than those being farmed. The Commission also agreed to delete ‘at the farm level’ to clarify that any population (farmed or wild) could be sampled.

In the sixth paragraph, the Commission did not agree that continuous sampling could be used as there is 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 pathogenic agent. However, the Commission recognised that obtaining a three month interval between surveys might be challenging in some circumstances, however, it was considered to be better placed in the third paragraph of ‘Requirements for targeted surveillance’ in Article 1.4.13. The Commission agreed to amend the text to include flexibility for these specific situations by adding: ‘In situations where seasonal conditions do not permit a gap of at least three months between surveys, the maximum possible time gap should be allowed to elapse between one survey and the next.’

In the last paragraph, the Commission did not agree to add ‘effective in accordance with the *Aquatic Code* for a specific pathogen’ to address detection of possible lingering infection. According to the requirements in disease-specific chapters, all aquatic animals are required to have been destroyed and then restocked. Depopulation is the first step in establishing that the pathogenic agent has been eliminated; only through a stepwise process including depopulation, cleaning, disinfection and fallowing followed by targeted testing can elimination of a pathogenic agent be confirmed.

#### **Article 1.4.11.**

At the end of the first paragraph, the Commission did not agree to add ‘Absence of susceptible species is not a pathway to prove freedom for compartments’ as the application of the pathways is outlined in Table 1.1. and declaration of freedom at a compartment level would not be required for trade in species that are not considered susceptible.

The Commission reiterated that it did not agree with comments also made on other articles to add compartments 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.

The Commission did not agree to remove the second paragraph as it is a requirement to ensure that no susceptible species have been introduced for the pathway to be utilised and basic biosecurity conditions must be maintained to ensure consistency with Article 1.4.9.

In point 2 a), the Commission agreed to add ‘reports which provide evidence regarding’ as it added clarity.

In the penultimate paragraph, the Commission did not agree to delete ‘pathogenic agent’ and add ‘susceptible species’ as it was not considered an improvement.

The Commission reminded Members that each pathway is intended to support a claim of freedom independent of other pathways. The Commission considered that Article 1.4.3. and this article emphasise that pathway 1 would only be applicable for commencing production of a new species,



that is listed as susceptible in Article X.X.2. of the disease-specific chapters, in a country or zone where it has been demonstrated that no susceptible species were previously present. Once a new species has been introduced, further declarations of freedom would require the use of pathway 3- Targeted surveillance. The use of pathway 1 would be chosen depending on the circumstances of the specific situation.

#### **Article 1.4.12.**

In the first sentence of the first paragraph and in point 2, the Commission did not agree with a comment to add compartment as an applicable level of application for pathway 2 – Historical freedom. The Commission considered that while historical health records may support a self-declaration of freedom, targeted surveillance is required to demonstrate that biosecurity measures are effective. Targeted surveillance is a fundamental requirement to establish free status of a compartment.

In point 1, the Commission agreed with a comment to add ‘or zone’ and to add cross-references to Articles 1.4.6. and 1.4.7. to ensure consistency within the Chapter.

In the first paragraph of ‘Requirements for passive surveillance’, the Commission did not agree to add guidance on how 95% confidence could be quantified and found equivalent to other pathways. Instead, the Commission agreed to delete the paragraph as it was considered that the information was found in Article 1.4.9.

In the second sentence of the second paragraph of ‘Requirements for passive surveillance’, the Commission agreed to delete ‘cover’ and add ‘represent’ to emphasise that the Early Detection Systems should be representative of the populations of susceptible species in the country or zone.

In the section, ‘Need for targeted surveillance’, the Commission did not agree to add ‘(i.e. population under sufficient surveillance, species susceptible to show clinical signs, environmental conditions conducive to clinical expression)’, as it was considered to not be an improvement and created unnecessary repetition within the chapter.

#### **Article 1.4.13.**

The Commission did not agree to change the title of pathway 3 to ‘Surveillance when Historical freedom cannot be demonstrated’ as all four pathways are available and the most suitable would be chosen by a competent authority depending on the circumstances.

In the third paragraph of ‘Requirements for targeted surveillance’, the Commission did not agree to remove the second sentence, as it would remove the guidance on the duration of the survey required to obtain freedom. The Commission noted that there was consensus for the 2-year duration for surveys from the extensive consultation completed.

With the addition of the new sentence as a result of comments on Article 1.4.10., the Commission split the third paragraph of ‘Requirements for targeted surveillance’ into two paragraphs for readability.

In the first sentence of the new fourth paragraph of ‘Requirements for targeted surveillance’, the Commission agreed with a comment to:

- delete ‘or greater’ after ‘95% confidence’ and to add ‘would be detected if present at or above’ after ‘pathogenic agent’ to clarify that the surveillance sensitivity (confidence) calculates the probability of detecting a pathogenic agent if present;

- add ‘Over the period of targeted surveillance, the combined’ and ‘in the country, zone or compartment’ after ‘design prevalence’ as it was considered to provide additional clarity and guidance.

In the new fourth paragraph of ‘Requirements for targeted surveillance’, the Commission agreed with a comment to add a cross-reference Article 1.4.16. for establishing the design prevalence. However, the Commission did not agree to refer to the relevant disease-specific chapter of the *Aquatic Manual* as design prevalence is not presented in the *Aquatic Manual* and would always need to be determined based on the circumstances of the survey, in addition to disease-specific factors.

In ‘Other sources of data’, the Commission did not agree with a comment to add a requirement for quantification of the passive surveillance system as it was considered to not be an improvement and too complex for most Members to implement. The Commission also considered that the ‘other sources of data’ should not be the core evidence to support the claim of freedom. It is up to the Competent Authority to demonstrate that the information used to support the claim of freedom is sufficiently rigorous and the Commission considered that there was sufficient guidance in the proposed chapter to support Members to do this.

#### **Article 1.4.14.**

In the first sentence in the first paragraph of point 2, in 2a) and in the first sentence of point 3, the Commission did not agree with a comment to remove the requirement for depopulation of infected populations and to add a requirement for pathogen eradication or containment as this pathway concerns the return to freedom after a disease outbreak. The Commission considered that a return to freedom could not be achieved without depopulation either by slaughter or moving the animals to an infected area outside the zone or compartment. Depopulation is the first step in establishing that the pathogenic agent has been eliminated; only through a stepwise process including depopulation, cleaning, disinfection and fallowing followed by targeted testing can elimination of a pathogenic agent be confirmed.

In point 2 b), the Commission did not agree with a comment to add ‘vessels’, or ‘staff’ as it considered that the point did not need to be inclusive of all possible pathways of exposure.

In the fourth sentence of the last paragraph of point 2, the Commission agreed with a comment to delete ‘is not present’ and add ‘would not be detected if present at’ as the surveillance sensitivity (confidence) calculates the probability the pathogen would be detected if present. Similarly in the final sentence of point 3, the Commission also agreed to delete ‘is not present above’ and add ‘would be detected above’.

#### **Article 1.4.15.**

In point 2, the Commission did not agree with a comment to delete points a) and b), as it agreed that targeted surveillance is required at the zone or compartment level except when they occur within a country that is declared free. The Commission noted that there are several reasons why a compartment may be established within a free country such as to prevent the introduction of other diseases for which the country is not free, to obtain a higher level of assurance of disease freedom, in preparation for possible future disease outbreaks within the country or for valuable broodstock populations.

#### **Article 1.4.16.**

In the second sentence of the second paragraph of point 1, the Commission agreed with a comment to delete ‘Exotic’ as the defined term ‘disease’ was more appropriate.

In the third paragraph, the Commission agreed with a comment to delete ‘risk of infection’ and add ‘likelihood of exposure’ as this will determine whether clustering might occur and whether a multi-stage survey is required.

In the third paragraph, the Commission agreed to delete ‘is relatively small, and’ as the size of population is not a factor for choosing a single vs multi-stage surveys. The main factor is homogeneity of the likelihood of exposure.

In the fifth paragraph of point 3, the Commission agreed with a comment to delete ‘below’ and add ‘above’ to correct an error.

In the same paragraph, the Commission did not agree to

- delete ‘infection’ and add ‘disease’ as it considered that infection was the most appropriate term to be used;
- rephrase the paragraph as the proposed changes did not improve clarity.

The Commission did not agree to delete the sixth paragraph of point 3, as it considered that the text was useful and the terminology used appropriate.

In the third sentence of point 3b), the Commission agreed with a comment to delete ‘that can remain sub-clinical’ and add ‘less contagious’ as it is not because an infection is sub-clinical that it is less contagious (i.e. lower prevalence).

In point 3 b) i), the Commission did not agree with a comment to remove the point, as it considered that a default value needed to be provided and that a higher design prevalence could be used, if appropriately justified.

In point 4, the Commission agreed with a comment that the list of risk factors for disease introduction, exposure and establishment could be expanded and added ‘exposure to recent stressors’ to point c) and added a new point e) ‘evidence of morbidity or mortality’ as these are additional factors that could identify high risk populations.

In the second paragraph of point 5, the Commission did not agree with a comment to require validation of test methods prior to initiation of targeted surveillance as it was considered too restrictive for implementation by all Members. The Commission also informed Members that guidance on validation of diagnostic assays and approaches for diagnostic sensitivity and specificity should be included in the *Aquatic Manual* only.

In the first paragraph of point 6, the Commission agreed with a comment to expand the introductory wording of the paragraph for clarity. However, the Commission did not agree with a comment to remove Table 1.2. as Members have found it useful. Nor did it agree to make generalised recommendations for acceptable diagnostic test sensitivity and specificity by type of assay as there are many factors involved.

In the eighth paragraph of point 6, the Commission did not agree to insert the formula and an example of software that could be used by Members for sample size calculations as it considered that it would not be appropriate to recommend specific resources in the *Aquatic Code*. The Commission recommended Members seek advice or support from one of the two Epidemiology and Risk Assessment of Aquatic Animal Diseases Collaborating Centres. The Contact information for the Collaborating centres can be found on the OIE website (<https://www.oie.int/en/what-we-offer/expertise-network/collaborating-centres/#ui-id-3>).

In the fifth sentence of the eighth paragraph of point 6, the Commission did not agree to add ‘pathogenic agent would be detected if present at a’ prior to ‘prevalence’ as it considered it would change the meaning of the information provided.

In point 7, the Commission did not agree with a comment to:

- delete ‘discrete populations of wild susceptible species’ and delete ‘defined stocks within a population’ as it was not considered to be an improvement;
- add ‘or stocks’ after ‘discrete populations’, delete ‘defined within a wild population stocks’ and add ‘individual animals within a defined wild population stock’ as stocks and population were considered to be the same.

In point 7, the Commission agreed to delete ‘discrete’ before ‘populations’ as it was not clear how these words represent different sampling stages and that it could be confusing to Members to have wild populations described as both ‘discrete populations’ and ‘defined stocks’. In response to comments on point 8, the Commission agreed to delete the point on Discounting as the paragraph was considered not relevant to the chapter.

#### **Article 1.4.17.**

In the first paragraph, the Commission added ‘and may be supplemented with targeted surveillance if necessary (as described in Article 1.4.12.).’ to align with the types of primary and secondary surveillance information described in Table 1.1. for each pathway for self-declaration of freedom from disease.

In the final paragraph, the Commission agreed with a comment that there are various approaches to surveillance sensitivity estimation and combination and that scenario tree modeling is just one approach. The Commission rephrased the paragraph to indicate scenario tree modeling is just an example of how multiple sources of information can be combined.

#### **Article 1.4.18.**

In the third sentence of the third paragraph, the Commission did not agree to delete ‘lower’ and add ‘different’ as it considered a higher standard of evidence may interfere with notification requirements.

The Commission noted that due to the extensive number of amendments being proposed compared to the current text in the *Aquatic Code*, the revised Chapter 1.4. would be proposed for adoption as clean text. However, the Commission agreed to also provide a version of Chapter 1.4., for Member information only, that shows the changes made to the draft revised chapter during this meeting. This marked version is presented in [Annex 6](#).

The revised version of Chapter 1.4. Aquatic Animal Health Surveillance is presented as [Annex 7](#) and will be proposed for adoption at the 89th General Session in May 2022.

#### *4.1.4.2. 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, Chile, Chinese Taipei, Colombia, New Caledonia, New Zealand, Switzerland, the USA, and the EU.

#### **February 2022 meeting**

The Commission reminded Members that if the model Articles X.X.4. to X.X.8. for disease-specific chapters to address declaration of freedom from [Pathogen X], are adopted at the 89th General

Session in May 2022, these amendments will be applied to all disease-specific chapters in the 2022 edition of the *Aquatic Code*.

In response to a general comment and to comments on Article X.X.7., the Commission did not agree to add compartment as an applicable level of application for pathway 1, as it considered that targeted surveillance is required to demonstrate that biosecurity measures are effective and that it is a fundamental element to establish a free status for a compartment (see Item 4.1.4.1.).

#### **Article X.X.5.**

In response to a comment requesting a definition of ‘shared water body’, the Commission did not agree as it considered that it would not be an improvement. The Commission considered that the reference to ‘shared water bodies’ in Article X.X.5. refers to natural epidemiological linkages that could not be broken through implementation of basic biosecurity conditions that apply to trade, movement of product etc, and that this concept is generally understood.

In the second paragraph, the Commission agreed with a comment to add ‘if it can demonstrate that’ at the end of the sentence as it would be expected that in a declaration Members would demonstrate how the requirements for freedom have been met. This change has also been proposed for Articles X.X.6. and X.X.7. and within the draft Chapter 9.X.

In point 4 b), the Commission did not agree to remove the requirement for depopulation and add a requirement for pathogen eradication as this point concerns the return to freedom after a disease outbreak. The Commission considered that a return to freedom could not be achieved without depopulation either by slaughter or moving the animals to an infected area outside the zone or compartment. The Commission also did not agree with a comment to make this change in Article X.X.6. point 4 b) and X.X.7. point 2 a) and in Chapter 1.4. (see Item 4.1.4.1.).

In point 4 d) ii), the Commission did not agree to delete ‘affected *aquaculture establishments* were not epidemiologically connected to wild populations of *susceptible species*’ and to add ‘wild susceptible species were not linked to the disease event that occurred’ as it was not considered to be an improvement.

#### **Article X.X.7.**

In point 2 b), the Commission added ‘aquatic’ prior to ‘animal’ to align with an amendment to draft Chapter 9.X. (see Item 4.1.6.).

The Commission noted that due to the extensive number of amendments being proposed compared to the current text in the *Aquatic Code*, the revised model Articles X.X.4. to X.X.8. for disease-specific chapters to address declaration of freedom from [Pathogen X] would be proposed for adoption as clean text. However, the Commission agreed to also provide a version of the revised model Articles, for Member information only, that shows the changes made to the draft revised model Articles during this meeting. This marked version is presented in [Annex 8](#).

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 9](#) and will be proposed for adoption at the 89th General Session in May 2022.

### **4.1.5. Safe Commodities – Articles X.X.3 of 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/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 agreed to begin with a review of Section 9. and developed an example article, Article 9.8.3. Infection with white spot syndrome virus, to demonstrate the suggested approach, noting that it was difficult to propose a uniform model Article X.X.3. because of differences in time/temperature treatments as well as products listed in Article X.X.3. between disease-specific chapters. The Commission circulated the example article, Article 9.8.3., for comment in its September 2020 report.

#### *4.1.5.1. Revised Articles 9.X.3. for crustacean disease-specific chapters*

Comments were received from Colombia, New Caledonia, Switzerland, Thailand, the UK, the EU and AU-IBAR.

#### *Background*

At its February 2021 meeting, the Aquatic Animals Commission considered comments on the example Article 9.8.3. and applied these amendments to Article 9.X.3. for 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. were amended 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. The revised Articles 9.X.3. were circulated for comment in the Commission's February 2021 report.

At its September 2021 meeting, the Commission reviewed comments and revised the proposed Articles 9.X.3. to improve clarity including re-ordering the aquatic animal products. The Commission also reviewed the use of 'meal' throughout the *Aquatic Code* and agreed that the addition of a specific time/temperature heat treatment for meal proposed in Articles 9.X.3. did not impact the definition of meal in the Glossary. The revised Articles 9.X.3. were circulated for comments in the Commission's September 2021 report.

#### **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 (Item 5.1.5., page 24).

#### **February 2022 meeting**

The Commission noted that the convention for the inclusion of numbers within the *Aquatic Code* is based on the Oxford dictionary, i.e. to write in full numbers from one to ten and for numbers above ten to use a numerical format, e.g. 100.

The Commission wished to inform Members that it has included work to review the safe commodity assessments for all listed diseases on its work plan. This will ensure that the thermal treatments for inactivation of listed pathogens are based on current scientific evidence (see February 2022 Aquatic Animals Commission report Part B).

The Commission reiterated that the proposed amendments have been made to specify the time/temperature treatments required to inactivate the pathogenic agent. The Commission noted that this is a change from the current commodity-based approach and was made in response to Member comments that some of the levels of thermal treatment in the current text were inconsistent or not commercially feasible as they would diminish product quality.

The Commission agreed with a comment that some of the proposed time/temperatures could be challenging to practically implement and reminded Members that equivalent time/temperature

combinations could be used (e.g. longer times at lower temperatures or shorter times at higher temperatures) where supported by evidence. The Commission also agreed that there is limited scientific information on the inactivation of many aquatic animal pathogenic agents and it encourages research by Members on inactivation of OIE listed pathogenic agents.

In point 1 of Article 9.X.3., the Commission agreed with a comment not to specify any product types such as cooked, pasteurised or retorted, noting that these were only examples and that any aquatic animal product should be considered safe if it has undergone the time/temperature treatment, as specified. The Commission noted that this approach will be applied to all the other revised Articles X.X.3.

In point 1 of Article 9.1.3. of Chapter 9.1, Acute hepatopancreatic necrosis disease (AHPND), the Commission did not agree with a comment that the reference to the specific strain of *Vibrio parahaemolyticus* (Vp) should be deleted. The Commission agreed that this would be in contradiction to Article 9.1.1. which indicates the causative agent for AHPND. However, the Commission noted that there is scientific literature which indicates that other *Vibrio* species may cause AHPND and it will request that the AHPND reference laboratories provide a recommendation on this issue for the Commission's September meeting.

In response to a comment on point 1 of Article 9.5.3. requesting the use of the current time/temperature published in the *Aquatic Code* for inactivation of infection with IMNV, the Commission noted that the inactivation time/temperature previously adopted (and current in the 2021 version of the *Aquatic Code*) was an error as it did not reflect the information presented in the '[Safe commodity assessments for OIE listed aquatic animal diseases](#)' published in 2016. The Commission investigated if there was any additional scientific information on inactivation of IMNV to support an alternative time/temperature combination, however, there is none and the commission agreed that there was no evidence to support an alternative at this time.

The revised Articles 9.X.3. for crustacean disease-specific chapters are presented as [Annex 10](#) and will be proposed for adoption at the 89th General Session in May 2022.

#### *4.1.5.2. Revised Articles 10.X.3. for fish disease-specific chapters*

Comments were received from Australia, Canada, Colombia, New Caledonia, Switzerland, Thailand, the UK and the EU.

#### *Background*

At its September 2021 meeting, the Aquatic Animals Commission reviewed and amended, as appropriate, Articles 10.X.3. of the disease-specific chapters of Section 10. Diseases of fish, of the *Aquatic Code* while ensuring alignment with proposed amendments to Articles 9.X.3. (see Item 4.1.5.1.).

The time/temperature treatments provided in Articles 10.X.3. of all fish disease-specific chapters were amended in line with the information provided in the [Safe commodity assessments for OIE listed aquatic animal diseases](#) published in 2016.

The Commission agreed not to include time/temperature heat treatments for *Gyrodactylus salaris* given that *G. salaris* would not survive in heat treated products such as pasteurised or retorted products because the parasite would be inactivated. The revised Articles 10.X.3. were circulated for comment in the Commission's September 2021 report.

### Previous Commission reports where this item was discussed:

September 2021 (Item 5.1.5.2., page 25).

### February 2022 meeting

As described for item 4.1.5.1, the Commission wished to inform Members that it has included work to review the safe commodity assessments for all listed diseases on its work plan to ensure that the time/temperatures for inactivation of listed pathogens is based on current scientific evidence (see February 2022 Aquatic Animals Commission report Part B).

In response to a general comment, the Commission agreed in principle that the articles relating to safe commodities (Articles X.X.3. and X.X.12.) in the disease-specific chapters should be sequential within the disease-specific chapters. The Commission noted that while the current order of articles is not ideal, a rearrangement of articles would have to be addressed through a broader review of the article structure of disease-specific chapters. This could be completed pending prioritisation of that work against other items within the Commission's workplan.

The Commission did not agree with a general comment to combine Articles X.X.3. and X.X.12., and noted that each article has a different scope. Article X.X.3. lists aquatic animal products that are considered safe for importation for any purpose regardless of the specified disease status of the exporting country, zone or compartment. Article X.X.12. lists aquatic animal products that are considered safe for retail trade for human consumption regardless of the specified disease status of the exporting country, zone or compartment. The assessments for products listed in Article X.X.3 and X.X.12 against the criteria in Chapter 5.4. Criteria to assess the safety of aquatic animal commodities, are available on the OIE website: '[Safe commodity assessments for OIE listed aquatic animal diseases](#)' published in 2016.

The Commission applied any relevant changes made in Article 9.X.3. to ensure harmonisation across all Articles X.X.3, as appropriate.

In point 1 of Article 10.3.3. of Chapter 10.3, Infection with *Gyrodactylus salaris*, in response to a comment, the Commission deleted 'pasteurised or retorted' to align with proposed changes in other 10.X.3 articles and added 'that have been heat treated and are hermetically sealed'. The Commission agreed that a specific time/temperature treatment was not required because, as an ectoparasitic helminth with a direct lifecycle, live birth and no resistant life stages, *G. salaris* would not survive in any heat treated, hermetically sealed product.

In points 6 and 7 of Article 10.3.3., the Commission did not agree to add a requirement for eviscerated fish, fillets and steaks to originate from fish held for 14 days in 25 parts per thousand (ppt) seawater prior to harvest and processing. The Commission noted that a 14 day holding period to inactivate *G. salaris* was not specified in the safe commodity assessment for this product (2016 [Safe commodity assessments for OIE listed aquatic animal diseases](#)). The Commission explained that the 14-day period indicated in Article 10.3.10. Infection with *Gyrodactylus salaris* is for trade of live fish, not for chilled eviscerated fish.

On point 1 of Article 10.5.3., Infection with salmonid alphavirus, a comment was made requesting that the proposed time/temperature for inactivation be reverted to the current text in the *Aquatic Code* as it was more practical for implementation. The Commission reiterated that this article was updated to be consistent with the information presented in the [Safe commodity assessments for OIE listed aquatic animal diseases](#), published in 2016. The Commission also reminded Members that equivalent time/temperature combinations can be used where supported by evidence.

The revised Articles 10.X.3. for fish disease-specific chapters are presented as [Annex 11](#) and will be proposed for adoption at the 89th General Session in May 2022.



#### 4.1.6. Draft Chapter 9.X. Infection with decapod iridescent virus 1

Comments were received from Australia, Canada, Chinese Taipei, Colombia, Korea (Rep. of), New Caledonia, Switzerland and the EU.

##### *Background*

Following the listing of infection with decapod iridescent virus 1 (DIV1) in Article 1.3.1. of Chapter 1.3. Diseases listed by the OIE, adopted in May 2021, the Aquatic Animals Commission developed a draft Chapter 9.X. Infection with decapod iridescent virus 1.

The format of the draft Chapter 9.X. was based on the article structure of other disease-specific chapters in Section 9 and included proposed horizontal amendments such as the model Articles X.X.4. to X.X.8. and Articles 9.X.3. The Commission noted that the proposed article structure for Article 9.X.3., and Articles 9.X.4. to 9.X.8., is based on model articles that will be proposed for adoption in May 2022.

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 also 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 Health Surveillance, would be appropriate for infection with DIV1. The Commission noted that if the revised Chapter 1.4. is adopted in May 2022, an assessment of these periods would be required for all listed diseases, including infection with DIV1. The draft Chapter 9.X. was circulated for comment in the Commission's September 2021 report.

##### **Previous Commission reports where this item was discussed:**

September 2021 report (Item 5.1.6., page 25).

##### **February 2022 meeting**

In point 1 of Article 9.X.3. the Commission applied relevant changes made in Article 9.X.3. to ensure harmonisation across all Articles X.X.3, as appropriate (see Item 4.1.5.1.).

In line 4 of Article 9.X.5. the Commission applied relevant changes to ensure harmonisation model Article X.X.4 to X.X.8 (see Item 4.1.4.2.).

In point 2 a) of Article 9.X.7. the Commission did not agree to replace 'aquatic animals' with 'susceptible aquatic animals with DIV1'. The Commission noted that point 2 is for the specific situation of regaining self-declaration of freedom after a disease incursion and that that all aquatic animals within the compartment would have to be killed and disposed of to achieve the outcome of re-gaining a self-declaration of freedom.

In point 2 b) of Article 9.X.7. the Commission agreed to add 'aquatic' before 'animal' for clarity. This amendment was also applied to the model articles for application to all disease-specific chapters (see Item 4.1.4.2.).

In the title of Article 9.X.12., the Commission did not agree to add 'bait' after 'animal feed' as the definition of feed in the Glossary would include bait.

The new draft Chapter 9.X. Infection with decapod iridescent virus 1, is presented as [Annex 12](#) and will be proposed for adoption at the 89th General Session in May 2022.

#### 4.1.7. Susceptible species – Section 10. Diseases of Fish

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

Comments were received from Colombia, Switzerland and the EU.

##### *Background*

At its September 2021 meeting, the Aquatic Animals Commission agreed to present the list of susceptible species in Article 10.1.2. in a table format, in line with the agreed convention to list susceptible species in a table format if there are more than ten susceptible species. The revised Article 10.1.2. was circulated for comment in the Commission's September 2021 report.

##### **Previous Commission reports where this item was discussed:**

September 2021 (Item 5.1.7., page 26).

##### **February 2022 meeting**

The Commission reviewed comments received and did not propose any amendments, noting that Members were supportive of the proposed changes.

The revised Article 10.1.2. of Chapter 10.1. Infection with epizootic haematopoietic necrosis virus, is presented as [Annex 13](#) and will be proposed for adoption at the 89th General Session in May 2022.

##### 4.1.7.2. Article 10.7.2. of Chapter 10.7. Infection with koi herpesvirus

Comments were received from Colombia, Switzerland and the EU.

##### *Background*

At its September 2021 meeting, 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 assessed as susceptible by the *ad hoc* Group of Susceptibility of fish species (<https://www.oie.int/en/what-we-do/standards/standards-setting-process/ad-hoc-groups/#ui-id-3>). The Commission proposed to add common carp X crucian carp hybrids (*Cyprinus carpio* x *Carassius carassius*) to Article 10.7.2. and circulated this proposal for comment.

##### **Previous Commission reports where this item was discussed:**

September 2021 (Item 5.1.8., page 26).

##### **February 2022 meeting**

The Commission reviewed comments received and did not propose any amendments noting that Members were supportive of the proposed changes.

The revised Article 10.7.2. of Chapter 10.7. Infection with koi herpesvirus, is presented as [Annex 14](#) and will be proposed for adoption at the 89th General Session in May 2022.

#### 4.1.8. Susceptible species – Section 11. Diseases of molluscs

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

Comments were received from Chinese Taipei, Colombia, Switzerland and the EU.

##### *Background*

At its September 2021 meeting, the Aquatic Animal Commission considered the June 2021 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 abalone herpesvirus in accordance with Chapter 1.5. Criteria for listing species as susceptible to infection with a specific pathogen. The *ad hoc* Group report can be found on the OIE website at <https://www.oie.int/en/what-we-do/standards/standards-setting-process/ad-hoc-groups/#ui-id-3>.

The Commission agreed to amend the list of susceptible species in Article 11.1.2. in line with recommendations of the *ad hoc* Group. They also agreed to amend Article 11.1.1. to ensure consistency with other mollusc disease-specific chapters with respect to the inclusion of the name and taxonomy of the pathogenic agent. Articles 11.1.1. and 11.1.2. were circulated for comment in the Commission's September 2021 report.

**Previous Commission reports where this item was discussed:**

September 2021 (Item 5.1.9.1., page 26).

**February 2022 meeting**

The Commission reviewed comments received and did not propose any amendments noting that Members were supportive of the proposed changes.

The revised Articles 11.1.1. and 11.1.2. of Chapter 11.1. Infection with abalone herpesvirus, are presented as [Annex 15](#) and will be proposed for adoption at the 89th General Session in May 2022.

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

Comments were received from Chinese Taipei, Colombia, Switzerland, the USA and the EU.

*Background*

At its February 2021 meeting, the Aquatic Animals Commission considered 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. Criteria for listing species as susceptible to infection with a specific pathogen. The *ad hoc* Group report can be found on the OIE website at <https://www.oie.int/en/what-we-do/standards/standards-setting-process/ad-hoc-groups/#ui-id-3>.

The Commission agreed to amend the list of susceptible species in Article 11.2.2. in line with the recommendations of the *ad hoc* Group. They also agreed to amend Article 11.2.1. to ensure consistency with other mollusc disease-specific chapters with respect to the inclusion of the name and taxonomy of the pathogenic agent. Articles 11.2.1. and 11.2.2. were circulated for comment in the Commission's February 2021 report.

At its September 2021 meeting, the Commission noted Member's support on the proposed amendments. No further amendments were made to Articles 11.2.1. and 11.2.2. that were circulated for comment in the Commission's September 2021 report.

**Previous Commission reports where this item was discussed:**

February 2021 (Part B: Item 1.5., page 10); September 2021 (Item 5.1.9.2., page 27).

**February 2022 meeting**

The Commission did not agree with a comment to reorder the species in Article 11.2.2. alphabetically by scientific name so that the *Ostrea* species and *Crassostrea* species are grouped together, as the convention is to order susceptible species alphabetically by English common names. Changing this approach would require horizontal changes in all disease-specific chapters of the *Aquatic Code* and in corresponding disease-specific chapters of the *Aquatic Manual*. The Commission noted that it will look further into the issue within the context of other items prioritised on its workplan.

In response to a comment to include *Ostrea equestris* in Article 11.2.2. of Chapter 11.2. Infection with *Bonamia exitiosa*, the Commission consulted the *ad hoc* Group on Susceptibility of mollusc species to OIE listed diseases. The *ad hoc* Group applied the criteria outlined in their November December 2020 report (<https://www.oie.int/en/what-we-do/standards/standards-setting-process/ad-hoc-groups/#ui-id-3>) for the susceptibility of mollusc species to infection with *Bonamia exitiosa*.

The Commission noted that the *ad hoc* Group had considered scientific evidence that supported that *O. equestris* and *Ostrea stentina* are distinct species and the ramifications for the susceptible species assessments. The Commission agreed with the recommendations of the *ad hoc* Group to include *O. equestris* in Article 11.2. and delete *O. stentina* as it no longer met the criteria for listing as susceptible to infection with *Bonamia exitiosa*. Relevant sections of Chapter 2.4.2., Infection with *Bonamia exitiosa* were also amended in line with the recommendations of the *ad hoc* Group (see Item 5.1.4.2.)

The *ad hoc* Group assessment of *O. equestris* and reassessment of *O. stentina* for listing as susceptible to infection with *Bonamia exitiosa* can be found in [Annex 17](#).

The revised Articles 11.2.1. and 11.2.2. of Chapter 11.2. Infection with *Bonamia exitiosa*, are presented as [Annex 16](#) and will be proposed for adoption at the 89th General Session in May 2022.

## 5. OIE MANUAL OF DIAGNOSTIC TESTS FOR AQUATIC ANIMALS

### 5.1. Texts proposed for adoption in May 2022

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](mailto:AAC.Secretariat@oie.int)).

At the last meeting in September 2021, the Commission had proposed amendments to the explanatory text in Section 4. Diagnostic methods, introducing Table 4.1. *OIE recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals*. The Commission reviewed comments received from Members and Reference Laboratory experts, and finalised the text. All chapters proposed for adoption would include the new explanatory text.

#### 5.1.1. Chapter 2.3.0. General information (diseases of fish)

Comments were received from Colombia, Switzerland, and the EU.

##### *Background*

At its September 2021 meeting, 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 Section 2.5. was circulated for comment in the Commission's February 2021 report.

**Previous Commission reports where this item was discussed:**

September 2021 (Item 6.1.2., page 31).

**February 2022 meeting**

The Commission agreed to include a new sentence on the need to further investigate negative molecular results when clinical signs indicate the presence of a specific disease or when other positive test results indicate that a false negative result may have been obtained.

The revised Chapter 2.3.0. General information (diseases of fish), is presented as [Annex 18](#) and will be proposed for adoption at the 89th General Session in May 2022.

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

Comments were received from Australia, Canada, Chile, China (People's Rep. of), Colombia, Norway, Switzerland, Thailand, the UK and the EU.

*Background*

At its September 2020 meeting, the Aquatic Animals Commission reviewed Chapter 2.3.4. 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. The revised chapter was circulated for comment in the Commission's September 2020 report.

At its September 2021 meeting, the Commission amended the proposed chapter after considering Member comments. The Commission did not agree to jointly describe infection with HPR-deleted ISAV and HPR0 ISAV rather than consider them separately in the chapter. The Commission confirmed that the clinical expression of disease, epidemiology and control measures differ which justified leaving their descriptions separate. The revised chapter was circulated for comment in the Commission's September 2021 report.

**Previous Commission reports where this item was discussed:**

September 2020 (Item 5.4., Page 15); September 2021 (Item 6.1.4., page 32).

**February 2022 meeting**

A Member had submitted a recently published short communication on the first report of successful isolation of a HPR0-like variant of ISAV using cell culture and asked that the chapter be reviewed in the light of this finding. The Commission carefully reviewed the publication, which is an experimental study and noted that the finding, which is significant, requires further investigation. The Commission made reference to this finding at appropriate places within the chapter.

Another Member commented that since HPR-deleted and HPR0 variants of ISAV were listed in 2013, new scientific experience and information on these variants has been gathered and published. The Member asked that the Commission consider reviewing the assessment of these variants against the listing criteria, particularly the HPR0 variant. The Commission advised Members that it will consider the assessment of ISAV in its broader work plan in the future, however, noted that it is important that any changes to the listing are considered carefully to ensure stability of reporting requirements and trade standards. Members are encouraged to provide any relevant information for its consideration.

In Section 2.1.1. Aetiological agent, the Commission agreed that the differences between the North American clade and European clade are not only limited to segment 6 and included a reference to this finding. The Commission also agreed to include a sentence and a reference stating that deleted ISAV variants have been found without virulence marker on segment 5. A Member proposed including a sentence in this section on the newly isolated and cultured HPR0-like variant. The Commission agreed that the sentence would fit better in Section 4.3. Cell culture for isolation.

In Section 2.1.3. Survival and stability outside the host, the Commission supported a proposal to include a sentence on the difficulty of estimating how long the virus remains infectious in the natural environment.

Given the publication on the isolation and cultivation of HPR0-like ISAV mentioned above, the Commission agreed to delete a sentence stating that HPR0 ISAV has not been isolated in cell culture from Section 2.2.4. Distribution of the pathogen in the host. However, a new sentence mentioning this single report was included in Section 4.3. Cell culture, clarifying that experimental studies in fish for this variant have not yet been published.

In Section 2.3.1. Mortality, morbidity and prevalence, the Commission did not agree to delete a statement that HPR0 ISAV has not been associated with clinical disease in Atlantic salmon based on the recent publication as this single report of an experimental study needs more investigation and *in-vivo* validation.

In Section 2.3.3. Gross pathology, the Commission agreed to remove from the list of findings that have been described to be consistent with infection with HPR-deleted ISAV the point i) yellowish or blood-tinged fluid in peritoneal and pericardial cavities. These findings are from a single study on Coho salmon conducted in 2001, it has not been possible to verify the findings, and Coho salmon are not considered a susceptible species.

In Section 2.3.4. Modes of transmission and life cycle, the Commission clarified that except for a single report, HPR0 ISAV has not been isolated in cell culture.

In Section 2.3.6. Geographical distribution, the Commission did not accept a suggestion to reinstate a statement that the HPR0 ISAV variant has been reported in all countries where infection with HPR-deleted ISAV has occurred as this is not confirmed. Information on disease occurrence can be found in the OIE-WAHIS.

In Section 3.1. Selection of populations and individual specimens, a Member proposed to separate the surveillance activities from the sampling of specimens. The Commission felt that the existing information is clear as written and did not agree to the change.

In Section 3.2.1. Detection of HPR-deleted ISAV, the Commission agreed to remove 'gill' from the list of organs or tissues to be sampled as only internal organs should be used for diagnostic testing for HPR-deleted ISAV.

In Section 3.4. Non-lethal sampling, the Commission agreed to insert a sentence and a reference stating that gill swabs are recommended for non-lethal sampling for HPR0.

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 to be consistent with amendment to the template agreed at the meeting in September 2021.

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, purpose 'C Confirmatory diagnosis of a suspect result from surveillance or presumptive diagnosis', the Commission agreed to change the rating of cell culture from '+++' to '++' for all life stages, the rating of the reverse-transcription PCR from '+' to '++' for early life stages and juveniles, and from '++' to '+' for adults, to add the rating '++' to all life stages for the real-time PCR, and to give the level of validation of these three tests as '1'. The ratings are consistent with the case definitions given in Section 6. Corroborative diagnostic criteria. The Commission also agreed to change the level of validation of the real-time RT-PCR from '3' to '1' for the purpose B. Presumptive diagnosis of clinically affected

animals, to be consistent with the method recommended in Section 4.4.1. of the chapter and to change the level of validation from NA (not available) to ‘1’ for immunohistochemistry and IFAT for purpose C.

In Section 4.3. Cell culture for isolation, based on earlier comments (see Section 2.1.1 and Section 2.2.4), text and a reference were added on the recent publication of the isolation of a HPR0-like variant of the ISAV using cell culture, but clarifying that experimental studies in fish for this variant have not yet been published.

In Section 6. Corroborative diagnostic criteria, the Commission did not accept to alter the introductory paragraph as the text is standard approved text from the template.

In Section 6.1.2. Definition of confirmed case in apparently healthy animals, the Commission did not agree to include cell culture in the criteria because it is not recommended in Table 4.1 for apparently healthy animals. In Section 6.3. Diagnostic sensitivity and specificity for diagnostic tests, a Member had proposed to include data from a published real-time RT-PCR. As the method is different from the one recommended in Section 4.4.1. of the chapter, the Commission did not agree to include it.

Finally, as none of the test methods are validated to at least level 2, the Commission deleted the data in Tables 6.3.1. For presumptive diagnosis of clinically affected animals and 6.3.2. For surveillance of apparently healthy animals.

The revised Chapter 2.3.4. Infection with HPR-deleted or HPR0 infectious salmon anaemia virus, is presented as [Annex 19](#) and will be proposed for adoption at the 89th General Session in May 2022.

#### 5.1.3. Chapter 2.3.6. Infection with koi herpesvirus

Comments were received from Australia, China (People’s Rep. of), Chinese Taipei, Colombia, Japan, Switzerland, Thailand, the UK, the USA and the EU.

##### *Background*

At its September 2020 meeting, the Aquatic Animals Commission reviewed Chapter 2.3.6. Infection with koi herpesvirus (KHV), which had been updated by the OIE Reference Laboratory experts and reformatted using the new disease chapter template. The revised chapter was circulated for comment in the Commission’s September 2020 report.

At its September 2021 meeting, the Commission reiterated 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. The revised chapter was circulated for comment in the Commission’s September 2021 report.

##### **Previous Commission reports where this item was discussed:**

September 2020 (Item 5.5., page 15); September 2021 (Item 6.1.5., page 35).

##### **February 2022 meeting**

The Commission noted that several of the comments received and issues raised by Members are based on a paper by Engelsma *et al.* (2013). To address these comments, the Commission agreed to stress throughout the chapter that the strains detected by Engelsma *et al.* were novel strains of cyprinid herpesvirus closely related to KHV. The Commission also agreed to use the same terminology in the chapter that is used in the paper, for example to refer to KHV ‘strains’ rather than ‘genotypes’.

In Section 1. Scope, the Commission agreed to delete reference to ‘all genotypes’ of the pathogenic agent. The Commission also agreed to remove the references from this section following the style of the *Aquatic Manual*, and to delete the sentence on the use of the abbreviation ‘KHV’ as it is stated in the first sentence of the scope.

In Section 2.1.1. Aetiological agent, the Commission agreed to clarify that Engelsma *et al.* (2013) detected novel strains of cyprinid herpesvirus closely related to KHV. These strains may represent low or non-pathogenic variants of CyHV-3, but further investigation is required to establish the true genetic relationship between these strains and KHV. The Commission also agreed to update the description of the KHV genome, which has now been fully determined.

In Section 2.2.6. Vectors, the Commission agreed to include species of migratory wild duck as species in which KHV has been detected by PCR in areas where fish and ducks coexist, along with a reference supporting this finding.

In Section 2.3.4. Modes of transmission and life cycle, the Commission included the intestine as one of the portals of virus entry in carp, along with a supporting reference.

In Section 2.4.1. Vaccination, the Commission agreed to add the reference to the original publication of studies in Japan showing that oral administration of a liposome-based vaccine containing inactivated KHV was effective in protecting carp against clinical disease.

A Member commented that a sentence in Section 3.2 Selection of organs or tissues, stating that KHV DNA was detected with high probability from the encephalon of the surviving fish at 120 days post-infection was incorrect as the researchers had used material from a number of organs. The Commission reviewed the reference and confirmed that the virus was detected with the highest probability from the brain of surviving fish at 120 days post infection. The comment was thus rejected.

A Member questioned the ratings of the conventional nested PCR 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, which is based on Engelsma *et al.* (2013). The Commission, in consultation with the OIE Reference Laboratory experts, agreed to change the ratings from ‘++’ to ‘+’ for the purposes ‘A Surveillance of apparently healthy animals’ and ‘C Confirmatory diagnosis of a suspect result from surveillance or presumptive diagnosis’, and the ratings from ‘+++’ to ‘++’ for the purpose ‘B. Presumptive diagnosis of clinically affected animals’. The Commission also decided to change the level of validation from ‘1’ to ‘NA’ (not available) for all three purposes as no validation data are published.

For the conventional PCR, the Commission agreed to change the level of validation from ‘1’ to ‘3’ for the purposes ‘B. Presumptive diagnosis of clinically affected animals’ and ‘C Confirmatory diagnosis of a suspect result from surveillance or presumptive diagnosis’ and to include a footnote indicating the references supporting the change and clarifying that other conventional PCR assays are validated to level 1.

In Section 4.4.2. Real-time PCR, clarified the finding of Engelsma *et al.* (2013) that real-time PCR methods for the detection of KHV DNA in fresh tissue samples do not detect novel strains of cyprinid herpesvirus closely related to KHV.

In Section 4.4.3. Conventional PCR, and in line with the changes proposed to Table 4.1., the Commission agreed to remove text specifically recommending the Engelsma *et al.* (2013) method. The method remains listed in Table 4.4.2.1. Primer and probe sequences and cycling conditions for the KHV real-time PCR, as it is still listed in Table 4.1.



The Commission did not agree to include an antibody ELISA in Section 4.10. Other methods, as antibody tests are unreliable for this disease, and consequently the Commission does not recommend them for use.

In Section 5. Test(s) recommended for surveillance to demonstrate disease freedom in apparently healthy populations, the Commission agreed to refer to ‘novel strains of cyprinid herpesvirus closely related to KHV’ rather than to ‘KHV variants’ in accordance with the decision to use the findings as described in Englesma *et al.* (2013). Following the decision to no longer specifically recommend the conventional nested PCR published by Englesma *et al.* (2013), the Commission also agreed to delete the sentence referring to it.

In Section 6.2.2. Definition of confirmed case in clinically affected animals, the Commission did not agree to a proposal to delete all the criteria apart from a ‘positive result by conventional PCR or conventional nested PCR and sequencing of the amplicon’. The current text is consistent with the tests and their ratings in Table 4.1.

Finally, the Commission amended Table 6.3.1. For surveillance of clinically affected/apparently healthy animals by clarifying the published references on which the data is based. Table 6.3.2. Surveillance of apparently healthy animals was deleted as no information is currently available.

Reference:

ENGELSMA M.Y., WAY K., DODGE M.J., VOORBERGEN-LAARMAN M., PANZARIN V., ABBADI M., EL-MATBOULI M., FRANK SKALL H. KAHNS S. & STONE D.M (2013). Detection of novel strains of Cyprinid herpesvirus closely related to koi herpesvirus. *Dis. Aquat. Org.*, **107**, 113–120.

The revised Chapter 2.3.6. Infection with koi herpesvirus, is presented as [Annex 20](#) and will be proposed for adoption at the 89th General Session in May 2022.

#### 5.1.4. Susceptible species of Section 2.4. Diseases of molluscs

##### 5.1.4.1. Sections 2.2.1. and 2.2.2. of Chapter 2.4.1. Infection with abalone herpesvirus (susceptible species)

Comments were received from Chinese Taipei, Colombia, Switzerland and the EU.

##### *Background*

At its September 2021 meeting, the Aquatic Animals Commission reviewed the June 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 abalone herpesvirus.

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 (see also Item 4.1.8.1.). Articles 11.1.1. and 11.1.2. were circulated for comment in the Commission’s September 2021 report.

##### **Previous Commission reports where this item was discussed:**

September 2021 (Item 6.1.7.1., page 39).

##### **February 2022 meeting**

The Commission reviewed comments received and did not propose any amendments noting that Members were supportive of the proposed changes.

The revised Sections 2.2.1. and 2.2.2. of Chapter 2.4.3. Infection with abalone herpesvirus, are presented as [Annex 21](#) and will be proposed for adoption at the 89th General Session in May 2022.

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

Comments were received from Colombia, Switzerland, the USA 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 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 on Susceptibility of mollusc species to infection with OIE listed diseases.

At its September 2021 meeting, the Commission noted Member's support on the proposed amendments. No further amendments were made to Section 2.2.1. and 2.2.2. that were circulated for comment in the Commission's February 2021 report.

**Previous Commission reports where this item was discussed:**

February 2021 (Part B: Item 3.2., page 13); September 2021 (Item 6.1.7.2., page 39).

**February 2022 meeting**

In response to a comment to include *Ostrea equestris* in Section 2.2.1. of Chapter 2.4.2. Infection with *Bonamia exitiosa*, the Commission consulted the *ad hoc* Group on Susceptibility of mollusc species to OIE listed diseases. The *ad hoc* Group applied the criteria outlined in their November December 2020 report (<https://www.oie.int/en/what-we-do/standards/standards-setting-process/ad-hoc-groups/#ui-id-3>) for the susceptibility of mollusc species to infection with *Bonamia exitiosa*.

The commission noted that the *ad hoc* Group had considered scientific evidence that support that *O. equestris* and *Ostrea stentina* are distinct species and the ramifications for the susceptible species assessments. The Commission agreed with the recommendations of the *ad hoc* Group to include *Ostrea equestris* and delete *Ostrea stentina* from Section 2.2.1. Susceptible host species as *Ostrea stentina* no longer met the criteria for listing as susceptible to infection with *Bonamia exitiosa*. The Commission agreed to add *Ostrea stentina* to Section 2.2.2. Species with incomplete evidence for susceptibility. Article 11.2.2. of Chapter 11.2. Infection with *Bonamia exitiosa*, were also amended in line with the recommendations of the *ad hoc* Group (see Item 4.1.8.2.).

The *ad hoc* Group assessment of *Ostrea equestris* and reassessment of *Ostrea stentina* for listing as susceptible to infection with *Bonamia exitiosa* can be found in [Annex 17](#).

The revised Sections 2.2.1. and 2.2.2. of Chapter 2.4.2. Infection with *Bonamia exitiosa* are presented as [Annex 22](#) and will be proposed for adoption at the 89th General Session in May 2022.

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.../Annexes

## MEETING OF THE OIE AQUATIC ANIMAL HEALTH STANDARDS COMMISSION

Virtual meeting, 24 & 27 January, 16-23 February 2022

### List of participants

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## USER'S GUIDE

### A. Introduction

- 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*.
  - 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.
  - 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.
  - 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 ~~pathogen~~ pathogenic 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, handling, disposal and treatment 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**

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## GLOSSARY

### 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*.

### 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.~~

### BASIC BIOSECURITY CONDITIONS (CLEAN VERSION)

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

### 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*.

### 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.~~

#### **EARLY DETECTION SYSTEM (CLEAN VERSION)**

means a system, as described in Article 1.4.7., which ensures the rapid recognition of signs that are suspicious of a *listed disease*, or an *emerging disease*, 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 an investigation by the *Aquatic Animal Health Services* with minimal delay.

#### **PASSIVE SURVEILLANCE**

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

#### **PASSIVE SURVEILLANCE (CLEAN VERSION)**

means *aquatic animal* health surveillance typically based on observations of clinical or behavioural signs of *disease*, or an assessment of mortality or production data, which are generated by an *early detection system* or from other information which is available to the *Competent Authority*.

#### **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*.

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## CHAPTER 1.3.

### DISEASES LISTED BY 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 necrosisvirus
- Infection with *Gyrodactylus salaris*
- Infection with HPR-deleted or HPR0 infectious salmon anaemia virus
- Infection with infectious haematopoietic necrosisvirus
- 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.

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## 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 aquatic animals 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 virus has been classified in the family *Amnoonviridae*, Genus *Tilapinevirus* and given the species name *Tilapia tilapinevirus* (ICTV, 2018). The host range is not well known but several species of tilapines are known to be susceptible (Eyngor *et al.*, 2014; Waiyarnit *et al.*, 2021) and the giant gourami (*Osphronemus goramy*) has shown evidence of susceptibility (Jaemwimol *et al.*, 2018). TiLV has also been detected in other species, however without clinical signs (Piamsomboon *et al.*, 2021). Tilapia is the second most important 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 fillets and other tilapia products are traded globally. There are no treatments for infection with tilapia lake virus however there are vaccines under development (Zeng *et al.*, 2021; Mai *et al.*, 2022).

#### **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; Castañeda *et al.*, 2020; Contreras *et al.*, 2021; 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, live tilapia have been traded internationally to establish populations for production in new regions, and extensive trade in live tilapia continues. The current driver for international trade is the dissemination of improved genetic strains (although the 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, product-specific risks have not been considered in this assessment (Castañeda *et al.*, 2020).

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; Castañeda *et al.*, 2020; Contreras *et al.*, 2021; 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 countries are likely to be 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*
<b>Americas</b>					
Colombia				1	
Mexico		20	1		
Peru		5	2	1	
USA			3		
<b>Asia</b>					
Chinese Taipei	9				
India			3		
Malaysia	2	2			
Philippines	1		1		
Thailand	1				
<b>Europe</b>					
Israel	16 (Tilapia syncytial hepatitis)				
<b>Total</b>	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 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; Skornik *et al.*, 2021). 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 aquatic animals 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; Kabuusu *et al.*, 2017). 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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## 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*~~Authorities~~ ~~is~~ ~~are~~ 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 Articles ~~s~~ 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 Articles ~~s~~ 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 Articles ~~s~~ 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 Articles ~~s~~ 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~~~~Authorities~~ 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~~~~Authorities~~ 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* including when water bodies are shared with other countries or are under the control of different ~~Competent Authorities~~. 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 at the country or zone.

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* at the country or zone level, 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 at the country, zone or compartment level. 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* for a country, zone or compartment.

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 <del>s</del> Secondary evidence to claim freedom (if required)	Applicable level of application
1. Absence of <i>susceptible species</i>	<u>Active surveillance</u> <u>Surveys,</u> <u>historical data,</u> <u>import records,</u> <u>environmental information</u>	None	Country, zone
2. Historical freedom	<i>Passive surveillance</i>	<i>Targeted surveillance</i> (in populations where <i>passive surveillance</i> is not appropriate)	Country, zone
3. <u>Targeted surveillance</u>	<i>Targeted surveillance</i>	<i>Passive surveillance</i> (in appropriate populations)	Country, zone, compartment
4. Returning to freedom	<i>Targeted surveillance</i>	<i>Passive surveillance</i> (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 verify 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~~ 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, zone or compartment:

- 1) the quality of *Aquatic Animal Health Services* can be substantiated to meet the requirements of Chapter 3.1.:
- 2) *basic biosecurity conditions* (which include an early detection system) 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;~~
- ~~43)~~ there has been no vaccination of susceptible *aquatic animals* for the specific *disease* for at least from the period that implementation of the basic biosecurity conditions have been applied prior to self-declaration;
- ~~54)~~ the *Aquatic Animal Health Services* have sufficient capacity and expertise to investigate and report *disease* events to the Competent Authority;
- ~~65)~~ 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 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;~~

21) an *early detection system* (as described in Article 1.4.7.);

32) 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*, thea Competent 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 thea Competent Authority underpins is important to generate evidence for claims of disease freedom and to provide assurance that a change in disease status would be rapidly discovered, 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)~~ have broad awareness 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 *listed disease and emerging* disease occurrence;
- 3) the *Aquatic Animal Health Services* have capacity to undertake rapid and effective *disease* investigation based on a national chain of command led by a Competent Authority;
- 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 suspicions of the occurrence of listed diseases or emerging diseases occurrence to thea 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, thea 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*, veterinarians 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, the~~ 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.

# Requirements for passive surveillance

- 4) 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*.
- 1) 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 points 1a), b) and 4 a) 1 d) b) 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, veterinarians* 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 fisheries and aquatic fauna surveys), 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.
- 54) 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*.
- 65) *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.

#### 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*); ~~or~~
  - b) the *disease* would manifest clinically and be detected by the country's *early detection system* (see pathway 2 – historical freedom); ~~and/or~~
  - c) by the time *targeted surveillance* commenced (see pathway 3 – *Targeted 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 recommended in the disease-specific chapters:
    - 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* (i.e. periods of the year when prevalence and intensity of infection are highest and most conducive to detection 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 to reduce the likelihood of disease introduction by the same or similar routes. Mitigation measures should be implemented 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 ~~testingsurvey in the compartment~~ testing survey in the compartment is required to demonstrate that eradication has been successful and to ~~testensure~~ test 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:~~

- ~~1a)~~ reports which provide evidence regarding 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);
- ~~2b)~~ documentation from the relevant *Competent Authority* showing that those *susceptible species* have not been imported into the country or *zone*;
- ~~3c)~~ 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 thea *Competent Authority* wishing to establish freedom ahead of farming a new species.

Article 1.4.12.

### Pathway 2 – Historically 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 or zone 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-requirements for basic biosecurity conditions of Article 1.4.6., early detection system of Article 1.4.7. and passive surveillance 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, thea *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-represent all the *susceptible species* populations in the country or *zone*. If thea *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 Pathway 2 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 ~~– the default minimum period~~) need to be conducted three or more months apart to declare freedom unless disease-specific evidence supports an alternative strategy. In situations where seasonal conditions do not permit a gap of at least three months between surveys, the maximum possible time gap should be allowed to elapse between one survey and the next.

The ~~Over the period of *targeted surveillance*, the combined~~ 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~~ would be detected if present at or below ~~above~~ the design *prevalence* in the country, zone or compartment. 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 as described in Article 1.4.16). Surveys should be designed in accordance with the recommendations provided in Article 1.4.16.

~~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 zones* 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 production 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~~ informed 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 zones* 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 4.34., and synchronously followed as described in Chapter 4.67., for a period determined by the biophysical properties of the *pathogenic agent* (i.e. survival in the environment), a *surveillance* programme within the *protection zones* and *infected zones* should commence. The programme should include both farmed and wild populations of *susceptible species* in the *protection zones* and *infected zones*. A *risk-based* approach to the design of the survey is recommended (~~refer to as described in~~ 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~~ would be detected if 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 followed as described in Chapter 4.34.~~ and followed as described in Chapter 4.67., 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 sixth 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~~ would be detected 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~~ a *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 data/information* 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 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 likelihood of exposure/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 selected first-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~~ above 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 based on the epidemiology of the disease. If not specified for the particular disease, i 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 less contagious 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 disease 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 *disease* exposure (e.g. to ~~quarantine facilities, aquatic animal processing facilities, or ports~~);
- c) environmental or husbandry conditions that are permissive for *disease* establishment (e.g. temperature, salinity, production system type, habitat type, *exposure to recent stressors*);
- d) conditions that are conducive for development of clinical *disease*; including the species or life stages that are most susceptible to clinical *disease*;
- e) *evidence of morbidity or mortality*.

## 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

*In surveys conducted to demonstrate the absence or presence of an infection, 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 1.4-2 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.~~

## 98. 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.) and may be supplemented with targeted surveillance if necessary (as described in Article 1.4.12.). *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. If combining evidence from different sources including passive surveillance and targeted surveillance, a Competent Authority may choose to use various approaches, such as a scenario tree modelling approach.

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* provides s 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*.

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[Return to Agenda](#)

## (CLEAN VERSION)

## 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 a *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 a *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. *Competent Authorities* are 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 Articles 1.4.5. to 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 Articles 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 described in detail in Articles 1.4.11. to 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 Articles 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*.

*Competent Authorities* 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**

*Competent Authorities* 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, a *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* including when water bodies are shared with other countries or are under the control of different *Competent Authorities*. 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 at the country or *zone*.

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* at the country or *zone* level, that is supported primarily by *passive surveillance* 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 at the country, *zone* or *compartment* level. 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* for a country, *zone* or *compartment*.

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

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 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 (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 verify that *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* 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.

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 country, *zone* or *compartment*:

- 1) the quality of *Aquatic Animal Health Services* can be substantiated to meet the requirements of Chapter 3.1.:
- 2) *basic biosecurity conditions* (which include an *early detection system*) as described in Article 1.4.6. are in place;
- 3) there has been no vaccination of susceptible *aquatic animals* for the specific *disease* from the implementation of the *basic biosecurity conditions* prior to self-declaration;
- 4) the *Aquatic Animal Health Services* have sufficient capacity and expertise to investigate and report *disease* events to a *Competent Authority*;
- 5) a *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 specific *disease* and for detection of the *disease* should it occur. The requirements for *basic biosecurity conditions* include:

- 1) an *early detection system* (as described in Article 1.4.7.);
- 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*, a *Competent Authority* should describe how all of the requirements for *basic biosecurity conditions* relevant to its declaration, are continuously met.

#### Article 1.4.7.

##### Early detection system

The *early detection system* of a *Competent Authority* is important to generate evidence for claims of disease freedom and to provide assurance that a change in disease status would be rapidly discovered.

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

- 1) observers (e.g. the personnel of *aquaculture establishments*, processors, transportation services) have broad awareness 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 *listed disease* and *emerging disease* occurrence;
- 3) the *Aquatic Animal Health Services* have capacity to undertake rapid and effective *disease* investigation based on a national chain of command led by a *Competent Authority*;
- 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*, *aquatic animal health professionals* and others with an occupational role with *aquatic animals* have a legal obligation to report suspicion of the occurrence of *listed diseases* or *emerging diseases* to a *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*, *veterinarians* and others to initiate the necessary steps of *passive surveillance*. Specifically, a *Competent Authority* should be able to demonstrate that efforts have been made to make 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*, *veterinarians* 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*. The sensitivity of an *early detection system* (i.e. the likelihood of *pathogenic agent* detection following introduction) 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

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* information to be utilised for a *self-declaration of freedom from disease*.

- 1) 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 signs of the *disease* at least seasonally;

- b) observation of signs of the *disease*, which may include increased mortality, would lead to investigation and, where appropriate, reporting to a *Competent Authority*;
  - c) populations of susceptible farmed *aquatic animals* should be under sufficient observation, such that, if 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 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 it would be observed and reported in adjacent farmed 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* or unexplained increased mortality and reporting them to a *Competent Authority*. For wild populations, the requirements of points 1a), b) and d) may not be met under most circumstances and, therefore, *passive surveillance* will be insufficiently sensitive. If a *Competent Authority* utilises *passive surveillance* 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* will result in detection of the *disease* should it occur.
- 3) Awareness of signs of *disease* and the necessary level of observation is best demonstrated through examples of reporting by farmers, *aquatic animal health professionals*, *veterinarians* and others to a *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. fisheries and aquatic fauna surveys), submissions to laboratories, *aquaculture establishment* records (e.g. mortality, medicine use, etc.).
- 4) Evidence from published literature will generally be sufficient to demonstrate the environmental conditions 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*.
- 5) *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 a *Competent Authority* 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 sufficient duration, so that, by the end of the period, should the *disease* have been introduced before the *basic biosecurity conditions* began:
  - a) the specific *pathogenic agent* would not remain present in the environment (see pathway 1 – absence of *susceptible species*); or
  - b) the *disease* would manifest clinically and be detected by the country's *early detection system* (see pathway 2 – historical freedom); or
  - c) by the time *targeted surveillance* commenced (see pathway 3 – *Targeted 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 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 of *basic biosecurity conditions* required prior to a self-declaration, for all *listed diseases*, 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 *listed disease* based on its epidemiology (e.g. agent stability in the environment, presence of resistant life stages, *vectors*), and 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 of *basic biosecurity conditions* required 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 is considered to be less than 30% (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 longer than ten years, as appropriate. An evaluation of the following factors will determine whether a period longer than ten years is recommended in the disease-specific chapters:
- 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*;
  - v) environmental conditions that influence levels of *infection* and clinical expression, including seasonality of the *disease* (i.e. periods of the year when *prevalence* and intensity of *infection* are highest and most conducive to detection);
  - 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 of *basic biosecurity conditions* required prior to commencement of *targeted surveillance* will 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, the epidemiology of a *disease* and nature of production systems may limit the 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 to reduce the likelihood of *disease* introduction by the same or similar routes. Mitigation measures should be implemented 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. 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 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 survey in the *compartment* is required to demonstrate that eradication has been successful and to 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, of the local *aquatic animal* fauna (including wild populations) demonstrated by the following forms of evidence:
  - a) reports which provide evidence regarding the absence of the *susceptible species* in the country or *zone* from structured surveys (e.g. of fisheries and aquatic fauna surveys, historical fisheries data);
  - b) documentation from the relevant *Competent Authority* showing that those *susceptible species* have not been imported into the country or *zone*;
  - 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 a *Competent Authority* wishing to establish freedom ahead of farming a new species.

#### Article 1.4.12.

### Pathway 2 – Historical 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* information generated by a country's *early detection system*. For this pathway to be utilised, the following conditions should be met:

- 1) the country or *zone* has *basic biosecurity conditions* in place, including an *early detection system*, that is sufficiently sensitive to detect the *disease* should it occur, and the requirements for *basic biosecurity conditions* of Article 1.4.6., *early detection system* of Article 1.4.7. and *passive surveillance* 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*

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, a *Competent Authority* needs to provide evidence that its *early detection system* meets the conditions described in Article 1.4.7. and the requirements for *passive surveillance* in Article 1.4.8. The *early detection system* needs to represent all the *susceptible species* populations in the country or *zone*. If a *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* 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. Pathway 2 should only be utilised as the basis of a *self-declaration of freedom from disease*, if it is based primarily on *passive surveillance* 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 targeted surveillance

For many *diseases*, there will be significant temporal variability in the *prevalence* and intensity of *infection* (and therefore likelihood of detection by *targeted surveillance*). For example, the likelihood of detection may be greatest for a particular life stage, or during periods of the year when *pathogenic agent* replication and transmission are 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 – the default minimum period) need to be conducted three or more months apart to declare freedom unless disease-specific evidence supports an alternative strategy. In situations where seasonal conditions do not permit a gap of at least three months between surveys, the maximum possible time gap should be allowed to elapse between one survey and the next.

Over the period of *targeted surveillance*, the combined number of *aquaculture establishments* and *aquatic animals* sampled should be sufficient to generate at least 95% confidence that the *pathogenic agent* would be detected if present at or above the design *prevalence* in the country, *zone* or *compartment*. 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% (a higher design *prevalence* can only be used if justified by epidemiological evidence as described in Article 1.4.16.). Surveys should be designed in accordance with the recommendations provided in Article 1.4.16.

#### Other sources of data

This pathway to *disease* freedom should be based primarily on the results of *targeted surveillance*. However, the submission may also include an analysis of the *passive surveillance* information to provide supplemental evidence. This evidence may be used for defined populations of *susceptible species* where *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 demonstrate that eradication has been successful and to ensure the reviewed *basic biosecurity conditions* are effective.

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 zones* 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 production 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 informed 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 zones* 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 4.4., and synchronously followed as described in Chapter 4.7., for a period determined by the biophysical properties of the *pathogenic agent* (i.e. survival in the environment), a *surveillance* programme within the *protection zones* and *infected zones* should commence. The programme should include both farmed and wild populations of *susceptible species* in the *protection zones* and *infected zones*. A *risk-based* approach to the design of the survey is recommended (as described in Article 1.4.6.). The following *aquaculture establishments* or populations should be preferentially selected for sampling:

- a) establishments which have been restocked following depopulation;
- b) establishments and wild populations at greatest *risk* of exposure to *infection* during the *outbreak*, i.e. in close 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* would be detected if 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, as described in Chapter 4.4. and fallowed as described in Chapter 4.7., 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 sixth 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 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* would be detected 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 a *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.

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* information 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 *susceptible species* to the *disease* in a country, *zone* or *compartment*, to which the *surveillance* results apply. *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 can be considered to be homogenous with regards to likelihood of exposure, 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. *aquaculture establishments* or villages) are selected. At the second stage, animals are selected for testing from each of the first-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 above 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 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 based on the epidemiology of the *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 less contagious 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 *disease* introduction, exposure and establishment. The identification of appropriate *risk* factors may include consideration of:

- a) the possible pathways of *disease* introduction (e.g. through *aquatic animals*, *aquatic animal products*, *feed*, *fomites*, *vectors* and *water*);
- b) proximity of susceptible populations to sources of *disease* exposure (e.g. to *aquatic animal* processing facilities, or ports);
- c) environmental or husbandry conditions that are permissive for *disease* establishment (e.g. temperature, salinity, production system type, habitat type, exposure to recent stressors);
- d) conditions that are conducive for development of clinical *disease*; including the species or life stages that are most susceptible to clinical *disease*;

- e) evidence of morbidity or mortality.

## 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

In surveys conducted to demonstrate the absence or presence of an *infection*, 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 1.2. 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 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. 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.) and may be supplemented with *targeted surveillance* if necessary (as described in Article 1.4.12.). *Passive surveillance* information can also be used to provide additional support for *disease* freedom, 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.

If combining evidence from different sources including *passive surveillance* and *targeted surveillance*, a *Competent Authority* may choose to use various approaches, such as a scenario tree modelling approach.

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* provides 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 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*.

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## 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 it can demonstrate that:

- 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 4.34.) have been completed followed by following as described in Chapter 4.67.; 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 it can demonstrate that:

- 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 4.34.) have been completed followed by following 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 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 it can demonstrate that:

- 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 4.3-4.) have been completed, and the *compartment* has been followed as described in Chapter 4.6-7. 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 *aquatic 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.~~

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## (CLEAN VERSION)

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

## Article X.X.4.

**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.

**Country free from infection with [PATHOGEN X]**

If a country shares water bodies with other countries, it can only make a self-declaration of freedom from infection with [PATHOGEN X] if 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 it can demonstrate that:

- 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 [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 *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 [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 4.4.) 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:
  - 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 *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.

##### 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 it can demonstrate that:

- 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 [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. 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 *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.

#### **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 it can demonstrate that:

- 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 *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* 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 4.4.) 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 *aquatic 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.

#### **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.

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# 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 importation 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 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) 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, for a time/temperature equivalent that has been demonstrated to inactivate VpAHPND;
  - 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 VpAHPND);
  - 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 VpAHPND);
  - 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 VpAHPND;
  - d) crustacean oil;
  - e) 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 VpAHPND);
  - f) 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 VpAHPND, 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) *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 *VpAHPND*;
- 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 *VpAHPND*;
- 3) crustacean oil;
- 4) chemically extracted chitin.

[...]

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(TRACK CHANGES 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

- 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 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.:
- a1) 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 inactivate *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)b2) 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)e5) 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) *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.

[...]

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(TRACK CHANGES 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**

- 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 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.:~~
- a1) 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 *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) e) 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) *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.

[...]

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(TRACK CHANGES 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**

- 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 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.:
  - a1) 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, (for a time/temperature equivalent that has been demonstrated to inactivate 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;
- e) 3) crustacean oil.
- d) e) crustacean meal that has been heat treated at a core temperature of at least 100°C for at 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 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*:

- a) *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 IHNV;
- 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 IHNV;
- c) crustacean oil.

[...]

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(TRACK CHANGES 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**

- 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 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) ~~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, ~~for a time/temperature equivalent that has been demonstrated to inactivate 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);
  - e) 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)~~ 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) *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.

[...]

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(TRACK CHANGES 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**

- 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 these aquatic animal products listed below, Competent Authorities should not require any sanitary measureseconditions 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.:
- a1) 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) 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) *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.

[...]

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(TRACK CHANGES 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**

- 4) ~~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.:~~
- a1) ~~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) *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.

[...]

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## (TRACK CHANGES 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**

- ~~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 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.:
- a1) 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);~~
  - e) ~~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)e) 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)d4)~~ 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) *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.

[...]

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(TRACK CHANGES 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

- 4) ~~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.:
- a1) 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);~~
- e) ~~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) *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.

[...]

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[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 importation or transit of aquatic animal products for any purpose regardless of the infection with EHNV status of the exporting country, zone or compartment

- 4) 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) 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 time/temperature equivalent that has been demonstrated to inactivate 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) fish oil;
  - e) fish meal;
  - f) 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) *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.

[...]

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(TRACK CHANGES 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**

- 4) 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*);~~
  - e)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 time/temperature equivalent that has been demonstrated to inactivate *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) *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.

[...]

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(TRACK CHANGES 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**

4) 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 that have been heat treated and are hermetically sealed;

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*);~~

e)2) mechanically dried eviscerated fish;

e)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) *aquatic animal products* that have been heat treated and are hermetically sealed;
- 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.

[...]

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(TRACK CHANGES 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.

- 4) 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);~~
  - e)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 any time/temperature equivalent that has been demonstrated to inactivate 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;
  - D4) 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) *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.

[...]

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(TRACK CHANGES 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**

- 4) 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 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);
  - e) 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 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;
  - d) 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) *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.

[...]

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(TRACK CHANGES 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 IHN status of the exporting country, zone or compartment**

- 4) 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 IHN, regardless of the infection with IHN 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 IHN;
    - 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 IHN);
    - 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 IHN);
    - c) 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 inactivate IHN);
  - 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 IHN;
  - d) fish oil;
  - e) fish meal;
  - f) 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 IHN 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 IHN, 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 IHN 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 IHN, regardless of the infection with IHN status of the *exporting country, zone or compartment*:

- 1) *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 IHN;
- 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 IHN;
- 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 IHN;
- 4) fish oil;
- 5) fish skin leather.

[...]

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(TRACK CHANGES 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**

- 4) 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);
    - e) 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) 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) 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) *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.

[...]

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(TRACK CHANGES 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**

- 4) 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 121°C 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 90°C for at least ten minutes (or any time/temperature equivalent that has been demonstrated to inactivate RSIV);
  - e) 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 100°C for at least 30 minutes or any time/temperature equivalent that has been demonstrated to inactivate 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) 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) *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.

[...]

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(TRACK CHANGES 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

- 4) 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) 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 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;
  - d) 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) *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 importation or transit of aquatic animal products for any purpose regardless of the infection with VHSV status of the exporting country, zone or compartment**

- 4) 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);
  - e) 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) naturally dried, eviscerated fish (i.e. sun-dried or wind-dried);
  - e) 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) *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.

[...]

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## 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*, *Competent Authorities* should not require any *sanitary measures* 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 it can demonstrate that:

- 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 following 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 it can demonstrate that:

- 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 it can demonstrate that:

- 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 *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 *aquatic 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 inactivate 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.1214., 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.

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## 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*).~~

<u>Family</u>	<u>Scientific name</u>	<u>Common name</u>
<u>Esocidae</u>	<u><i>Esox lucius</i></u>	<u>Northern pike</u>
<u>Galaxiidae</u>	<u><i>Galaxias olidus</i></u>	<u>Mountain galaxias</u>
<u>Ictaluridae</u>	<u><i>Ameiurus melas</i></u>	<u>Black bullhead</u>
<u>Melanotaeniidae</u>	<u><i>Melanotaenia fluviatilis</i></u>	<u>Crimson spotted rainbow fish</u>
<u>Percidae</u>	<u><i>Perca fluviatilis</i></u>	<u>European perch</u>
	<u><i>Sander lucioperca</i></u>	<u>Pike-perch</u>
<u>Percichthyidae</u>	<u><i>Macquaria australasica</i></u>	<u>Macquarie perch</u>
<u>Poeciliidae</u>	<u><i>Gambusia holbrooki</i></u>	<u>Eastern mosquito fish</u>
	<u><i>Gambusia affinis</i></u>	<u>Mosquito fish</u>
<u>Salmonidae</u>	<u><i>Oncorhynchus mykiss</i></u>	<u>Rainbow trout</u>
<u>Terapontidae</u>	<u><i>Bidyanus bidyanus</i></u>	<u>Silver perch</u>

[...]

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## 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*).

[...]

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## 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 laevis*), hybrids of greenlip x blacklip abalone (*Haliotis laevis* x *Haliotis rubra*) and small abalone (*Haliotis diversicolor*). *Haliotis diversicolor* (subspecies *aquaticus* and *supertexta*), *Haliotis laevis*, *Haliotis rubra* and hybrids of *Haliotis laevis* x *Haliotis rubra*. These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

[...]

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## 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*), crested oyster (*Ostrea equestris*), 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.~~

[...]

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## **ASSESSMENT OF *OSTREA EQUESTRIS* AND REASSESSMENT OF *OSTREA STENTINA* AS SUSCEPTIBLE SPECIES TO INFECTION WITH *BONAMIA EXITIOSA***

### *Background*

In response to a comment questioning whether *Ostrea stentina* and *Ostrea equestris* should be considered as distinct species, the Aquatic Animals Commission requested that the *ad hoc* Group on Susceptibility of mollusc species to infection with OIE listed diseases (the *ad hoc* Group) review any new scientific information and provide its opinion on this. If the species were distinct, the Commission requested that the *ad hoc* Group determine the impact on the species proposed for inclusion in Article 11.2.2. of Chapter 11.2. Infection with *Bonamia exitiosa*.

The November - December 2020 report on the susceptibility of mollusc species to infection with *Bonamia exitiosa* can be found on the [OIE website](#).

The November-December 2020 *ad hoc* Group report noted that “According to WoRMS, *Ostrea stentina* and *Ostrea equestris* are considered distinct species, however there are some papers (Hill *et al.*, 2010; Shilts *et al.*, 2007) that consider them synonyms.” Based on this information the *ad hoc* Group considered the two species synonyms for their assessment of species susceptible to infection with *Bonamia exitiosa*.

At its February 2021 meeting, based on the *ad hoc* Group’s recommendation that *Ostrea stentina* and *Ostrea equestris* were synonyms, the Aquatic Animals Commission proposed to include *Ostrea stentina* in Article 11.2.2. of Chapter 11.2. of the *Aquatic Code*.

### ***Ad hoc* Group review of evidence and recommendations (January 2022)**

The *ad hoc* Group reviewed the scientific evidence regarding the taxonomic status of flat oysters to resolve whether *Ostrea equestris* and *Ostrea stentina* are synonyms or distinct species for the purposes of assessing susceptibility to infection with *Bonamia exitiosa*.

The status of the complex *Ostrea equestris* / *Ostrea stentina* / *Ostrea aypouria* has been controversial for many years. Studies investigating this issue have been based on both morphological and phylogenetical data. The *ad hoc* Group reviewed new evidence on phylogenetic analysis using genetic distances estimated from COI sequences and contacted several experts to support its recommendation to the Aquatic Animals Commission.

In light of new scientific evidence and personal communications, the *ad hoc* Group recommended that *Ostrea stentina* and *Ostrea equestris* be considered distinct species. The *ad hoc* Group also noted that the two species had a different geographic distribution. *Ostrea equestris* is distributed in the Americas (North and South) and the western Pacific (New Zealand), while *Ostrea stentina* is distributed in the eastern Atlantic (Tunisia, Spain).

Based on the recommendation that *Ostrea equestris* and *Ostrea stentina* are distinct species, the *ad hoc* Group assessed *Ostrea equestris* and reassessed *Ostrea stentina* for listing as susceptible to infection with *Bonamia exitiosa*.

### **Methodology**

- The AHG applied criteria, as outlined in Article 1.5.3 of the *Aquatic Code*), to assess *Ostrea equestris* and reassess *Ostrea stentina* in order to determine susceptibility to infection with *Bonamia exitiosa*. The same methodology and considerations outlined in the *ad hoc* Group report (<https://www.oie.int/app/uploads/2021/11/a-ahg-susceptibility-of-mollusc-species-to-infection-with-oie-listed-diseases-november-december-2020.pdf>) was applied to these assessments.

## Assessments of host susceptibility to infection with *Bonamia exitiosa*

### Summary

The *ad hoc* Group agreed that *Ostrea equestris* met the criteria for listing as susceptible to infection with *Bonamia exitiosa* in accordance with Chapter 1.5. of the *Aquatic Code*. *Ostrea equestris* was proposed to be included in Article 11.2.2. of the *Aquatic Code*. The outcomes of this assessment are shown in Table 1.

The *ad hoc* Group agreed that *Ostrea stentina* had incomplete evidence of susceptibility and proposed it be removed from Article 11.2.2. of the *Aquatic Code* and be included in Section 2.2.2. of Chapter 2.4.2., Infection with *Bonamia exitiosa* of the *Aquatic Manual*. The outcomes of this re-assessment are shown in Table 1.

Table 1. Assessments for *O.equestris* and *O.stentina* for susceptibility to infection with *B. exitiosa* .

Family	Scientific name	Common name	Stages 1: Route of infection	Stage 2: Pathogen identification	Stage 3: Evidence for infection				Individual Outcome	References
					A	B	C	D		
Overall Score 1										
Ostreidae	<i>Ostrea equestris</i>	Crested oyster <sup>1</sup>	N <sup>2</sup>	PCR and sequencing (18S & ITS)	YES	ND	YES	YES	1	Hill <i>et al.</i> , 2014
Overall Score 2										
Ostreidae	<i>Ostrea stentina</i>	Dwarf oyster	N	PCR & sequencing (18S & ITS)	YES	ND	ND	YES	1	Hill <i>et al.</i> , 2010

### Assessment Table Key

N: Natural infection  
 E: Experimental (non-invasive)  
 EI: Experimental (invasive)  
 YES: Demonstrates criterion is met.  
 NO: Criterion is not met.  
 ND: Not determined.

<sup>1</sup> The common names of mollusc species are in accordance with FAOTERM (<http://www.fao.org/faoterm/collection/faoterm/en/>). Where the common mollusc name was not found in FAOTERM, the naming was done in accordance with <https://www.sealifebase.ca>.

<sup>2</sup> Samples were investigated from geographically separated locations and time periods.

## Species specific comments

*Ostrea equestris*: Only one paper was available for the assessment (Hill *et al.*, 2014) but was determined by the *ad hoc* Group to provide sufficient information to demonstrate that the criteria for susceptibility be scored as a '1' as there were multiple collections of oysters from different locations and time periods.

*Ostrea stentina*: Only one study (Hill *et al.*, 2010) was available for the assessment and within that study there was only one sample collected from one location at one time point. The *ad hoc* Group was unable to find any additional studies or evidence to corroborate the *O. stentina* assessment. Consequently, even though the assessment criteria were met for the one individual animal sampled and that the paper was assigned an outcome of '1', based on the limited data presented in Hill *et al.*, 2010, the *ad hoc* Group assessed *Ostrea stentina* as an overall score of '2'

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## 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. Negative molecular results should be investigated further when clinical signs indicate the presence of a specific disease, or other positive test results have indicated that a false negative result may have been obtained.

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## CHAPTER 2.3.4.

## INFECTION WITH HPR-DELETED OR HPR0 INFECTIOUS SALMON ANAEMIA VIRUS

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### 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, 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. 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 (Cardenas *et al.*, 2019; 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). However, deleted ISAV variants have been found without this virulence marker on segment 5 (Cardenas *et al.*, 2019). 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 either 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-infectivity 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	<i>Caligus rogercresseyi</i>	sea lice	Ito <i>et al.</i> , 2015
Cyclopteridae	<i>Cyclopterus lumpus</i>	lumpfish	Ito <i>et al.</i> , 2015
Cyprinidae	<i>Cyprinus carpio</i>	common carp	Ito <i>et al.</i> , 2015
Gadidae	<i>Gadus morhua</i>	Atlantic cod	MacLean <i>et al.</i> , 2003; Snow & Raynard, 2005
	<i>Pollachius virens</i>	saithe	Snow <i>et al.</i> , 2002
	<i>Pollachius virens</i>	pollack	Ito <i>et al.</i> , 2015
Mytilidae	<i>Mytilus edulis</i>	blue mussel	Molloy <i>et al.</i> , 2014; Skar & Mortensen, 2007
Pleuronectidae	<i>Hippoglossus hippoglossus</i>	Atlantic halibut	Ito <i>et al.</i> , 2015
Salmonidae	<i>Oncorhynchus tshawytscha</i>	Chinook salmon	Rolland & Winton, 2003
	<i>Carassius auratus</i>	goldfish	Ito <i>et al.</i> , 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  $\alpha$ -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; iii-iv) focal or diffusely dark red liver (a thin fibrin layer may be present on the surface); iv) 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; vi) swollen, dark red kidney with blood and liquid effusing from cut surfaces; and vii) 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.~~

Except for a single report by Ditlecadet *et al.* (2021), 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- $\beta$ -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<sup>-2</sup> and 50 Jm<sup>-2</sup>, respectively, while the corresponding value for ISAV in wastewater from a fish-processing plant was approximately 72 Jm<sup>-2</sup>. Ozonated seawater (4 minutes with 8 mg ml<sup>-1</sup>, 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<sup>-1</sup>) 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, demonstrates 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, and 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. Gill swabs are recommended for non-lethal sampling for HPR0 (Aamelfort *et al.*, 2016).

### 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 RNA later~~, 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.

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

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 pathogen detection 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 animal life stage.

The designations used in the Table indicate:

**Ratings against for purposes of use.** For each recommended assay a qualitative rating **against for** 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, availability cost, timeliness, and sample throughput and operability. For a specific purpose of use, assays are rated as:

Key:

+++ = Most suitable Methods —are most suitable with desirable performance and operational characteristics.

++ = Suitable Method(s) are suitable with acceptable performance and operational characteristics under most circumstances.

+ = Less suitable Methods —are suitable, but performance or operational characteristics may significantly limit application under some circumstances.




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, detection of homologous sequences within the host genome). 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.

**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 <sup>1</sup> of a suspect result from surveillance or presumptive diagnosis			
	Early life stages <sup>2</sup>	Juveniles <sup>2</sup>	Adults	LV	Early life stages <sup>2</sup>	Juveniles <sup>2</sup>	Adults	LV	Early life stages <sup>2</sup>	Juveniles <sup>2</sup>	Adults	LV
Gross signs					+	+	+	1				
Histopathology <sup>3</sup>					++	++	++	1				
Cell or artificial media culture					++	++	++	1	++ 	++ 	++ 	NA <u>1</u>
Real-time RT-PCR	+++	+++	+++	1	+++	+++	+++	<u>3-1</u>	<u>++</u>	<u>++</u>	<u>++</u>	<u>1</u>
Conventional RT-PCR	+	+	+	1	++	++	++	1	<u>+</u>	<u>+</u>	<u>+</u>	NA <u>1</u>
Amplicon sequencing <sup>4</sup>									+++	+++	+++	NA
<i>In-situ</i> hybridisation												
Immunohistochemistry					++	++	++	1	++	++	++	NA <u>1</u>
IFAT on kidney imprints or blood smears					++	++	++	1	+++	+++	+++	NA <u>1</u>
Bioassay												
LAMP												
Ab-ELISA												
Ag-ELISA												
Other antigen detection methods <sup>5</sup>												

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.

<sup>1</sup>For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). <sup>2</sup>Susceptibility of early and juvenile life stages have been defined is described in Section 2.2.3.

<sup>3</sup>Histopathology and cytopathology can be validated if the results from different operators have been statistically compared. <sup>4</sup>Sequencing of the PCR product.

Shading indicates the test is inappropriate or should not be used for this purpose.

#### 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<sup>-1</sup>) and 2-mercapto-ethanol (40 µM) (this latter supplement may be omitted).

For virus isolation, cells grown in 25 cm<sup>2</sup> 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.**

**Until 2022, for HPR0 ISAV, there had been no reports of cultivation of HPR0 ISAV and no reports of virulence markers in the F segment. However in 2022, a HPR0-like ISAV variant within the North American clade was described and cultured on ASK and SHK-1 cells (Ditlecadet *et al.*, 2022). This variant was found to have F virulence markers, but experimental studies in fish for this variant have not yet been published.**

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	7	155	Snow <i>et al.</i> , 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)	45 × 15 seconds @ 95°C and 1 minute @ 60°C	8	104	Snow <i>et al.</i> , 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 ~~g~~ 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~~ species specific IgG (diluted 4/200 in 2.5% BSA in Tris buffer) for 60 minutes, followed by ABC-AP (diluted 4/400 in Tris buffer) for 45 minutes. Following a final wash, Fast Red (1 mg ml<sup>-1</sup>) and Naphthol AS-MX phosphate (0.2 mg ml<sup>-1</sup>) 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 MAb 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 obtained 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<sup>-1</sup> 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 <sup>3</sup>

Apparently healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link(s) to an infected population. Geographical proximity to, or movement of animals or animal products or equipment, etc., from a known infected population equate to an epidemiological link. Alternatively, healthy populations are sampled in surveys to demonstrate disease freedom.

##### 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

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.

<sup>3</sup> For example transboundary commodities.

#### *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 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 of internal organs 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 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 <i>et al.</i> , 1995

DSe: = diagnostic sensitivity, DSp = diagnostic specificity, *n* = number of samples used in the study,  
PCR: = 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
Real-time PCR	Surveillance	Salmonids	Gills, Kidney, and heart	Salmo salar and other salmonids	Non available	Non available	Cell culture	Snow <i>et al.</i> , 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.

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## 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 the KHV genome 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.*, 2008a). 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 of which 156 are 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.

The conventional polymerase chain reaction (PCR) developed by Engelsma *et al.* (2013) detected novel strains of cyprinid herpesvirus closely related to KHV. These strains may represent low or non-pathogenic variants of CyHV-3, but further investigation is required to establish the true genetic relationship between these strains, and KHV.

#### 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	<i>Acipenser gueldenstaedtii</i>	Atlantic sturgeon
	<i>Acipenser ruthenus</i> × <i>Huso huso</i>	hybrid sterlet × beluga
	<i>Acipenser oxyrinchus</i>	Russian sturgeon
Cyprinidae	<i>Leuciscus idus</i>	blue back ide
	<i>Rutilus rutilus</i>	common roach
	<i>Tinca tinca</i>	Tench
	<i>Hypophthalmichthys molitrix</i>	silver carp
Gammaridae	<i>Gammarus pulex</i>	scud (crustacean)
Nemacheilidae	<i>Barbatula barbatula</i>	stone loach
Percidae	<i>Gymnocephalus cernuus</i>	Eurasian ruffe
	<i>Perca fluviatilis</i>	European perch
Salmonidae	<i>Oncorhynchus mykiss</i>	rainbow trout
Unionidae	<i>Anodonta cygnea</i>	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	<i>Intellagama lesueurii</i>	Eastern water dragon
Ambassidae	<i>Ambassis agassizii</i>	olive perchlet
Anguillidae	<i>Anguilla australis</i>	short-finned eel
Ariidae	<i>Neosalanx gillii</i>	salmon catfish
Chelidae	<i>Emydura macquarii</i>	Macquarie short-necked turtle
Clupeidae	<i>Nematalosa erebi</i>	bony bream
Eleotridae	<i>Hypseleotris</i> sp.	carp gudgeon
Galaxiidae	<i>Galaxias maculatus</i>	common galaxias
Limnodynastidae	<i>Limnodynastes tasmaniensis</i>	spotted marsh frogs
Melanotaeniidae	<i>Melanotaenia duboulayi</i>	crimson-spotted rainbowfish
Mordaciidae	<i>Mordacia mordax</i>	short-headed lamprey ammocoetes
Mugilidae	<i>Mugil cephalus</i>	sea mullet
Parastacidae	<i>Cherax destructor</i>	common yabby
Pelodryadidae	<i>Litoria peronii</i>	Peron's tree frog
Percichthyidae	<i>Maccullochella peelii</i>	Murray cod
	<i>Macquaria ambigua</i>	golden perch
Plotosidae	<i>Tandanus tandanus</i>	eel-tailed catfish
Retropinna	<i>Retropinna semoni</i>	Australian smelt
Terapontidae	<i>Bidyanus bidyanus</i>	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%) compared 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 (Gilad *et al.*, 2003; 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 also been detected by PCR in swan mussels (*Anodonta cygnea*) and freshwater shrimp (*Gammarus pulex*) (Kielpinski *et al.*, 2010) and in migratory wild ducks of the genera *Anas*, *Mareca*, *Spatula* and *Oxyura* (Torres-Meza *et al.*, 2020) in areas where fish and ducks coexist. 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 endophthalmia (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 and the intestine are the major portal of virus entry in carp (Dishon *et al.*, 2005; Gilad *et al.*, 2004; Ilouze *et al.*, 2006; 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; Miyazaki *et al.*, 2008b). 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  $4.0 \times 10^3 \mu\text{Ws/cm}^2$ , temperatures above 50°C for 1 minute and by iodophor (200 mg litre<sup>-1</sup>) treatment for 30 seconds at 15°C (Kasai *et al.*, 2005). The following disinfectants are also effective for inactivation: iodophor at 200 mg litre<sup>-1</sup> for 20 minutes, benzalkonium chloride at 60 mg litre<sup>-1</sup> for 20 minutes, ethyl alcohol at 30% for 20 minutes and sodium hypochlorite at 200 mg litre<sup>-1</sup> 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 40:1. 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 pathogen detection~~ 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 animal life stage.

~~The designations used in the Table indicate:~~

**Ratings against for purposes of use.** For each recommended assay a qualitative rating against for 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, availability, cost, timeliness, and sample throughput and operability. For a specific purpose of use, assays are rated as:

**Key:**

- +++ = ~~Most suitable Methods~~ are most suitable with desirable performance and operational characteristics.
- ++ = ~~Suitable Method(s)~~ are suitable with acceptable performance and operational characteristics under most circumstances.
- + = ~~Less suitable Methods~~ are suitable, but performance or operational characteristics may significantly limit application under some circumstances.
- 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, detection of homologous sequences within the host genome). 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.



**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 <sup>1</sup> of a suspect result from surveillance or presumptive diagnosis			
	Early life stages <sup>2</sup>	Juveniles <sup>2</sup>	Adults	LV	Early life stages <sup>2</sup>	Juveniles <sup>2</sup>	Adults	LV	Early life stages <sup>2</sup>	Juveniles <sup>2</sup>	Adults	LV
Wet mounts												
Histopathology <sup>3</sup>						++	++	1				
Cell or artificial media culture						++	++	1				
Real-time PCR	+++	+++	+++	4 3	+++	+++	+++	4 3				
Conventional PCR					++	+++	+++	4-3 <sup>5</sup>	++	++	++	4-3 <sup>5</sup>
Conventional nested PCR	+ 4	+ 4	+ 4	4 NA	++ 4	++ 4	++ 4	4 NA	+ 4	+ 4	+ 4	4 NA
Amplicon sequencing <sup>4</sup>									+++	+++	+++	1
In-situ hybridisation												
Bioassay												
LAMP						+++	+++	1				
IFAT						+	+	1				
ELISA												
Other antigen detection methods <sup>5</sup>												
Other method <sup>5</sup>												

LV = level of validation, refers to the stage of validation in the OIE Pathway (chapter 1.1.2); **NA = not available**; PCR = polymerase chain reaction; LAMP = loop-mediated isothermal amplification; IFAT = indirect fluorescent antibody test; ELISA = enzyme-linked immunosorbent assay, respectively.

<sup>1</sup>For confirmatory diagnoses, methods need to be carried out in combination (see Section 6).

<sup>2</sup>Susceptibility of early and juvenile life stages have been defined is described in Section 2.2.3.

<sup>3</sup>Histopathology and cytopathology can be validated if the results from different operators have been statistically compared.

<sup>4</sup>Sequencing of the PCR product.

<sup>5</sup>Specify the test used: Bercovier et al. (2005) method as modified by Clouthier et al. (2017); other conventional PCR assays level 1.

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 for virological examination is described in Section 2.3.2. of 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 virus that has caused 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 cell culture material.
- ii) Extracted DNA is then amplified using the PCR protocols described below (Section 4.4.2. or 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 however, 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 do not detect novel strains of cyprinid herpesvirus closely related to KHV 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 increasing 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	78	Gilad <i>et al.</i> (2004) <sup>1</sup>
	KHV-163r: CGG-GTT-CTT-ATT-TTT-GTC-CTT-GTT (400 nM)	1 × 10 minutes @ 95°C		
	KHV-109p: 6FAM-CTT-CCT-CTG-CTC-GGC-GAG-CAC-G-TAMRA (80 nM)	40 × 15 seconds @ 95°C and 60 seconds @ 60°C		
Glucokinase	CgGluc-162f: ACT-GCG-AGT-GGA-GAC-ACA-TGA-T (400 nM)	40 × 15 seconds @ 95°C and 60 seconds @ 60°C	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)			

<sup>1</sup>The Gilad *et al.* (2004) (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. 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 conventional PCR protocols methods 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)	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 <i>et al.</i> (2013)

Primer sequence (5'→3') (concentration)	Cycling conditions	Amplicon size (bp)	References
Nested PCR:  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 × <del>45-5</del> minutes @ <del>94-95</del> °C  40 × <del>45-60</del> seconds @ 95°C, <del>45-60</del> seconds @ 55°C and 60 seconds @ 72°C  1 × <del>7-10</del> minutes @ 72°C	409	Bercovier <i>et al.</i> (2005) <sup>1</sup> <u>Clouthier <i>et al.</i> (2017)</u>
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 <i>et al.</i> (2002) Yuasa <i>et al.</i> (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 <i>et al.</i> , (2004)

<sup>1</sup>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~~has~~ been used in research applications for detection, confirmation, or identification of KHV. Although ~~this~~ these methods have~~has~~ not been thoroughly compared with other techniques and is not included in Table 4.1, they are it is a non-destructive (non-lethal) techniques and some laboratories may find them it useful in a research 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  $-20^{\circ}\text{C}$ ) for glass slides or a mixture of 30% acetone/70% ethanol, also stored at  $-20^{\circ}\text{C}$ , for plastic wells.
- v) Let the fixative act for 15 minutes. A volume of 0.5 ml/2 cm<sup>2</sup> 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  $-20^{\circ}\text{C}$ .
- 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  $37^{\circ}\text{C}$ .
- x) Rinse four times with PBST.
- xi) Treat the imprints with the antibody solution (prepared at step viii) for 1 hour at  $37^{\circ}\text{C}$  in a humid chamber and do not allow evaporation to occur. A volume of 0.25 ml/2 cm<sup>2</sup> well is adequate for imprints in cell culture plates.
- xii) Rinse four times with PBST.
- xiii) Treat the imprints for 1 hour at  $37^{\circ}\text{C}$  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<sup>2</sup> 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 fluorescence 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  $37^{\circ}\text{C}$  for 30 minutes. The sections are then washed in cold PBS before proceeding with steps viii–xvi above. Tissues collected for direct detection 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.* 2017b). 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 healthy 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 novel strains of cyprinid herpesvirus closely related to KHV 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 <sup>4</sup>

Apparently healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link(s) to an infected population. Geographic proximity to, or movement of animals or animal products or equipment, etc., from a known infected population equate to an epidemiological link. Alternatively, healthy populations are sampled in surveys to demonstrate disease freedom.

##### 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:

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<sup>4</sup> For example transboundary commodities.

- i) A positive result from a real-time PCR assay
- ii) A positive result from a conventional nested PCR assay.

### 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 ~~followed by~~ and sequencing of the amplicon
- ii) Detection of KHV in tissue samples by real time PCR followed 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 PCR assays, based on an analysis of field collections and experimentally infected carp (Amita *et al.*, 2002; Ito *et al.*, 2014a; 2014b) demonstrated 94–100% DSe and 100% DSp.

### 6.3.1. For surveillance of clinically affected apparently healthy animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (n)	DSp (n)	Reference test	Citation
Real-time PCR <sup>1</sup>	Diagnosis	Experimentally infected koi and apparently healthy wild common carp <sup>3</sup>	kidney	Common carp & koi ( <i>Cyprinus carpio</i> L.)	99	93	None; Bayesian latent class modelling	Clouthier <i>et al.</i> , 2017 <sup>4</sup>
PCR <sup>2</sup>	Diagnosis	Experimentally infected koi and apparently healthy wild common carp <sup>3</sup>	kidney	Common carp & koi ( <i>Cyprinus carpio</i> L.)	99	93	None; Bayesian latent class modelling	Clouthier <i>et al.</i> , 2017 <sup>4</sup>

DSe: = diagnostic sensitivity, DSp = diagnostic specificity.

<sup>1</sup>Gilad *et al.* (2004) method as modified by Clouthier *et al.* (2017);

<sup>2</sup>Bercovier *et al.* (2005) method as modified by Clouthier *et al.* (2017);

<sup>3</sup>Note that Clouthier *et al.* (2017) reported diagnostic performance for a combined dataset of clinically affected and apparently healthy populations.

<sup>4</sup>The diagnostic accuracy study did not include samples that were known to be positive for the KHV-like CyHV strains reported by Engelsma *et al.* (2013).

### 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 ( <i>Cyprinus carpio</i> L.)	99	93	None; Bayesian-latent class modelling	Clouthier <i>et al.</i> , 2017 <sup>1</sup>
PCR	Diagnosis	Experimentally infected koi and apparently healthy wild common carp	kidney	Common carp & koi ( <i>Cyprinus carpio</i> L.)	99	93	None; Bayesian-latent class modelling	Clouthier <i>et al.</i> , 2017 <sup>1</sup>

DSe: = diagnostic sensitivity, DSp = diagnostic specificity.

<sup>1</sup>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.

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## 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 laevis*), 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 laevis*

Blacklip abalone — *H. rubra*

Hybrid (greenlip × blacklip) — *H. laevis* × *H. rubra*

Diversicolor abalone or jukong 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 laevis*), hybrids of greenlip x blacklip abalone (*Haliotis laevis* 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*).

[...]

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## 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*), crusted oyster (*Ostrea equestris*), 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 dwarf oyster (*Ostrea stentina*)

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*).

[...]

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