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

Paris, 11–18 September 2018

The OIE Aquatic Animal Health Standards Commission (Aquatic Animals Commission) met at OIE Headquarters in Paris from 11 to 18 September 2018. The list of participants is presented in **Annex 1**.

The Aquatic Animals Commission thanked the following Member Countries for providing written comments on draft texts for the OIE *Aquatic Animal Health Code* (hereinafter referred to as the *Aquatic Code*) and OIE *Manual of Diagnostic Tests for Aquatic Animals* (hereinafter referred to as the *Aquatic Manual*) circulated after the Commission's September 2018 meeting: Australia, Canada, China (People's Rep. of), Chinese Taipei, Colombia, Fiji, Japan, Malaysia, Mexico, New Caledonia, New Zealand, Singapore, Switzerland, Thailand, the United States of America (USA), the Member States of the European Union (EU), and the African Union Interafrican Bureau for Animal Resources (AU-IBAR) on behalf of African Member Countries of the OIE.

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

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

The Aquatic Animals Commission encourages Member Countries to refer to previous reports when preparing comments on longstanding issues. The Commission also draws the attention of Member Countries to the reports of *ad hoc* Groups, which include important information, and encourages Member Countries to review these reports together with the report of the Commission, where relevant. These reports are readily available on the OIE website.

The table below summarises the texts as presented in the Annexes. Member Countries should note that texts in **Annexes 3 to 17** are presented for Member Countries' comments and **Annexes 18 to 22** are presented for Member Countries' information.

Comments on **Annexes 3 to 17** of this report must reach OIE Headquarters by the **4 January 2019** to be considered at the February 2019 meeting of the Aquatic Animals Commission. Comments received after the due date will not be submitted to the Commission for its consideration.

All comments should be sent to the OIE Standards Department at: standards.dept@oie.int.

The Aquatic Animals Commission again strongly encourages Member Countries to participate in the development of the OIE's international standards by submitting comments on this report, and prepare to participate in the process of adoption at the General Session. Comments should be submitted as Word files rather than pdf files because pdf files are difficult to incorporate into the Commission's working documents.

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

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A. MEETING WITH THE DIRECTOR GENERAL

The Aquatic Animals Commission met with Dr Monique Eloit, Director General on 11 September 2018. Dr Eloit congratulated both new and re-elected members on their election and welcomed them all to this, the first meeting of the newly elected Commission, and thanked them for their commitment to the work of this Commission.

Dr Eloit acknowledged the Member Countries' requests and high expectations for the OIE standard setting process. Noting the resource and financial constraints faced by the OIE to support *ad hoc* group meetings, she asked the Commission for its active consideration of these constraints in considering its work programme. Dr Eloit drew to the attention of the Commission members to the framework for the evaluation of the performance of Specialist Commissions which would be introduced at its meeting in February 2019. The Director General highlighted the importance of good coordination among the Specialist Commissions and their Secretariats and noted the high expectations for the Common Secretariat for which the Standards Department takes a leading role.

The Director General emphasised her commitment to exploring ways in which the OIE could better support the work of this Commission and OIE work in the area of aquatic animal health. She also noted that the OIE Global conference on Aquatic Animal Health provides an excellent opportunity to highlight activities in this area and raising awareness about the importance of this sector.

B. INDUCTION SESSION TO SUPPORT PERFORMANCE OF THE NEWLY ELECTED SPECIALIST COMMISSIONS

The Aquatic Animals Commission participated in a half day induction session that was facilitated by OIE staff. The purpose of the session, for new and previously elected members, was for members to get to know each other, to better understand how the work of the Commission fits into the mission of the OIE and to clarify the roles of Commission members and OIE Secretariat and other staff. Members acknowledged that this new initiative was very valuable for all concerned and will assist in ensuring the success of the work of the Commission. The OIE advised that it will continue to explore other novel ways of supporting the work of the Commission.

C. ADOPTION OF THE AGENDA

The draft agenda circulated prior to the meeting was discussed, updated, and agreed. The adopted agenda of the meeting is presented at **Annex 2**.

D. MEETING WITH THE PRESIDENT OF THE OIE TERRESTRIAL ANIMAL HEALTH STANDARDS COMMISSION

The President of the Aquatic Animals Commission met with the President of the Terrestrial Animal Health Standards Commission (hereinafter referred to as the Code Commission) during the week when both Commissions were meeting. The Presidents discussed issues of mutual interest in the *Aquatic* and *Terrestrial Codes*, notably: proposed amendments to Chapter 1.1., progress regarding proposed new and revised chapters in Section 4 of both Codes and the development of a guidance document on the application of the criteria for listing an OIE disease.

E. OIE *AQUATIC ANIMAL HEALTH CODE*

1. Texts circulated for Member Country comments at the February 2018 meeting

1.1. General comments

Comments were received from New Caledonia.

In response to a Member Country comment regarding the determination of equivalence for methods of inactivation of pathogenic agents, the Aquatic Animals Commission noted that this issue would be addressed by the *ad hoc* Group on Safe aquatic animal commodities when it is convened as scheduled in the work plan (**Annex 22**).

In response to a Member Country comment noting the importance of harmonisation between the two Codes, the Aquatic Animals Commission wished to assure Member Countries that there is active communication between them and the Code Commission to ensure alignment, where relevant.

1.2. Glossary

Comments were received from Australia, China (People's Rep. of), New Caledonia, Malaysia, Switzerland, EU and AU-IBAR.

Basic biosecurity conditions

The Aquatic Animals Commission reviewed Member Country comments for the revised definition for 'basic biosecurity conditions' and amended the text, where relevant.

The Aquatic Animals Commission did not agree with a Member Country comment to replace 'the disease' in point a) of the definition with 'mass mortality' or 'know/identify disease' as it considered that notifying mortality is part of the early detection system included in point b).

The Aquatic Animals Commission did not agree with a Member Country to add aquaculture establishment to the definition as basic biosecurity conditions is used in the *Aquatic Code* in the context of freedom from a disease in a country, zone or compartment.

The revised definition for 'basic biosecurity conditions' is presented in **Annex 3** for Member Country comment.

1.3. Criteria for listing species as susceptible (Chapter 1.5.)

Comments were received from Canada, China (People's Rep. of), New Zealand, Thailand, Switzerland, EU and AU-IBAR.

Article 1.5.2.

In response to a Member Country comment to reinstate the first sentence in Article 1.5.2. regarding susceptibility, the Aquatic Animals Commission agreed it was important to clarify that susceptibility may include clinical or non-clinical infection, and added this phrase to the end of the second paragraph. Species of aquatic animals are considered susceptible to infection with a pathogenic agent when the presence of a multiplying or developing pathogenic agent has been demonstrated by the occurrence of natural cases or by experimental exposure that mimics natural transmission pathways.

The Aquatic Animals Commission agreed with a Member Country comment to delete 'latent' from this article because latent infections would not provide sufficient evidence for demonstrating susceptibility of a species in accordance with the criteria.

Article 1.5.4.

The Aquatic Animals Commission did not agree with a Member Country to delete point 3 in Article 1.5.4. as it considered that all available evidence should be considered and evaluated as described in the paragraph of text that follows the three points classifying the evidence by transmission route.

Article 1.5.5.

The Aquatic Animals Commission did not agree to add 'validated' in Article 1.5.5. as it considered 'demonstrated to be equivalent' is sufficient.

Article 1.5.6.

The Aquatic Animals Commission did not agree with a Member Country comment to add 'that mimics natural pathways of transmission' because this point is to demonstrate that the pathogenic agent is viable and transmission pathways are irrelevant.

Article 1.5.7.

The Aquatic Animals Commission did not agree with a Member Country comment to delete 'for infection' in point 1 as it considered the text clear as written.

Article 1.5.8.

The Aquatic Animals Commission did not agree with a Member Country comment to replace 'partial information is available' with specific criteria and reminded Member Countries that the inclusion of species with incomplete evidence of susceptibility in Section 2.2.2. of the *Aquatic Manual* is to highlight where there are gaps in information. It also reminded Member Countries that trade measures cannot be applied for these species unless justified by a risk assessment.

Article 1.5.9.

The Aquatic Animals Commission noted that of the Member Country comments received, all but one supported the intent of the new Article 1.5.9. The Aquatic Animals Commission recognised that there remained a need to improve the readability of some of the text to ensure the purpose of this new article is clear. The Commission considered Member Country comments, made relevant amendments to clarify the text, and provided further explanation below.

The Aquatic Animals Commission noted that a number of Member Country comments received had been addressed in previous reports. The Aquatic Animals Commission have provided an extract of relevant text from their February 2018 meeting to address these comments.

"The criteria in Chapter 1.5. are used to determine which species or taxonomic groups of species are listed in the scope (Article X.X.2.) of each disease-specific chapter of the *Aquatic Code*. The Aquatic Animals Commission reminded Member Countries that the criteria would be applied by *ad hoc* groups and the outcomes of those assessments would be considered by the Commission and then provided to Member Countries for comment. The criteria are not intended to be applied by Member Countries to identify susceptible species for listed diseases.

The Aquatic Animals Commission noted that for some listed diseases, susceptible host species have long been listed in the *Aquatic Code* at a taxonomic ranking higher than species. For example, susceptible host species of infection with white spot syndrome virus have been listed at the ranking of Order since the disease-specific chapter was adopted in 1997 and susceptible host species of infection with *Aphanomyces astaci* have been listed at the ranking of Family since the disease-specific chapter was adopted in 1995. Application of the new criterion 1.5.9., once adopted, would result in greater scientific rigour being applied to determining host susceptibility at taxonomic rankings higher than species. The application of Article 1.5.9. to some diseases with a broad host range may result in susceptible host species being determined at lower taxonomic rankings than those currently included in Article X.X.2. of the *Aquatic Code*.

The Aquatic Animals Commission wished to remind Member Countries that the aim of the *Aquatic Code* is to prevent the spread of aquatic animal diseases and assure the sanitary safety of international trade in aquatic animals. Application of the current criteria in Chapter 1.5. to diseases with a proven broad host range (e.g. infection with *A. astaci* and infection with white spot syndrome virus) would result in a substantial reduction in the list of susceptible species for these diseases. As a consequence, the *Aquatic Code* measures for these diseases would not apply to many species that are likely to be susceptible. The Commission noted that this circumstance would be contrary to the purposes of the *Aquatic Code* and could lead to the spread of listed diseases. The Commission had agreed not to propose any amendments to the susceptible species for diseases with a broad host range (e.g. infection with *A. astaci* and infection with white spot syndrome virus) until such time that Member Countries have agreed on a suitable approach for Article 1.5.9.

The Aquatic Animals Commission wished to emphasise that Article 1.5.9. is intended to apply only to diseases that meet a threshold indicating that they have a broad host range. The Commission has recommended that this threshold for application of Article 1.5.9. be that a disease has at least one susceptible species from within each of three or more families. A disease that meets this threshold after the application of the criteria in Articles 1.5.1. to 1.5.8. (to determine susceptibility of individual species), would then be considered under Article 1.5.9. The Commission believes that this threshold restricts the application of Article 1.5.9. to an appropriate level such that Article 1.5.9. would only apply to diseases that truly do have a broad host range. The Commission considered a Member Country comment to set this threshold at the level of Genus (i.e. at least one susceptible species from within each of three or more genera); however, the Commission believes that this would result in Article 1.5.9. applying to diseases for which there might be only a small number of susceptible species and in such cases it would be preferable to list them individually."

The Aquatic Animals Commission did not agree with a Member Country comment to replace the ranking of Family 'with Genus or higher' in the first paragraph as the Commission did not consider that the text as written was inconsistent because the two levels of taxonomic ranking are applied for different purposes. Family is used to determine a threshold of low host specificity for application of Article 1.5.9. (as noted above) with Genus or higher as the outcome of the assessment.

In response to a Member Country comment seeking clarification for the meaning of the term 'refractory' used in this article, the Aquatic Animals Commission agreed to replace this term with 'non-susceptible' when used in this chapter to remove any ambiguity that this term may create.

The Aquatic Animals Commission did not agree with a Member Country comment to include specific guidelines for experimental procedures in Article 1.5.9.B because it considered that appropriately designed experimental procedures are a matter of good scientific practice and are available in relevant scientific literature.

In response to a Member Country comment to clarify what is meant by 'controlled challenge' the Aquatic Animals Commission amended the text to improve readability of this point.

The Aquatic Animals Commission did not agree with a Member Country comment to replace 'or' with 'and' in point C. (now point 3) between A. and B. because it considered that the standard of evidence required would be too high. However, the Commission agreed to amend point A. to strengthen the standard of evidence required to demonstrate that a species is not susceptible.

The revised Chapter 1.5. is presented in **Annexes 4A (clean) and 4B (track changes)** for Member Country comment.

1.4. Amendments to fish disease-specific chapters

1.4.1. Articles 10.5.1. and 10.5.2. Infection with salmonid alphavirus (Chapter 10.5.)

Comments were received from China (People's Rep. of), New Caledonia, EU and AU-IBAR.

In response to a Member Country comment the Aquatic Animals Commission clarified that proposed changes to susceptible species are consistent with findings of the *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases who undertake assessments against the criteria in Chapter 1.5. These changes include the deletion of brown trout (*Salmo trutta*) and addition of common dab *(Limanda limanda)* in Article 10.5.2. of the *Aquatic Code*. Common dab has been added to Section 2.2.1. *Susceptible host species* and brown trout has been added to Section 2.2.2. Species with incomplete evidence for susceptibility of the *Aquatic Manual*.

The Aquatic Animals Commission became aware of a new paper reporting susceptibility of Artic charr *(Salvelinus alpinus)* to infection with salmonid alphavirus (Lewisch *et al*., 2018). The Commission considered that the criteria for listing Arctic charr as a susceptible species to infection with SAV had been met (as per criteria in Chapter 1.5.) and proposed its inclusion in Article 10.5.2. The Commission requested that the *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases undertake an assessment of this species against the criteria in Chapter 1.5. The Commission would review their assessment, along with Member Country comments at its next meeting in February 2019.

Reference: Lewisch E., Frank T., Soliman H., Schachner O., Friedl A. & El-Matbouli M. First confirmation of salmonid alphavirus infection in Arctic charr *Salvelinus alpinus* and in Austria. *Dis. Aquat. Organ.*, 130, 71–76.

The relevant *ad hoc* Group report is available at:
http://www.oie.int//fileadmin/SST/adhocreports/Susceptibility%20of%20fish%20species%20to%20infection%20with%20OIE%20listed%20diseases/AN/A_AHG_Susceptibility_of_Fish_November_2017.pdf

Revised Articles 10.5.1. and 10.5.2. are presented in **Annex 5** for Member Country comment.

1.4.2. Article 10.7.2. Infection with koi herpesvirus (Chapter 10.7.)

Comments were received from New Caledonia, Switzerland, EU and AU-IBAR.

The Aquatic Animals Commission agreed with several Member Country comments to review the nomenclature used in Article 10.7.2. and proposed the following amendment: "all varieties and subspecies of common carp *(Cyprinus carpio)*" when referring to common carp.

Revised Article 10.7.2. is presented in **Annex 6** for Member Country comment.

1.4.3. Article 10.9.2. Infection with spring viraemia of carp virus (Chapter 10.9.)

Comments were received from Australia, Canada, New Zealand, New Caledonia, Switzerland, EU and AU-IBAR.

The Aquatic Animals Commission agreed with Member Country comments to review the nomenclature used in Article 10.9.2. and proposed the following amendment: "all varieties and subspecies of common carp *(Cyprinus carpio)*" when referring to common carp.

The Aquatic Animals Commission agreed with Member Country comments to amend the scientific name for zebrafish to *Danio rerio* as an incorrect name had been used.

Aquatic Animals Commission agreed with Member Country comments to amend the scientific name for Caspian white fish to *Rutilus kutum* based on the most recent nomenclature for this species (https://www.fishbase.de/summary/Rutilus-kutum.html).

The Aquatic Animals Commission agreed with Member Country comments to delete '(white amur)' after grass carp as it agreed that only one common name was necessary.

Revised Article 10.9.2. is presented in **Annex 7** for Member Country comment.

2. Other *Aquatic Code* chapters for review

2.1. Chapter 1.1. Notification of diseases, and provision of epidemiological information

The Aquatic Animals Commission reviewed comments received from the OIE World Animal Health and Information Analysis Department. The Commission agreed to await finalisation of amendments being proposed in the equivalent chapter in the *Terrestrial Code* by the Code Commission to ensure harmonisation between the two chapters.

2.2. Chapter 1.3. Diseases listed by the OIE

Infection with *Gyrodactylus salaris*

The OIE Reference Laboratory expert for *G. salaris* had made the Aquatic Animals Commission aware that the NCBI GenBank has reclassified gene sequences submitted as *G. thymalli* to *G. salaris*. The change appeared to be based on a paper by Fromm *et al.* (2014) which compared micro RNA (mRNA) from a limited number (7) of populations of *G. salaris* and *G. thymalli* and recommended synonymization of the two species, effectively reclassifying *G. thymalli* as *G. salaris*. The Commission acknowledged the work but noted that mRNA, to its knowledge, had not been used in taxonomy of Gyrodactylids to date, and the variation between other valid Gyrodacytlid species, and also between species belonging to other genera of Gyrodactylids, was not known. In addition, it was noted that there are clear phenotypic differences between *G. salaris* and *G. thymalli*, notably host predilection and pathogenicity in different host species. Synonymisation has serious implication for the management of *G. salaris* in countries and zones with a declared *G. salaris* free status. The Commission found there was not sufficient evidence currently to synonymise *G. salaris* and *G. thymalli* in the Code and Manual. However, the current guidance in the *Aquatic Manual* chapter for *G. salaris* requires that amplified CO1 fragments are sequenced and compared with other sequences using a BLAST search in GenBank/EMBL to distinguish *G. salaris* from *G. thymalli*. Given synonymisation of the two species by GeneBank, this guidance will be reconsidered when the *Aquatic Manual* chapter is revised using the new manual chapter template.

Reference: Fromm, B., Burow, S., Hahn, C., Bachmann, L., 2014. MicroRNA loci support conspecificity of *Gyrodactylus salaris* and *Gyrodactylus thymalli* (Platyhelminthes : Monogenea). *Int. J. Parasitol.* 1–7. https://doi.org/10.1016/j.ijpara.2014.05.010

Infection with *Marteilia refringens*

The Aquatic Animals Commission reviewed a paper by Kerr *et al*. (2018) suggesting that two genetic lineages within *Marteilia refringens* can be distinguished and formally defined as separate species: *Marteilia refringens* and *Marteilia pararefringens*. The Commission noted this proposal and that separation of the species would impact the scope of the disease-specific chapter. Infection with *Marteilia pararefringens* would not be within scope of the listed disease. The Commission requested that the Reference Laboratory expert provide further advice on this matter. It also invited Member Countries to provide any available information or comment on this subject.

Reference: Kerr, R., Ward, G. M., Stentiford, G. D., Alfjorden, A., Mortensen, S., Bignell, J. P., Feist, S.W., Villalba, A., Carballal, M. J., Cao, A., Arzul, I., Ryder, D. & Bass, D., 2018. *Marteilia refringens* and *Marteilia pararefringens* sp. nov. are distinct parasites of bivalves and have different European distributions. *Parasitology* 1-10. https://doi.org/10.1017/S003118201800063X

Tilapia lake virus

The Aquatic Animals Commission agreed not to undertake any further assessments of tilapia lake virus against the criteria for listing an aquatic animal disease until the *ad hoc* Group on tilapia lake virus has completed its work (see Item 3.2.).

Shrimp haemocyte iridescent virus (SHIV)

The Aquatic Animals Commission reviewed a paper by Qiu *et al.* (2017) on Shrimp haemocyte iridescent virus (SHIV) and agreed that the disease should not be considered against the criteria for listing until more information about the disease is available. The Commission noted that the Network of Aquaculture Centres in Asia-Pacific (NACA) would discuss the disease at its meeting in November 2018. The disease will be considered again by the Commission at its February 2019 meeting.

Reference: Qiu, L., Chen, M. M., Wang, R. Y., Xiao -Yuan Wan, X. Y
X., Yang, B. Y., Jian -Hai Xiang, J. H
iridescent virus (SHIV) isolated from white leg shrimp, *Litopenaeus vannamei*. *Archives of Virology* 163, 781–785. https://doi.org/10.1007/s00705-017-3642-4

Infectious spleen and kidney necrosis virus (ISKNV)

The Aquatic Animals Commission did not agree with a Member Country comment to replace infection with red sea bream iridovirus (RSIV) with infectious spleen and kidney necrosis virus (ISKNV) as the listed disease. The Commission consider RSIV and ISKNV as separate pathogenic agents. Although the two viruses are genetically quite similar, they are epidemiologically distinct. ISKNV would have to be assessed separately against the criteria for listing. The Commission requested that the *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases separately assess the host susceptibility of both pathogenic agents at its next meeting in November 2018.

2.3. Chapter 8.3. Infection with *Ranavirus* species

Comments were received from China (People's Rep. of).

The Aquatic Animals Commission agreed to amend ‘Infection with ranavirus’ with ‘Infection with *Ranavirus* species’ throughout the chapter where relevant to ensure the terminology is in line with the recently revised listed name in Chapter 1.3.

The Aquatic Animals Commission agreed with a Member Country comment to add ‘in amphibians’ and to delete the *Ranavirus* species exceptions (i.e. epizootic haematopoietic necrosis virus and European catfish virus) in Article 8.3.1. noting that although this disease appears in the section on amphibians it agreed this wording added clarity to the scope of this disease.

Revised Chapter 8.3. is presented in **Annex 8** for Member Country comment.

2.4. Acute hepatopancreatic necrosis disease (Chapter 9.1.)

The Aquatic Animals Commission noted that there were some errors in the use of AHPND throughout this chapter and, therefore, amended the use of AHPND and Vp_{AHPND} throughout the chapter, where relevant.

Revised Chapter 9.1. is presented in **Annex 9** for Member Country comment.

2.5. Article 10.2.1. of Infection with *Aphanomyces invadans* (Chapter 10.2.)

The Aquatic Animals Commission agreed to amend Article 10.2.1. to ensure consistency with other amended fish disease-specific chapters. It also amended some Family names in Article 10.2.2. to remove the use of italics as Family names for fish should not appear in italics.

The Aquatic Animals Commission noted that the Criteria for listing species as susceptible to infection with a specific pathogen would be applied to this disease once an approach to Article 1.5.9. has been agreed by Member Countries (see Item 1.3).

The revised Article 10.2.1. will be proposed for adoption in May 2019 and is presented in **Annex 18** for Member Country information.

2.6. Articles 10.3.4. and 10.3.5. Infection with *Gyrodactylus salaris* (Chapter 10.3.)

Comments were received from the EU.

The Aquatic Animals Commission considered a Member Country's comments that point 2 in Articles 10.3.4. and 10.3.5. is not sufficient for declarations of country, zone or compartment freedom for *Gyrodactylus salaris*, due to the existence of susceptible species that do not show clinical signs. The Member Country also questioned interpretation of the phrase "conditions conducive for clinical expression" and noted that this is an important criterion if a country were to claim historical freedom from a disease in accordance with point 2 in Articles 10.3.4. and 10.3.5.

The Aquatic Animals Commission noted that there may be conditions in some countries that are appropriate for application of point 2 in Articles 10.3.4. and 10.3.5. and agreed that it was important to maintain this pathway for those circumstances where it may suitably apply.

The Aquatic Animals Commission noted that the phrase "conditions conducive for clinical expression" includes characteristics of the host and pathogenic agent as well as environmental factors, and is one of several requirements for a country's early detection system to be effective. The outcome required is that, should the disease occur, the country's early detection system would be sufficiently sensitive to detect it.

The Aquatic Animals Commission wished to remind Member Countries that the pathways for making a self-declaration of freedom for a country, zone or compartment are being reviewed by the Commission and are the subject of a discussion paper (see Item 2.10.). The criteria for making self-declarations of freedom using historical freedom are being considered as part of that work and the Commission invites Member Countries to comment on this and related issues through this process.

2.7. Infection with infectious haematopoietic necrosis virus (Chapter 10.6.)

Comments were received from the EU.

The Aquatic Animals Commission agreed to amend the name of the pathogenic agent in Article 10.6.1. to "salmonid *Novirhabdovirus* (also known as infectious haematopoietic necrosis virus ([IHNV])" in accordance with the classification in the database of the International Committee of Taxonomy of Viruses (ICTV) (https://talk.ictvonline.org/taxonomy/p/taxonomy-history?taxnode_id=20171739).

The Aquatic Animals Commission reviewed the work of the *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases (see Item 3.1.) and agreed with their recommendations regarding the list of susceptible species in Article 10.6.2.

The Aquatic Animals Commission agreed with the following recommendations:

- Seven of the eight species currently listed in Article 10.6.2. that met the criteria for listing as susceptible species would remain in listed in Article 10.6.2.

- One species currently listed in Article 10.6.2. pink *(Oncorhynchus rhodurus)* that did not meet the criteria for listing as a susceptible species would be deleted from Article 10.6.2.

Six additional species that were assessed and met the criteria for listing as a susceptible species would be added to Article 10.6.2. These are: Arctic charr *(Salvelinus alpinus)*, brook trout *(Salvelinus fontinalis)*, brown trout *(Salmo trutta)*, cutthroat trout *(Onchorynchus clarkii)*, lake trout *(Salvelinus namaycush)*, marble trout *(Salmo marmoratus)*.

In response to a Member Country comment seeking clarification for the meaning of 'high health status' in point 2)a)ii) of Article 10.6.8. the Aquatic Animals Commission clarified that 'high health status' means the highest feasible disease status for a source population from a country not declared free.

The Aquatic Animals Commission did not agree with a Member Country comment to add point 4 from Article 10.6.11. to Article 10.6.10. regarding disposal of carcasses as Article 10.6.10. addresses processing of aquatic animals or aquatic animal products which generates waste materials not carcasses.

Revised Chapter 10.6. is presented in **Annex 10** for Member Country comment.

2.8. Model Article X.X.8.

Comments were received from AU-IBAR.

In response to a Member Country comment seeking clarification for the meaning of 'lifelong holding' the Aquatic Animals Commission clarified that 'lifelong holding' of aquatic animals only applies to 'live' aquatic animals because that is the definition of an aquatic animal. The Commission clarified that 'live' aquatic animals must remain in the quarantine facility for the duration of their lives.

To address the safe disposal of dead aquatic animals or products derived from them, the Aquatic Animals Commission proposed to add a new point b) in point 1 of Article X.X.8. to ensure that aquatic animals are killed and processed into a safe form before removal from the quarantine facility.

The Aquatic Animals Commission noted that this proposed amendment would be made in all disease-specific chapters once adopted.

Revised Article X.X.8. is presented in **Annex 11** for Member Country comment.

2.9. New draft chapter on Biosecurity for Aquaculture Establishments

As agreed at the Aquatic Animals Commission's February 2018 meeting, the Commission made further amendments to the draft chapter on Biosecurity for aquaculture establishments that had been prepared by the *ad hoc* Group on Biosecurity for aquaculture establishments who met in January 2018.

The Aquatic Animals Commission reminded Member Countries that this is a new chapter for inclusion in Section 4 of the *Aquatic Code* and follows the agreed work programme for revision of Section 4. This is the second new chapter of this section following the adoption of Chapter 4.3. on Disinfection of aquaculture establishments and equipment in 2017.

The new draft Chapter 4.X. is presented in **Annex 12** for Member Country comment.

2.10. Discussion paper on Approaches for determining periods required to demonstrate disease freedom

As agreed at the Aquatic Animals Commission's February 2018 meeting, the Commission has undertaken further work on the issue of demonstration of freedom and developed a discussion paper on Approaches for determining periods required to demonstrate disease freedom.

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

It is the intention of the Aquatic Animals Commission to use this discussion paper to engage Member Countries in exploring improvements to the standards of the *Aquatic Code* for demonstration of freedom. Any recommendations provided with the paper are for the purpose of stimulating discussion only and should not be considered the intended approaches of the Commission for revision of the *Aquatic Code*.

Member Countries are invited to comment on the paper and, for convenience, several discussion points have been provided throughout the text as a basis for Member Country responses. These discussion points are summarised in Table 3 in Section 7 of the document.

The discussion paper on Approaches for determining periods required to demonstrate disease freedom is presented in **Annex 13** for Member Country comment.

F. OIE *AD HOC* GROUP REPORTS

3.1. Report of the *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases

The Aquatic Animals Commission reviewed the report of the meeting of the *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases which was held from 2–4 May 2018. The Commission commended the *ad hoc* Group for their substantial work.

The OIE *ad hoc* Group had undertaken assessments of susceptible species to infection with infectious haematopoietic necrosis virus (IHNV) using the 'Criteria for listing species as susceptible to infection with a specific pathogen' (Chapter 1.5. of the *Aquatic Code*) for inclusion in the relevant articles of Chapter 10.6. of the *Aquatic Code* and Chapter 2.3.4. of the *Aquatic Manual*.

Member Countries are referred to Items 2.7. and 4.4. regarding proposed changes in Chapter 10.6. of the *Aquatic Code* and Chapter 2.3.4. of the *Aquatic Manual* respectively.

The Aquatic Animals Commission also requested that the *ad hoc* Group continue its work to review the list of susceptible species for the remaining fish disease-specific chapters.

The report of the OIE *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases is presented at **Annex 20** for Member Country information.

3.2. Report of the electronic *ad hoc* Group on Tilapia lake virus

The Aquatic Animals Commission reviewed the report of the *ad hoc* Group on Tilapia lake virus (TiLV) who worked electronically from February to September 2018 on the assessment of TiLV diagnostics and their validation. The Commission commended the *ad hoc* Group for their substantial work that continued to demonstrate excellent collaboration between a number of laboratories worldwide

The Aquatic Animals Commission thanked those Member Countries that had supported the work by providing positive TiLV control material for molecular test evaluation and inter-laboratory comparability studies.

The *ad hoc* Group was requested to continue this important work and report back to the next meeting of the Commission in February 2019.

The Aquatic Animals Commission also wanted to acknowledge the Ministry of Agriculture, Forestry and Fisheries of Japan (MAFF) for its generous financial donation to support the molecular test evaluations and inter-laboratory comparability studies that will be performed by members of the *ad hoc* Group.

The report of the electronic *ad hoc* Group on TiLV is presented at **Annex 21** for Member Country information.

Member Countries were reminded that *ad hoc* group reports are also available on a dedicated page of OIE website at http://www.oie.int/en/standard-setting/specialists-commissions-working-groups/scientific-commission-reports/ad-hoc-groups-reports/

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

4. Texts circulated for Member Country comment at the February 2018 meeting

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

The Aquatic Animals Commission informed Member Countries that Section 2.2.1. *Susceptible host species* and Section 2.2.2. *Species with incomplete evidence for susceptibility* in Chapter 2.3.9. *Spring viraemia of carp* will be amended when the work of applying the new *Aquatic Manual* template to this chapter is finalised.

4.1. Infection with yellow head virus genotype 1 (Chapter 2.2.9.)

Section 2.2.1. *Susceptible host species*

The list of susceptible species was amended to be in alphabetical order by common name.

Section 2.2.2. *Species with incomplete evidence for susceptibility*

The list of species of which there is incomplete evidence for susceptibility was amended to be in alphabetical order by common name.

In response to a Member Country comment, the Aquatic Animals Commission added the common names of species where they were missing based on FAOTERM and other sources, and placed them in alphabetical order by common name.

In response to a Member Country request to add references to Sections 2.2.1. and 2.2.2., the Aquatic Animals Commission reminded Member Countries that all the references are included in the report of the OIE *ad hoc* Group on Susceptibility of crustacean species to infection with OIE listed diseases (available at: http://www.oie.int/en/standard-setting/specialists-commissions-working-ad-hoc-groups/ad-hoc-groups-reports/).

Revised Sections 1, 2.2.1. and 2.2.2. of Chapter 2.2.9. is presented at **Annex 14** for Member Country comment.

4.2. Infection with salmonid alphavirus (Chapter 2.3.6.)

The Aquatic Animals Commission reviewed Member Country comments and amended the text, where relevant.

Section 2.1.1. *Aetiological agent, agent strains*

Table 2.1. was amended to be consistent with the revised list of susceptible species. Text referring to pancreas disease and sleeping disease that had been marked for deletion from the scope was edited and moved to Section 2.1.1.

Section 2.2.1. *Susceptible host species*

The list of susceptible species was amended as per proposed amendments in Article 10.5.2. (see Item 1.4.1.).

Section 2.2.2. *Species with incomplete evidence for susceptibility*

The Aquatic Animals Commission agreed to delete salmon louse *(Lepeophtheirus salmonis)* from the second paragraph because the species is already included in Section 2.2.6. *Vectors* of the chapter.

Section 2.2.8. *Known or suspected wild aquatic animal carriers*

In response to a Member Country comment questioning why common dab is still included in Section 2.2.8. *Known or suspected wild aquatic animal carriers* of the *Aquatic Manual* when considered a susceptible species, the Aquatic Animals Commission noted that while this is not incorrect it agreed that this text was not clear, and it noted that this would be revised when the new template for *Aquatic Manual* chapters is applied.

Section 4.3.1.1.2. *Reverse-transcription polymerase chain reaction (RT-PCR), real-time RT-PCR, and genotyping by sequencing*

The Aquatic Animals Commission noted that the description of the real-time reverse-transcription polymerase chain reaction (RT-PCR) lacked important details such as the number of cycles in the amplification step, the annealing temperatures, etc. and amended the description according to the review by the OIE Reference Laboratory.

Table 5.1. *Methods for targeted surveillance and diagnosis*

Following Member Country comments, the Aquatic Animals Commission amended the ranking of histopathology for both presumptive and confirmatory diagnosis in Table 5.1. from 'a' (method is the recommended method for reasons of availability, utility, and diagnostic specificity and sensitivity) to 'b' (method is a standard method with good diagnostic sensitivity and specificity) and 'd' (method is presently not recommended for this purpose), respectively.

Revised Chapter 2.3.6. is presented at **Annex 15** for Member Country comment.

4.3. Infection with koi herpesvirus (Chapter 2.3.7.)

Section 2.2.1. *Susceptible host species*

The list of susceptible species was amended as per proposed amendments in Article 10.7.2. (see Item 1.4.2.).

Given the large number of Member Country comments received, the Aquatic Animals Commission requested that these comments be sent to the OIE Reference Laboratories for their review and that they be considered at the same time that the chapter is reformatted using the new template. The Commission would review the revised text at its next meeting in February 2019.

Revised Sections 1, 2.2.1. and 2.2.2. of Chapter 2.3.7. are presented at **Annex 16** for Member Country comment.

5. Other *Aquatic Manual* issues

5.1. Acute hepatopancreatic necrosis disease (Chapter 2.2.1.)

A Member Country requested that their country be removed from the list of countries in the *Aquatic Manual* chapter for acute hepatopancreatic necrosis disease (AHPND) that have reported this disease. The Commission requested that the Member Country provide the evidence and rationale for disregarding the articles published in peer-reviewed journals that conclude, based on the research presented, that AHPND is present in the country concerned.

5.2. Infection with white spot syndrome virus (Chapter 2.2.8.)

A Member Country commented that the text in Section 2.2.5. *Persistent infection* of Chapter 2.2.8. *Infection with white spot syndrome virus* refers to carriers, but that there is no definition of carrier in the *Aquatic Code*. The Commission confirmed that this issue would be addressed when the chapter is next updated at which time it will be reformatted using the new template.

A Member Country proposed the addition of five new text references. The Aquatic Animals Commission stated that the chapter had been adopted this year and that further changes were not anticipated until the next revision using the new template when these references will be considered.

5.3. Infection with *Gyrodactylus salaris* (Chapter 2.3.3.)

The Aquatic Animals Commission agreed with a Member Country comment to change the word "strains" to "clades" in Section 2.2.1. *Aetiological agent, agent strains*.

In response to a Member Country comment that transient infections in non-susceptible species have been reported for *G. salaris*, contributing to spread of the disease, the Aquatic Animals Commission agreed to submit this comment to the OIE Reference Laboratory expert for consideration when the chapter is updated using the new template.

5.4. Infection with infectious haematopoietic necrosis virus (Chapter 2.3.4.)

Section 2.2.1. *Susceptible host species*

The list of susceptible species was amended following the recommendations by the *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases (see Item 3.2.).

Section 2.2.2. *Species with incomplete evidence for susceptibility*

The list of species with incomplete evidence for susceptibility was amended following the recommendations by the *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases (see Item 3.2.).

Section 2.2.6. *Vectors*

The Aquatic Animals Commission agreed with a recommendation from the *ad hoc* Group on susceptibility of fish species to infection with OIE listed disease to include the mayfly (*Callibaetis* sp.) and the salmon louse *(Lepeophtheirus salmonis)* in Section 2.2.6. *Vectors* of the chapter. Invertebrate species should be considered as potential vectors for transmission of infectious haematopoietic necrosis virus and not susceptible species as viral replication within insects is unlikely and may be difficult to determine.

Revised Chapter 2.3.4. is presented at **Annex 17** for Member Country comment.

5.5. Chapter 2.3.3. Infection with *Gyrodactylus salaris* and Chapter 2.3.5. Infection with HPR-deleted or HPR0 infectious salmon anaemia virus

The OIE Reference Laboratory experts had identified the need to further review and update these two chapters. As both chapters had been adopted in May 2018, the Aquatic Animals Commission proposed that the updates be put on hold until members of the *ad hoc* Group are available to assist with reformatted the chapters using the new template.

5.6. New draft chapter on Infection with *Batrachochytrium salamandrivorans*

The Aquatic Animals Commission wished to inform Member Countries that a new draft chapter on Infection with *Batrachochytrium salamandrivorans* is being developed by experts with experience of this pathogenic agent and that the Commission will consider the chapter at its next meeting in February 2019.

5.7. *Aquatic Manual* disease chapter template

A number of Member Countries proposed amendments to the list of countries given in Section 2.3.3. *Geographical distribution* in a number of disease-specific chapters of the *Aquatic Manual*. The Aquatic Animals Commission stressed that this section of the new disease chapter template requests information at the continental level along with a reference to the OIE World Animal Health Information System (WAHIS). It would therefore be addressed when the chapters are reformatted using the new template.

A Member Country asked if Section 2.4.1. *Vaccination* was necessary in the crustacean disease chapters. The Aquatic Animals Commission confirmed that the section would be kept in the crustacean disease chapters so as to keep the numbering of sections consistent among all the chapters and it would also be noted that no vaccines are available for crustacean diseases.

In response to a Member Country comment, the Aquatic Animals Commission amended the guidance in Section 2.3.2. *Clinical signs, including behavioural changes* of the template to request authors to include information, where relevant, on susceptible species that do not typically present clinical infection and host species in which clinical signs rarely occur.

In response to a request to include in Section 2.2. *Host factors*, a section on conditions conducive to clinical expression, the Aquatic Animals Commission affirmed that the issue would be addressed through its ongoing work on demonstration of freedom and consequential revisions to *Aquatic Code*.

The Aquatic Animals Commission did not agree with a Member Country comment to include photographs in the *Aquatic Manual* but would consider producing an Atlas of Aquatic Animal Diseases if funding for such a project could be found.

5.8. Review of chapters that had been updated and reformatted using the new disease chapter template

Two chapters had been revised and reformatted using the new template: Chapter 2.3.8. *Infection with red sea bream iridovirus* and Chapter 2.3.9. *Infection with spring viraemia of carp virus*.

In each case, a member of the *ad hoc* Group on the new *Aquatic Manual* template that developed the template assisted the OIE Reference Laboratory expert with the task. Based on feedback from the Aquatic Animals Commission, the *ad hoc* Group members and OIE experts will revise these chapters further, and they will be reviewed at the next meeting of the Commission in February 2019.

In addition, the *ad hoc* Group members and relevant OIE Reference Laboratory experts would commence work on applying the new chapter template to Chapter 2.2.7. *Infection with koi herpesvirus* and Chapter 2.3.10. *Infection with viral haemorrhagic septicaemia virus*.

H. OIE REFERENCE CENTRES

6. Applications for OIE Reference Centre status or changes of experts

An application had been received for an OIE Collaborating for Research, Detection, and Control of Mollusc Diseases. The Aquatic Animals Commission found that the activities and services proposed fitted more with the mandate of an OIE Reference Laboratory rather than a Collaborating Centre. The applicant would be requested to re-submit the application giving a clearer indication of the services the institute will offer as an OIE Collaborating Centre – a centre of expertise that would benefit OIE Member Countries- along with a 5-year plan of the proposed activities. The application would be reviewed at the next meeting of the Commission in February 2019.

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

Infection with salmonid alphavirus
Dr Hilde Sindre to replace Dr Torunn Taksdal at the Norwegian Veterinary Institute, Oslo (Norway).

I. OTHER ISSUES

7.1. Technical disease cards

7.1.1. Tilapia lake virus

The Aquatic Animals Commission reviewed the technical disease card for Tilapia lake virus and amended the sections on disease names and synonyms, geographical distribution, and confirmatory text methods in line with recent publications.

The Commission highlighted that the updated technical disease card is available on the OIE website at: http://www.oie.int/en/international-standard-setting/specialists-commissions-groups/aquatic-animal-commission-reports/disease-information-cards/

The revised technical disease card for Tilapia lake virus is presented at **Annex 19** for Member Country information.

7.1.2. Infection with *Batrachochytrium salamandrivorans*

The Aquatic Animals Commission reviewed the technical disease card for Infection with *Batrachochytrium salamandrivorans* and concluded that no amendments were necessary.

The technical disease card is available on the OIE website at: http://www.oie.int/en/international-standard-setting/specialists-commissions-groups/aquatic-animal-commission-reports/disease-information-cards/

J. OIE GLOBAL CONFERENCE ON AQUATIC ANIMAL HEALTH

The Aquatic Animals Commission continued to work on finalising the programme for the OIE Global Conference, ensuring that the programme would be engaging and relevant to all Member Countries. The Commission noted that the dates for the Conference had been changed and that the Conference will be held from **2–4 April 2019** in Santiago, Chile.

K. WORK PLAN OF THE OIE AQUATIC ANIMAL HEALTH STANDARDS COMMISSION FOR 2018/2019

The Aquatic Animals Commission reviewed and updated its work programme, taking into account Member Country comments, Headquarters' comments, progress with current programmes and completed work.

The revised 2018/2019 work programme is presented at **Annex 22** for Member Country information.

L. NEXT MEETING

The next meeting of the Aquatic Animals Commission is scheduled for 7–14 February 2019 inclusive.

/Annexes

Annex 1

MEETING OF THE OIE AQUATIC ANIMAL HEALTH STANDARDS COMMISSION

Paris, 11–18 September 2018

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Annex 2

MEETING OF THE OIE AQUATIC ANIMAL HEALTH STANDARDS COMMISSION

Paris, 11–18 September 2018

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Adopted agenda

A. MEETING WITH THE DIRECTOR GENERAL

B. INDUCTION SESSION TO SUPPORT PERFORMANCE OF THE NEWLY ELECTED SPECIALIST COMMISSIONS

C. ADOPTION OF THE AGENDA

D. MEETING WITH THE PRESIDENT OF THE OIE TERRESTRIAL ANIMAL HEALTH STANDARDS COMMISSION

E. OIE *AQUATIC ANIMAL HEALTH CODE*

1. Texts circulated for Member Country comments at the February 2018 meeting

   1.1. General comments

   1.2. Glossary

   1.3. Criteria for listing species as susceptible (Chapter 1.5.)

   1.4. Amendments to fish disease-specific chapters

      1.4.1. Articles 10.5.1. and 10.5.2. “Infection with salmonid alphavirus” (Chapter 10.5.)

      1.4.2. Article 10.7.2. “Infection with koi herpesvirus” (Chapter 10.7.)

      1.4.3. Article 10.9.2. “Infection with spring viraemia of carp virus” (Chapter 10.9.)

2. Other *Aquatic Code* chapters for review

   2.1. Notification of diseases, and provision of epidemiological information (Chapter 1.1.)

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

   2.3. Infection with *Ranavirus* species (Chapter 8.3.)

   2.4. Acute hepatopancreatic necrosis disease (Chapter 9.1.)

   2.5. Article 10.2.1. “Infection with *Aphanomyces invadans*” (Chapter 10.2.)

   2.6. Articles 10.3.4. and 10.3.5. “Infection with *Gyrodactylus salaris*” (Chapter 10.3.)

   2.7. Infection with infectious haematopoietic necrosis virus (Chapter 10.6.)

   2.8. Model Article X.X.8.

   2.9. New draft chapter on Biosecurity for Aquaculture Establishments

   2.10. Discussion paper on Approaches for determining periods required to demonstrate disease freedom

Annex 2 (contd)

F. OIE *AD HOC* GROUP REPORTS

3.1. Report of the *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases

3.2. Report of the electronic *ad hoc* Group on Tilapia lake virus

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

4. Texts circulated for Member Country comment at the February 2018 meeting

4.1. Infection with yellow head virus genotype 1 (Chapter 2.2.9.)

4.2. Infection with salmonid alphavirus (Chapter 2.3.6.)

4.3. Infection with koi herpesvirus (Chapter 2.3.7.)

5. Other *Aquatic Manual* issues

5.1. Acute hepatopancreatic necrosis disease (Chapter 2.2.1.)

5.2. Infection with white spot syndrome virus (Chapter 2.2.8.)

5.3. Infection with *Gyrodactylus salaris* (Chapter 2.3.3.)

5.4. Infection with infectious haematopoietic necrosis virus (Chapter 2.3.4.)

5.5. Chapter 2.3.3. "Infection with *Gyrodactylus salaris"* and Chapter 2.3.5. "Infection with HPR-deleted or HPR0 infectious salmon anaemia virus"

5.6. New draft chapter on Infection with *Batrachochytrium salamandrivorans*

5.7. *Aquatic Manual* disease chapter template

5.8. Review of chapters that had been updated and reformatted using the new disease chapter template

H. OIE REFERENCE CENTRES

6. Applications for OIE Reference Centre status or changes of experts

I. OTHER ISSUES

7. Technical disease cards

7.1.1. Tilapia lake virus

7.1.2. Infection with *Batrachochytrium salamandrivorans*

J. OIE GLOBAL CONFERENCE ON AQUATIC ANIMAL HEALTH

K. WORK PLAN OF THE OIE AQUATIC ANIMAL HEALTH STANDARDS COMMISSION FOR 2018/2019

L. NEXT MEETING

Annex 3

GLOSSARY

BASIC BIOSECURITY CONDITIONS

means a minimum set of conditions required to ensure *biosecurity* ~~applying to~~ for a particular *disease,* ~~and a particular *zone* or~~ in a country, *zone* or *compartment* that should include ~~required to ensure adequate disease security, such as:~~

a) compulsory notification of the *disease*~~,~~ ~~including~~ or suspicion of the *disease*~~,~~ ~~is compulsorily notifiable~~ to the *Competent Authority*; and

b) an *early detection system* ~~is in place~~ ~~within the *zone* or country~~; and

c) ~~import~~ requirements to prevent the introduction of the *pathogenic agent* ~~*disease*~~ into ~~the~~ a *free* ~~country~~ *country*, ~~or~~ *zone* or *compartment*, or the spread from *infected zones* and *protection zones,* in accordance with the relevant disease-specific chapter ~~as outlined in the *Aquatic Code*, are in place~~.

Annex 4A (Tracked changes)

CHAPTER 1.5.

CRITERIA FOR LISTING SPECIES AS SUSCEPTIBLE TO INFECTION WITH A SPECIFIC ~~PATHOGEN~~ PATHOGENIC AGENT

Article 1.5.1.

Purpose

In each disease-specific chapter, Article X.X.2. lists the *aquatic animal* species that have been found to be susceptible to *infection* with the relevant *pathogenic agent*. The recommendations of each disease-specific chapter apply only to the species listed in Article X.X.2.

The purpose of this chapter is to provide criteria for determining which species are listed as susceptible in Article ~~1.5.2.~~ X.X.2. of each disease-specific chapter in the *Aquatic Code*.

Article 1.5.2.

Scope

~~Susceptibility may include clinical or non-clinical *infection* but does not include species that may carry the *pathogenic agent* without replication~~.

Species of *aquatic animal*s are considered susceptible to *infection* with a *pathogenic agent* when the presence of a multiplying~~,~~ or developing ~~or latent~~ *pathogenic agent* has been demonstrated by the occurrence of natural *cases* or by experimental exposure that mimics natural transmission pathways. Susceptibility includes clinical or non-clinical *infection*.

The decision to list an individual ~~a~~ species as susceptible in a disease-specific chapter~~s~~ should be based on a finding that the evidence is definite in accordance with Article 1.5.3. ~~All species in a taxonomic group may be listed as susceptible when certain criteria are met in accordance with Article 1.5.9.~~ A taxonomic ranking higher than species is listed when the criteria in Article 1.5.9. are met.

~~However,~~ ~~possible~~ Possible susceptibility of a species is also important information and, in accordance with Article 1.5.8., these species are ~~this~~ ~~should also be~~ included in Section ~~2.2.1.~~ *2.2.2. Species with incomplete evidence for susceptibility* ~~entitled «Susceptible host species »~~ of the relevant disease-specific chapter of the *Aquatic Manual*~~.~~ ~~in accordance with Article 1.5.8.~~

Article 1.5.3.

Approach

A three-stage approach is outlined in this chapter to assess susceptibility of a species to *infection* with a specified *pathogenic agent* and is based on:

1) criteria to determine whether the route of transmission is consistent with natural pathways for the *infection* (as described in Article 1.5.4.);

2) criteria to determine whether the *pathogenic agent* has been adequately identified (as described in Article 1.5.5.);

3) criteria to determine whether the evidence indicates that presence of the *pathogenic agent* constitutes an *infection* (as described in Article1.5.6.).

Annex 4A (Tracked changes) (contd)

Article 1.5.4.

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

The evidence should be classified as transmission through:

1) natural occurrence; includes situations where *infection* has occurred without experimental intervention e.g. *infection* in wild or farmed populations; or

2) non-invasive experimental procedures; includes cohabitation with infected hosts, *infection* by immersion or ingestion; or

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

Consideration needs to be given to whether experimental procedures (e.g. inoculation, infectivity load) mimic natural pathways for *disease* transmission. Consideration should also be given to environmental factors as these may affect host resistance or transmission of the ~~pathogen~~ *pathogenic agent*.

Article 1.5.5.

Stage 2: criteria to determine whether the pathogenic agent has been adequately identified

The *pathogenic agent* should be identified and confirmed in accordance with the methods described in Section ~~7~~ 4 (diagnostic methods) ~~(corroborative diagnostic criteria)~~ of the relevant disease-specific chapter in the *Aquatic Manual*, or other methods that have been demonstrated to be equivalent.

Article 1.5.6.

Stage 3: criteria to determine whether the evidence indicates that presence of the pathogenic agent constitutes an infection

A combination of the following criteria should be used to determine *infection* (see Article 1.5.7.):

A. the *pathogenic agent* is multiplying in the host, or developing stages of the *pathogenic agent* are present in or on the host;

B. viable *pathogenic agent* is isolated from the proposed *susceptible species*, or infectivity is demonstrated by way of transmission to naive individuals;

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

D. the specific location of the ~~pathogen~~ *pathogenic agent* corresponds with the expected target tissues.

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

Article 1.5.7.

Outcomes of the assessment

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

1) transmission has been obtained naturally or by experimental procedures that mimic natural pathways for the *infection* in accordance with Article 1.5.4.;

AND

2) the identity of the *pathogenic agent* has been confirmed in accordance with Article 1.5.5.;

AND

3) there is evidence of *infection* with the *pathogenic agent* in the suspect host species in accordance with criteria A to D in Article 1.5.6.. Evidence to support criterion A alone is sufficient to determine *infection*. In the absence of evidence to meet criterion A, satisfying at least two of criteria B, C or D would be required to determine *infection*.

Article 1.5.8.

Species for which there is incomplete evidence for susceptibility

The decision to list a species as susceptible in Article 1.5.2. of each disease-specific chapter should be based on a finding that the evidence is definite.

However, where there is ~~insufficient~~ incomplete evidence to demonstrate susceptibility of a species through the approach described in Article 1.5.3. ~~because transmission does not mimic natural pathways of *infection*, or the identity of the *pathogenic agent* has not been confirmed, or *infection* is only partially supported~~, but partial information is available, these species ~~information~~ will be included in Section 2.2.2. *Species with incomplete evidence for susceptibility* of the relevant disease-specific chapter in the *Aquatic Manual*.

If there is ~~insufficient~~ incomplete evidence to demonstrate susceptibility of a species, the *Competent Authority* should, prior to the implementation of any import health measures for the species, ~~assess the risk of spread~~ undertake a *risk analysis* for the ~~pathogen~~ *pathogenic agent* under consideration, in accordance with the recommendations in Chapter 2.1.~~, prior to the implementation of import health measures~~.

Article 1.5.9.

Listing susceptible species at a taxonomic ranking of Genus or higher ~~than species~~ ~~Pathogenic agents with a broad host range~~

Some *pathogenic agents* have low host species specificity and can infect numerous species across multiple taxa. These *pathogenic agents* are eligible for assessment using this article if they have at least one *susceptible species* in three or more taxa at the ranking of Family. The outcome of applying this article may be that *susceptible species* are listed in Article X.X.2. of each disease-specific chapter at a ranking of Genus or higher. ~~For *pathogenic agents* with that have a broad host range, it may be appropriate for the outcome of the assessment of susceptibility to can be made at a taxonomic ranking higher than species (e.g. genus, family). For a *pathogenic agent* to be considered to have a broad host range, and thus be a potential candidate for listing *susceptible species* at a taxonomic ranking of genus or higher, there must be at least one *susceptible species* within each of three or more host families. It may be appropriate for the outcome of the assessment to be made at a taxonomic classification higher than species for a pathogenic agent that has a broad host range. A pathogenic agent will be considered to have a broad host range when it has been demonstrated as susceptible in at least three families.~~

For *pathogenic agents* that have a ~~broad host range~~ low host species specificity, ~~1)A~~ a decision to conclude susceptibility of species at ~~for~~ a taxonomic ranking of Genus or higher ~~level above species~~ should only be made where:

~~A. susceptibility has been demonstrated in at least one species from within each of three or more families;~~

~~AND~~

Annex 4A (Tracked changes) (contd)

~~B~~A1) more than one species within the ~~family~~ taxonomic ranking has been found to be susceptible in accordance with the approach described in Article 1.5.3. ~~criteria above~~;

AND

~~C~~B2) no species within the taxonomic ~~group~~ ranking has been found to be ~~refractory~~ non-susceptible to *infection*;

AND

~~C~~3) ~~The~~ the ~~taxa~~ taxonomic ranking is at ~~chosen should be~~ the lowest level supported by ~~this~~ evidence of points A and B.

~~2)~~ Evidence ~~that a~~ of non-susceptibility of a species ~~is refractory~~ to *infection* ~~may include~~ includes:

A. absence of *infection* in a species exposed to the *pathogenic agent* in natural settings where the ~~pathogen~~ *pathogenic agent* is known to be present and ~~it~~ has ~~causes~~ caused *infection* in co-located *susceptible species*;

OR

B. absence of *infection* in species exposed to the *pathogenic agent* through ~~a controlled challenges using~~ appropriately designed experimental procedures.

CHAPTER 1.5.

CRITERIA FOR LISTING SPECIES AS SUSCEPTIBLE TO INFECTION WITH A SPECIFIC PATHOGENIC AGENT

Article 1.5.1.

Purpose

In each disease-specific chapter, Article X.X.2. lists the *aquatic animal* species that have been found to be susceptible to *infection* with the relevant *pathogenic agent*. The recommendations of each disease-specific chapter apply only to the species listed in Article X.X.2.

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

Article 1.5.2.

Scope

Species of *aquatic animal*s are considered susceptible to *infection* with a *pathogenic agent* when the presence of a multiplying or developing *pathogenic agent* has been demonstrated by the occurrence of natural *cases* or by experimental exposure that mimics natural transmission pathways. Susceptibility includes clinical or non-clinical *infection*.

The decision to list an individual species as susceptible in a disease-specific chapter should be based on a finding that the evidence is definite in accordance with Article 1.5.3. A taxonomic ranking higher than species is listed when the criteria in Article 1.5.9. are met.

Possible susceptibility of a species is also important information and, in accordance with Article 1.5.8., these species are included in Section *2.2.2. Species with incomplete evidence for susceptibility* of the relevant disease-specific chapter of the *Aquatic Manual*.

Article 1.5.3.

Approach

A three-stage approach is outlined in this chapter to assess susceptibility of a species to *infection* with a specified *pathogenic agent* and is based on:

1) criteria to determine whether the route of transmission is consistent with natural pathways for the *infection* (as described in Article 1.5.4.);

2) criteria to determine whether the *pathogenic agent* has been adequately identified (as described in Article 1.5.5.);

3) criteria to determine whether the evidence indicates that presence of the *pathogenic agent* constitutes an *infection* (as described in Article 1.5.6.).

Article 1.5.4.

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

The evidence should be classified as transmission through:

1) natural occurrence; includes situations where *infection* has occurred without experimental intervention e.g. *infection* in wild or farmed populations; or

2) non-invasive experimental procedures; includes cohabitation with infected hosts, *infection* by immersion or ingestion; or

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

Consideration needs to be given to whether experimental procedures (e.g. inoculation, infectivity load) mimic natural pathways for *disease* transmission. Consideration should also be given to environmental factors as these may affect host resistance or transmission of the *pathogenic agent*.

Article 1.5.5.

Stage 2: criteria to determine whether the pathogenic agent has been adequately identified

The *pathogenic agent* should be identified and confirmed in accordance with the methods described in Section 4 (diagnostic methods) of the relevant disease-specific chapter in the *Aquatic Manual*, or other methods that have been demonstrated to be equivalent.

Article 1.5.6.

Stage 3: criteria to determine whether the evidence indicates that presence of the pathogenic agent constitutes an infection

A combination of the following criteria should be used to determine *infection* (see Article 1.5.7.):

A. the *pathogenic agent* is multiplying in the host, or developing stages of the *pathogenic agent* are present in or on the host;

B. viable *pathogenic agent* is isolated from the proposed *susceptible species*, or infectivity is demonstrated by way of transmission to naive individuals;

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

D. the specific location of the *pathogenic agent* corresponds with the expected target tissues.

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

Article 1.5.7.

Outcomes of the assessment

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

1) transmission has been obtained naturally or by experimental procedures that mimic natural pathways for the *infection* in accordance with Article 1.5.4.;

AND

2) the identity of the *pathogenic agent* has been confirmed in accordance with Article 1.5.5.;

AND

3) there is evidence of *infection* with the *pathogenic agent* in the suspect host species in accordance with criteria A to D in Article 1.5.6. Evidence to support criterion A alone is sufficient to determine *infection*. In the absence of evidence to meet criterion A, satisfying at least two of criteria B, C or D would be required to determine *infection*.

Article 1.5.8.

Species for which there is incomplete evidence for susceptibility

The decision to list a species as susceptible in Article 1.5.2. of each disease-specific chapter should be based on a finding that the evidence is definite.

However, where there is incomplete evidence to demonstrate susceptibility of a species through the approach described in Article 1.5.3., but partial information is available, these species will be included in Section 2.2.2. *Species with incomplete evidence for susceptibility* of the relevant disease-specific chapter in the *Aquatic Manual*.

If there is incomplete evidence to demonstrate susceptibility of a species, the *Competent Authority* should, prior to the implementation of any import health measures for the species, assess the risk of spread of the *pathogenic agent* under consideration, in accordance with the recommendations in Chapter 2.1.

Article 1.5.9.

Listing susceptible species at a taxonomic ranking of Genus or higher

Some *pathogenic agents* have low host species specificity and can infect numerous species across multiple taxa. These *pathogenic agents* are eligible for assessment using this article if they have at least one *susceptible species* in three or more taxa at the ranking of Family. The outcome of applying this article may be that *susceptible species* are listed in Article X.X.2. of each disease-specific chapter at a ranking of Genus or higher.

For *pathogenic agents* that have a low host species specificity, a decision to conclude susceptibility of species at a taxonomic ranking of Genus or higher should only be made where:

1) more than one species within the taxonomic ranking has been found to be susceptible in accordance with the approach described in Article 1.5.3.;

AND

2) no species within the taxonomic ranking has been found to be non-susceptible to *infection*;

AND

3) the taxonomic ranking is at the lowest level supported by evidence of points A and B.

Evidence of non-susceptibility of a species to *infection* includes:

A. absence of *infection* over time demonstrated through *targeted surveillance* of a species exposed to the *pathogenic agent* in natural settings where the *pathogenic agent* is causing clinical *disease* in co-located populations of *susceptible species*;

OR

B. absence of *infection* in species exposed to the *pathogenic agent* through appropriately designed experimental procedures.

Annex 5

CHAPTER 10.5.

INFECTION WITH SALMONID ALPHAVIRUS

Article 10.5.1.

General provisions

For the purposes of the *Aquatic Code*, infection with salmonid alphavirus means *infection* with any ~~subtype~~ genotype of the *pathogenic agent* salmonid alphavirus (SAV), of the Genus *Alphavirus* and Family *Togaviridae*.

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

Article 10.5.2.

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.: Arctic charr *(Salvelinus alpinus)*, Atlantic salmon *(Salmo salar)*, ~~brown trout *(Salmo trutta)*~~, common dab *(Limanda limanda)* and rainbow trout *(Onchorynchus mykiss)*. ~~These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally~~.

[…]

Annex 6

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* ~~*carpio*~~~~)~~, and common carp hybrids (e.g. *Cyprinus carpio* x *Carassius auratus*)~~, ghost carp (*Cyprinus carpio goi*)~~, ~~and koi carp (*Cyprinus carpio koi*)~~ ~~and common carp hybrids (e.g. *Cyprinus carpio* x *Carassius auratus*)~~. ~~These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.~~

[…]

Annex 7

CHAPTER 10.9.

INFECTION WITH SPRING VIRAEMIA OF CARP VIRUS

[…]

Article 10.9.2.

Scope

The recommendations in this chapter apply to the following species that meet the criteria for listing as susceptible in accordance with Chapter 1.5.: all varieties and subspecies of common carp *(Cyprinus carpio ~~carpio~~)* bighead carp *(Aristichthys nobilis)*, bream *(Abramis brama)*, Caspian white fish *(Rutilus ~~frisii~~kutum)*, ~~common carp *(Cyprinus carpio carpio)*~~, fathead minnow *(Pimephales promelas)*, golden shiner *(Notemigonus crysoleucas)*, goldfish *(Carassius auratus)*, grass carp ~~(white amur)~~*(Ctenopharyngodon idella~~idellus~~)*, ~~and koi carp *(Cyprinus carpio koi)*~~, ~~crucian carp *(Carassius carassius)*~~, roach *(Rutilus rutilus)*, sheatfish (also known as European or wels catfish~~or wels~~) *(Silurus glanis)*, ~~silver carp *(Hypophthalmichthys molitrix)*~~, ~~bighead carp *(Aristichthys nobilis)*~~, ~~grass carp (white amur) *(Ctenopharyngodon idellus)*~~, ~~goldfish *(Carassius auratus)*~~, ~~orfe *(Leuciscus idus)*~~ and zebrafish ~~*(Sander vitreus)*~~ (*Danio rerio*) ~~[i]tench *(Tinca tinca)*. These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally~~.

[…]

Annex 8

CHAPTER 8.3.

INFECTION WITH *RANAVIRUS* SPECIES

Article 8.3.1.

For the purposes of the *Aquatic Code*, infection with ~~ranavirus~~*Ranavirus* species means *infection* with any member virus species of the Genus *Ranavirus* and Family *Iridoviridae* in amphibians~~ with the exception of epizootic haematopoietic necrosis virus and European catfish virus~~.

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

Article 8.3.2.

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 species of the Orders ~~*Anura*~~Anura (frogs and toads) and ~~*Caudata*~~ Caudata (salamanders and newts). ~~The recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.~~

Article 8.3.3.

Importation or transit of aquatic animal products for any purpose regardless of the infection with ~~ranavirus~~ *Ranavirus* species status of the exporting country, zone or compartment

1) *Competent Authorities* should not require any conditions related to *Ranavirus* species, regardless of the infection with ~~ranavirus~~ *Ranavirus* species 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 8.3.2. that are intended for any purpose and comply with Article 5.4.1.:

   a) heat sterilised hermetically sealed amphibian 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 ~~all virus species of the genus~~*Ranavirus* species~~[with the exception of epizootic haematopoietic necrosis virus and European catfish virus])~~;

   b) cooked amphibian products that have been subjected to heat treatment at 65°C for at least 30 minutes (or any time/temperature equivalent that has been demonstrated to inactivate ~~all virus species of the genus~~ *Ranavirus* species~~with the exception of epizootic haematopoietic necrosis virus and European catfish virus])~~;

   c) pasteurised amphibian 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 ~~all virus species of the genus~~ *Ranavirus* species ~~[with the exception of epizootic haematopoietic necrosis virus and European catfish virus])~~;

   d) mechanically dried amphibian products (i.e. a heat treatment at 100°C for at least 30 minutes or any time/temperature equivalent that has been demonstrated to inactivate~~all virus species of the genus~~ *Ranavirus* species ~~[with the exception of epizootic haematopoietic necrosis virus and European catfish virus])~~.

2) When authorising the importation or transit of *aquatic animal products* derived from a species referred to in Article 8.3.2., other than those referred to in point 1 of Article 8.3.3., *Competent Authorities* should require the conditions prescribed in Articles 8.3.7. to 8.3.12. relevant to the infection with ~~ranavirus~~ *Ranavirus* species 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 8.3.2. but which could reasonably be expected to pose a *risk* of transmission of ~~ranavirus~~ *Ranavirus* species, 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.

Article 8.3.4.

Country free from infection with ~~ranavirus~~ *Ranavirus* species

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from infection with ~~ranavirus~~ *Ranavirus* species if all the areas covered by the shared water bodies are declared countries or *zones* free from infection with ~~ranavirus~~ *Ranavirus* species (see Article 8.3.5.).

As described in Article 1.4.6., a country may make a *self-declaration of freedom* from infection with ~~ranavirus~~ *Ranavirus* species if:

1) none of the *susceptible species* referred to in Article 8.3.2. are present and *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

2) any of the *susceptible species* referred to in Article 8.3.2. are present and the following conditions have been met:

   a) there has been no occurrence of infection with ~~ranavirus~~ *Ranavirus* species for at least the last ten years despite conditions that are conducive to its clinical expression (as described in the corresponding chapter of the *Aquatic Manual*); and

   b) *basic biosecurity conditions* have been continuously met for at least the last ten years;

OR

3) the infection with ~~ranavirus~~ *Ranavirus* species status prior to *targeted surveillance* is unknown but the following conditions have been met:

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

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

OR

4) it previously made a *self-declaration of freedom* from infection with ~~ranavirus~~ *Ranavirus* species and subsequently lost its free status due to the detection of ~~ranavirus~~ *Ranavirus* species but the following conditions have been met:

   a) on detection of *Ranavirus* species, 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 *Ranavirus* species, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; and

   c) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place since eradication of infection with ~~ranavirus~~ *Ranavirus* species; and

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

In the meantime, part or all of the unaffected area may be declared a free *zone* provided that such a part meets the conditions in point 3 of Article 8.3.5.

Article 8.3.5.

Zone or compartment free from infection with ~~ranavirus~~ *Ranavirus* species

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

As described in Article 1.4.6., a *zone* or *compartment* within the *territory* of one or more countries not declared free from infection with ~~ranavirus~~ *Ranavirus* species may be declared free by the *Competent Authority* of the country concerned if:

1) none of the *susceptible species* referred to in Article 8.3.2. are present in the *zone* or *compartment* and *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

2) any of the *susceptible species* referred to in Article 8.3.2. are present in the *zone* or *compartment* and the following conditions have been met:

   a) there has been no occurrence of infection with ~~ranavirus~~ *Ranavirus* species for at least the last ten years despite conditions that are conducive to its clinical expression (as described in the corresponding chapter of the *Aquatic Manual*); and

   b) *basic biosecurity conditions* have been continuously met for at least the last ten years;

OR

3) the infection with ~~ranavirus~~ *Ranavirus* species status prior to *targeted surveillance* is unknown but the following conditions have been met:

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

   b) *targeted surveillance*, as described in Chapter 1.4., has been in place, in the *zone* or *compartment*, for at least the last two years without detection of *Ranavirus* species;

OR

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

   a) on detection of *Ranavirus* species, 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 *Ranavirus* species, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; and

   c) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place since eradication of infection with ~~ranavirus~~ *Ranavirus* species; and

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

Annex 8 (contd)

Article 8.3.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from infection with ~~ranavirus~~ *Ranavirus* species following the provisions of points 1 or 2 of Articles 8.3.4. or 8.3.5. (as relevant) may maintain its status as free from infection with ~~ranavirus~~ *Ranavirus* species provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from infection with ~~ranavirus~~ *Ranavirus* species following the provisions of point 3 of Articles 8.3.4. or 8.3.5. (as relevant) may discontinue *targeted surveillance* and maintain its free status provided that conditions that are conducive to clinical expression of infection with ~~ranavirus~~ *Ranavirus* species, as described in the corresponding chapter of the *Aquatic Manual*, and *basic biosecurity conditions* are continuously maintained.

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

Article 8.3.7.

Importation of aquatic animals or aquatic animal products from a country, zone or compartment declared free from infection with ~~ranavirus~~ *Ranavirus* species

When importing *aquatic animals* of a species referred to in Article 8.3.2., or *aquatic animal products* derived thereof, from a country, *zone* or *compartment* declared free from infection with ~~ranavirus~~ *Ranavirus* species, 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 8.3.4. or 8.3.5. (as applicable) and 8.3.6., the place of production of the *aquatic animals* or *aquatic animal products* is a country, *zone* or *compartment* declared free from infection with ~~ranavirus~~ *Ranavirus* species.

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 point 1 of Article 8.3.3.

Article 8.3.8.

Importation of aquatic animals for aquaculture from a country, zone or compartment not declared free from infection with ~~ranavirus~~ *Ranavirus* species

When importing, for *aquaculture*, *aquatic animals* of a species referred to in Article 8.3.2. from a country, *zone* or *compartment* not declared free from infection with ~~ranavirus~~ *Ranavirus* species, 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 the *quarantine* facility the *aquatic animals* are killed and processed into one or more of the *aquatic animal products* referred to in point 1) of Article 8.3.3. or other products authorised by the *Competent Authority*; and

   ~~*b)*~~ *c)* the treatment of all transport water, equipment, effluent and waste materials to inactivate *Ranavirus* species in accordance with Chapters 4.3., 4.7. 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 ~~ranavirus~~ *Ranavirus* species;

b) in the *importing country*:

i) import the F-0 population into a *quarantine* facility;

ii) test the F-0 population for *Ranavirus* species 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* under conditions that are conducive to the clinical expression of infection with ~~ranavirus~~ *Ranavirus* species, and sample and test for *Ranavirus* species in accordance with Chapter 1.4. of the *Aquatic Code* and Chapter 2.1.2. of the *Aquatic Manual*;

v) if *Ranavirus* species ~~is~~ are not detected in the F-1 population, it may be defined as free from infection with ~~ranavirus~~ *Ranavirus* species and may be released from *quarantine*;

vi) if *Ranavirus* species are 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 8.3.9.

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 ~~ranavirus~~ *Ranavirus* species

When importing, for processing for human consumption, *aquatic animals* of a species referred to in Article 8.3.2., or *aquatic animal products* derived thereof, from a country, *zone* or *compartment* not declared free from infection with ~~ranavirus~~ *Ranavirus* species, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, require that:

1) the consignment is delivered directly to, and held in, *quarantine* or containment facilities until processing into one of the products referred to in point 1 of Article 8.3.3. or in point 1 of Article 8.3.12., or other products authorised by the *Competent Authority*; and

2) all water (including ice), equipment, *containers* and packaging material used in transport are treated to ensure inactivation of *Ranavirus* species or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and

3) all effluent and waste materials are treated to ensure inactivation of *Ranavirus* species or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

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 8.3.10.

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 ~~ranavirus~~ *Ranavirus* species

When importing *aquatic animals* of a species referred to in Article 8.3.2., 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 ~~ranavirus~~ *Ranavirus* species, 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 point 1 of Article 8.3.3. 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 *R~~r~~anavirus* species or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and

3) all effluent and waste materials are treated to ensure inactivation of *R~~r~~anavirus* species or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

Article 8.3.11.

Importation of aquatic animals intended for use in laboratories or zoos from a country, zone or compartment not declared free from infection with ~~ranavirus~~ *Ranavirus* species

When importing, for use in laboratories or zoos, *aquatic animals* of a species referred to in Article 8.3.2. from a country, *zone* or *compartment* not declared free from infection with ~~ranavirus~~ *Ranavirus* species, 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 *R~~r~~anavirus* species or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. 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 *R~~r~~anavirus* species or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.; and

4) the carcasses are disposed of in accordance with Chapter 4.7.

Article 8.3.12.

Importation (or transit) of aquatic animal products for retail trade for human consumption regardless of the infection with ~~ranavirus~~ *Ranavirus* species status of the exporting country, zone or compartment

1) *Competent Authorities* should not require any conditions related to *R~~r~~anavirus* species, regardless of the infection with ~~ranavirus~~*Ranavirus* species status of the *exporting country*, *zone* or *compartment*, when authorising the importation (or transit) of the following *aquatic animal products* that have been prepared and packaged for retail trade and comply with Article 5.4.2.:

   – no *aquatic animal products* listed.

2) When importing *aquatic animal products*, other than those referred to in point 1 above, derived from a species referred to in Article 8.3.2. from a country, *zone* or *compartment* not declared free from infection with ~~ranavirus~~*Ranavirus* species, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

CHAPTER 9.1.

ACUTE HEPATOPANCREATIC NECROSIS DISEASE

Article 9.1.1.

For the purposes of the *Aquatic Code*, acute hepatopancreatic necrosis disease (AHPND) means *infection* with strains of *Vibrio parahaemolyticus* (Vp_{AHPND}), of the Family Vibrionaceae, that contain a ~70-kbp plasmid with genes that encode homologues of the *Photorhabdus* insect-related (Pir) toxins, PirA and PirB.

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

Article 9.1.2.

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.: giant tiger prawn *(Penaeus monodon)* and whiteleg shrimp *(Penaeus vannamei)*.

Article 9.1.3.

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

1) *Competent Authorities* should not require any 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., which are intended for any purpose and comply with Article 5.4.1.:

   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 Vp_{AHPND};

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

   c) crustacean oil;

   d) crustacean *meal*;

   e) chemically extracted chitin.

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

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

Article 9.1.4.

Country free from AHPND

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

Annex 9 (contd)

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

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

OR

2) any of the *susceptible species* referred to in Article 9.1.2. are present and the following conditions have been met:

   a) there has been no occurrence of AHPND for at least the last ten years despite conditions that are conducive to its clinical expression (as described in the corresponding chapter of the *Aquatic Manual*); and

   b) *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

3) the AHPND status prior to *targeted surveillance* is unknown but the following conditions have been met:

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

   b) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of Vp_{AHPND} ~~AHPND~~;

OR

4) it previously made a *self-declaration of freedom* from AHPND and subsequently lost its free status due to the detection of Vp_{AHPND} ~~AHPND~~ but the following conditions have been met:

   a) on detection of Vp_{AHPND} ~~AHPND~~, 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 Vp_{AHPND} ~~AHPND~~, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; and

   c) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place since eradication of AHPND; and

   d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of Vp_{AHPND} ~~AHPND~~.

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

Article 9.1.5.

Zone or compartment free from AHPND

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

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

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

OR

2) any of the *susceptible species* referred to in Article 9.1.2. are present in the *zone* or *compartment* and the following conditions have been met:

   a) there has not been any occurrence of AHPND for at least the last ten years despite conditions that are conducive to its clinical expression (as described in the corresponding chapter of the *Aquatic Manual*); and

   b) *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

3) the AHPND status prior to *targeted surveillance* is unknown but the following conditions have been met:

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

   b) *targeted surveillance*, as described in Chapter 1.4., has been in place, in the *zone* or *compartment*, for at least the last two years without detection of Vp_{AHPND} ~~AHPND~~;

OR

4) it previously made a *self-declaration of freedom* for a *zone* from AHPND and subsequently lost its free status due to the detection of Vp_{AHPND} ~~AHPND~~ in the zone but the following conditions have been met:

   a) on detection of Vp_{AHPND} ~~AHPND~~, 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 Vp_{AHPND} ~~AHPND~~, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; and

   c) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place since eradication of AHPND; and

   d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of Vp_{AHPND} ~~AHPND~~.

Article 9.1.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from AHPND following the provisions of points 1 or 2 of Articles 9.1.4. or 9.1.5. (as relevant) may maintain its status as free from AHPND provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from AHPND following the provisions of point 3 of Articles 9.1.4. or 9.1.5. (as relevant) may discontinue *targeted surveillance* and maintain its free status provided that conditions are conducive to clinical expression of AHPND, as described in the corresponding chapter of the *Aquatic Manual*, and *basic biosecurity conditions* are continuously maintained.

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

Article 9.1.7.

Importation of aquatic animals or aquatic animal products from a country, zone or compartment declared free from AHPND

When importing *aquatic animals* of a species referred to in Article 9.1.2., or *aquatic animal products* derived thereof, from a country, *zone* or *compartment* declared free from AHPND, 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* or a *certifying official* approved by the *importing country*. The *international aquatic animal health certificate* should state that, on the basis of the procedures described in Articles 9.1.4. or 9.1.5. (as applicable) and 9.1.6., the place of production of the *aquatic animals* or *aquatic animal products* is a country, *zone* or *compartment* declared free from AHPND.

Annex 9 (contd)

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 point 1 of Article 9.1.3.

Article 9.1.8.

Importation of aquatic animals for aquaculture from a country, zone or compartment not declared free from AHPND

When importing, for *aquaculture*, *aquatic animals* of a species referred to in Article 9.1.2. from a country, *zone* or *compartment* not declared free from AHPND, 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 the *quarantine* facility the *aquatic animals* are killed and processed into one or more of the *aquatic animal products* referred to in point 1) of Article 9.1.3. or other products authorised by the *Competent Authority*; and

   ~~*b)*~~*c)* the treatment of transport water, equipment, effluent and waste materials to inactivate Vp_{AHPND} in accordance with Chapters 4.3., 4.7. 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 AHPND.

   b) In the importing country:

      i) import the F-0 population into a *quarantine* facility;

      ii) test the F-0 population for Vp_{AHPND} in accordance with Chapter 1.4. to determine their suitability as broodstock;

      iii) produce a first generation (F-1) population in *quarantine*;

      iv) culture F-1 population in *quarantine* under conditions that are conducive to the clinical expression of AHPND (as described in Chapter 2.2.1. of the *Aquatic Manual*) and test for Vp_{AHPND} in accordance with Chapter 1.4.;

      v) if Vp_{AHPND} is not detected in the F-1 population, it may be defined as free from AHPND and may be released from *quarantine*;

      vi) if Vp_{AHPND} 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 9.1.9.

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

When importing, for processing for human consumption, *aquatic animals* of a species referred to in Article 9.1.2., or *aquatic animal products* derived thereof, from a country, *zone* or *compartment* not declared free from AHPND, 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 processed into one of the products referred to in point 1 of Article 9.1.3. or in point 1 of Article 9.1.11., or other products authorised by the *Competent Authority*; and

2) all containers and water used in transport are treated to ensure inactivation of Vp_{AHPND} or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and

3) all processing effluent and waste materials are treated to ensure inactivation of Vp_{AHPND} or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

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 animals* or *aquatic animal products* being used for any purpose other than for human consumption.

Article 9.1.10.

Importation of aquatic animals or aquatic animal products intended for uses other than human consumption including animal feed, or for agricultural, industrial, research or pharmaceutical use, from a country, zone or compartment not declared free from AHPND

When importing, for use in animal feed or for agricultural, industrial, research or pharmaceutical use, *aquatic animals* of a species referred to in Article 9.1.2., or *aquatic animal products* derived thereof, from a country, *zone* or *compartment* not declared free from AHPND, 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 point 1 of Article 9.1.3. or other products authorised by the *Competent Authority*; and

2) all containers and water used in transport are treated to ensure inactivation of Vp_{AHPND} or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and

3) all processing effluent and waste materials are treated to ensure inactivation of Vp_{AHPND} or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

Article 9.1.11.

Importation of aquatic animals intended for use in laboratories or zoos from a country, zone or compartment not declared free from AHPND

When importing, for use in laboratories or zoos, *aquatic animals* of a species referred to in Article 9.1.2. from a country, *zone* or *compartment* not declared free from AHPND, 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 Vp_{AHPND} or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. 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 Vp_{AHPND} or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.; and

4) the carcasses are disposed of in accordance with Chapter 4.7.

Article 9.1.12.

Importation (or transit) of aquatic animal products for retail trade for human consumption regardless of the AHPND status of the exporting country, zone or compartment

1) *Competent Authorities* should not require any conditions related to Vp_{AHPND} ~~AHPND~~, regardless of the AHPND status of the *exporting country*, *zone* or *compartment*, when authorising the importation (or transit) of frozen peeled shrimp (shell off, head off) that have been prepared and packaged for retail trade and comply with Article 5.4.2.

   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.

Annex 9 (contd)

For these *aquatic animal products* Member Countries may wish to consider introducing internal measures to address the *risks* associated with the *aquatic animal products* 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 9.1.2. from a country, *zone* or *compartment* not declared free from AHPND, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

CHAPTER 10.6.

INFECTION WITH INFECTIOUS HAEMATOPOIETIC NECROSIS VIRUS

Article 10.6.1.

For the purposes of the *Aquatic Code*, infection with infectious haematopoietic necrosis virus means *infection* with the *pathogenic agent* salmonid *Novirhabdovirus* (also known as infectious haematopoietic necrosis virus ~~([IHNV])~~ of the Genus *Novirhabdovirus* and Family *Rhabdoviridae*.

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

Article 10.6.2.

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.: Arctic charr *(Salvelinus alpinus)*, Atlantic salmon *(Salmo salar)*, brook trout *(Salvelinus fontinalis)*, brown trout *(Salmo trutta)*, chinook salmon *(Oncorhynchus tshawytscha)*, chum salmon *(Oncorhynchus keta)*, coho salmon *(Oncorhynchus kisutch)*, cutthroat trout *(Onchorynchus clarkii)*, lake trout *(Salvelinus namaycush)*, masou salmon *(Oncorhynchus masou)*, marble trout *(Salmo marmoratus)*, rainbow trout ~~or steelhead~~ *(Oncorhynchus mykiss)*, ~~the Pacific salmon species (chinook [*Oncorhynchus tshawytscha*], sockeye [*Oncorhynchus nerka*], chum [*Oncorhynchus keta*], masou [*Oncorhynchus masou*], pink [*Oncorhynchus rhodurus*] and coho [*Oncorhynchus kisutch*])~~, and sockeye salmon (*Oncorhynchus nerka*) ~~Atlantic salmon *(Salmo salar)*. These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.~~

Article 10.6.3.

Importation or transit of aquatic animal products for any purpose regardless of the infection with IHNV status of the exporting country, zone or compartment

1) *Competent Authorities* should not require any conditions related to IHNV, regardless of the infection with IHNV status of the *exporting country*, *zone* or *compartment*, when authorising the importation or transit of the following *aquatic animal products* derived from a species referred to in Article 10.6.2. that are intended for any purpose and comply with Article 5.4.1.:

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

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

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

   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 IHNV status of the *exporting country*, *zone* or *compartment*.

3) When considering the importation or transit of *aquatic animal products* derived from a species not referred to in Article 10.6.2. but which could reasonably be expected to pose a *risk* of transmission of IHNV, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this analysis.

Article 10.6.4.

Country free from infection with IHNV

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from infection with IHNV if all the areas covered by the shared water bodies are declared countries or *zones* free from infection with IHNV (see Article 10.6.5.).

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

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

OR

2) any of the *susceptible species* referred to in Article 10.6.2. are present and the following conditions have been met:

   a) there has been no occurrence of infection with IHNV for at least the last ten years despite conditions that are conducive to its clinical expression (as described in the corresponding chapter of the *Aquatic Manual*); and

   b) *basic biosecurity conditions* have been continuously met for at least the last ten years;

OR

3) the infection with IHNV status prior to *targeted surveillance* is unknown but the following conditions have been met:

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

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

OR

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

   a) on detection of IHNV, 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 IHNV, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; and

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

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

   In the meantime, part or all of the unaffected area may be declared a free *zone* provided that such a part meets the conditions in point 3 of Article 10.6.5.

Article 10.6.5.

Zone or compartment free from infection with IHNV

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

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

1) none of the *susceptible species* referred to in Article 10.6.2. are present in the *zone* or *compartment* and *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

2) any of the *susceptible species* referred to in Article 10.6.2. are present in the *zone* or *compartment* and the following conditions have been met:

   a) there has been no occurrence of infection with IHNV for at least the last ten years despite conditions that are conducive to its clinical expression (as described in the corresponding chapter of the *Aquatic Manual*); and

   b) *basic biosecurity conditions* have been continuously met for at least the last ten years;

OR

3) the infection with IHNV status prior to *targeted surveillance* is unknown but the following conditions have been met:

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

   b) *targeted surveillance*, as described in Chapter 1.4., has been in place, in the *zone* or *compartment*, for at least the last two years without detection of IHNV;

OR

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

   a) on detection of IHNV, 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 IHNV, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; and

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

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

Annex 10 (contd)

Article 10.6.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from infection with IHNV following the provisions of points 1 or 2 of Articles 10.6.4. or 10.6.5. (as relevant) may maintain its status as free from infection with IHNV provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from infection with IHNV following the provisions of point 3 of Articles 10.6.4. or 10.6.5. (as relevant) may discontinue *targeted surveillance* and maintain its free status provided that conditions that are conducive to clinical expression of infection with IHNV, as described in the corresponding chapter of the *Aquatic Manual*, and *basic biosecurity conditions* are continuously maintained.

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

Article 10.6.7.

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

When importing *aquatic animals* of a species referred to in Article 10.6.2., or *aquatic animal products* derived thereof, from a country, *zone* or *compartment* declared free from infection with IHNV, 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 10.6.4. or 10.6.5. (as applicable) and 10.6.6., the place of production of the *aquatic animals* or *aquatic animal products* is a country, *zone* or *compartment* declared free from infection with IHNV.

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 point 1 of Article 10.6.3.

Article 10.6.8.

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

When importing for *aquaculture*, *aquatic animals* of a species referred to in Article 10.6.2. from a country, *zone* or *compartment* not declared free from infection with IHNV, 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 the *quarantine* facility the *aquatic animals* are killed and processed into one or more of the *aquatic animal products* referred to in point 1) of Article 10.6.3. or other products authorised by the *Competent Authority*; and

   ~~*b*~~*c)* the treatment of all transport water, equipment, effluent and waste materials to inactive IHNV in accordance with Chapters 4.3., 4.7. 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 IHNV.

b) In the *importing country:*

i) import the F-0 population into a *quarantine* facility;

ii) test the F-0 population for IHNV 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* under conditions that are conducive to the clinical expression of infection with IHNV, and sample and test for IHNV in accordance with Chapter 1.4. of the *Aquatic Code* and Chapter 2.3.4. of the *Aquatic Manual*;

v) if IHNV is not detected in the F-1 population, it may be defined as free from infection with IHNV and may be released from *quarantine*;

vi) if IHNV 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 10.6.9.

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 IHNV

When importing, for processing for human consumption, *aquatic animals* of a species referred to in Article 10.6.2. or *aquatic animal products* derived thereof, from a country, *zone* or *compartment* not declared free from infection with IHNV, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, require that:

1) the consignment is delivered directly to, and held in, *quarantine* or containment facilities until processing into one of the products referred to in point 1 of Article 10.6.3. or in point 1 of Article 10.6.12., or other products authorised by the *Competent Authority*; and

2) all water (including ice), equipment, *containers* and packaging material used in transport are treated to ensure inactivation of IHNV or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and

3) all effluent and waste materials from the holding of the *aquatic animals* are treated to ensure inactivation of IHNV or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

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 10.6.10.

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 IHNV

When importing *aquatic animals* of a species referred to in Article 10.6.2., 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 IHNV, 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 point 1 of Article 10.6.3. 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 IHNV or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and

3) all effluent and waste materials are treated to ensure inactivation of IHNV or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

Article 10.6.11.

Importation of aquatic animals intended for use in laboratories or zoos from a country, zone or compartment not declared free from infection with IHNV

When importing, for use in laboratories or zoos, *aquatic animals* of a species referred to in Article 10.6.2. from a country, *zone* or *compartment* not declared free from infection with IHNV, 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 IHNV or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. 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 IHNV or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.; and

4) the carcasses are disposed of in accordance with Chapter 4.7.

Article 10.6.12.

Importation (or transit) of aquatic animal products for retail trade for human consumption regardless of the infection with IHNV status of the exporting country, zone or compartment

1) *Competent Authorities* should not require any conditions related to IHNV, regardless of the infection with IHNV status of the *exporting country*, *zone* or *compartment*, when authorising the importation (or transit) of fish fillets or steaks (chilled) that have been prepared and packaged for retail trade and comply with Article 5.4.2.

   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 10.6.2. from a country, *zone* or *compartment* not declared free from infection with IHNV, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

Article 10.6.13.

Importation of disinfected eggs for aquaculture from a country, zone or compartment not declared free from infection with IHNV

1) When importing disinfected eggs of the species referred to in Article 10.6.2. for *aquaculture*, from a country, *zone* or *compartment* not declared free from infection with IHNV, the *Competent Authority* of the *importing country* should assess the *risk* associated with at least:

   a) the infection with IHNV status of the water to be used during the *disinfection* of the eggs;

   b) the prevalence of infection with IHNV in broodstock (ovarian fluid and milt); and

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

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

   a) the eggs should be disinfected prior to importing, in accordance with recommendations in Chapter 4.4. or those specified by the *Competent Authority* of the *importing country*; and

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

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

3) When importing disinfected eggs of the species referred to in Article 10.6.2. for *aquaculture*, from a country, *zone* or *compartment* not declared free from infection with IHNV, the *Competent Authority* of the *importing country* should require that the consignment be accompanied by an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* certifying that the procedures described in point 2 of this article have been fulfilled.

Annex 11

Model Article X.X.8. for all disease-specific chapters (or article 10.4.12. for infection with infectious salmon anaemia virus)

Importation of aquatic animals for aquaculture from a country, zone or compartment not declared free from 'infection with pathogen X'/ 'disease X'

When importing for *aquaculture*, *aquatic animals* of species referred to in Article X.X.2. from a country, *zone* or *compartment* not declared free from 'infection with pathogen X'/'*disease X'*, 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 the *quarantine* facility the *aquatic animals* are killed and processed into one or more of the *aquatic animal products* referred to in point 1) of Article X.X.3. or other products authorised by the *Competent Authority*; and

   ~~*b)*~~*c)* the treatment of all transport water, equipment, effluent and waste materials to inactive 'pathogen X' in accordance with Chapters 4.3., 4.7. 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 pathogen X'/ 'disease X'.

   b) In the *importing country*:

      i) import the F-0 population into a *quarantine* facility;

      ii) test the F-0 population for 'pathogen X' in accordance with Chapter 1.4. to determine their suitability as broodstock;

      iii) produce a first generation (F-1) population in *quarantine*;

      iv) culture F-1 population in *quarantine* under conditions that are conducive to the clinical expression of 'infection with pathogen X'/'disease X' (as described in Chapter X.X.X. of the *Aquatic Manual*) and test for 'pathogen X' in accordance with Chapter 1.4.;

      v) if 'pathogen X' is not detected in the F-1 population, it may be defined as free from 'infection with pathogen X'/'disease X' and may be released from *quarantine*;

      vi) if 'pathogen X' 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.

Annex 12

CHAPTER 4.X.

BIOSECURITY FOR AQUACULTURE ESTABLISHMENTS

Article 4.X.1.

Purpose

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

Article 4.X.2.

Scope

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

Article 4.X.3.

Introduction

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

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

The outcome achieved through the implementation of *biosecurity* at *aquaculture establishments* is improved health status of *aquatic animals* throughout the production cycle. The benefits include market access and increased productivity, directly through improved survival, growth rates and *feed* conversion and indirectly through the reduction in treatments and associated production costs.

Article 4.X.4.

General principles

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

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1) Planning is necessary to document the objectives of the *biosecurity plan*, the identified *risks* to be managed, the measures that will be put in place to manage the *disease risks*, required operating procedures and monitoring, as described in Articles 4.X.6. and 4.X.7.

2) Potential pathways for *pathogenic agents* to be transmitted into, spread within and released from the *aquaculture establishment* must be identified, as described in Articles 4.X.5. and 4.X.6., and giving consideration to the category of *aquaculture* production system and design of the *aquaculture establishment.*

3) *Risk analysis* should be undertaken to evaluate *biosecurity* threats and ensure the plan addresses *risks* appropriately and efficiently. The *risk analysis* may range from a simple to a complex analysis depending on the objectives of the *biosecurity plan* and the circumstances of the *aquaculture establishment* and *disease risks*, as described in Article 4.X.7.

4) *Biosecurity* measures to address identified *disease risks* should be evaluated based on their potential effectiveness, initial and ongoing costs (e.g. building works, maintenance), and management requirements, as described in Article 4.X.7.

5) Management practices should be integrated into the *aquaculture establishment*'s operating procedures and associated training are provided to personnel, as described in Articles 4.X.7. and 4.X.8.

6) A routine review schedule of the *biosecurity plan* and identified triggers for *ad hoc* review must be determined (e.g. changes to infrastructure, production techniques or *risk* profiles). Third party audit may be required where recognition of the *biosecurity* measures is required by customers, regulators or for market access, as described in Article 4.X.8.

Article 4.X.5.

Categories of aquaculture production systems

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

Open systems

Open *aquaculture* production systems have no control of water, environmental conditions and animals. These production systems may include stock enhancement of wild populations. As these systems cannot be considered 'establishments', they are not considered further in this chapter.

Semi-open

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

Semi-closed

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

Closed

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

Article 4.X.6.

Transmission pathways and associated risks

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

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

1. Aquatic animals

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

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

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

b) Quarantining introduced *aquatic animals* of unknown *disease* status from other farm populations in separate production units or dedicated *quarantine* facilities.

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

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

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

f) Isolating *aquatic animal* populations that display clinical signs of *disease* from other populations until the cause is known and the situation is resolved.

g) Removing sick or dead *aquatic animals* from production units as soon as possible and disposing of them in a biosecure manner in accordance with Chapter 4.7.

h) Where possible, preventing unintended movement of *aquatic animals* into, within or from the establishment.

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

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2. Aquatic animal products and waste

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

Movement of *aquatic animal products* and *aquatic animal* waste into, within and out of *aquaculture establishments* may pose a *risk* of *pathogenic agent* transmission. This is particularly the case when a susceptible population is exposed to *aquatic animal products* and *aquatic animal* waste derived from clinically or sub-clinically infected *aquatic animals*. High *risk* waste includes *aquatic animal* waste that constitutes, or is suspected of constituting, a high health *risk* to *aquatic animals*.

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

The *risk* of transmitting *pathogenic agents* via *aquatic animal products* and *aquatic animal* waste can be managed by:

a) determining the potential *disease risk* of aquatic animal products and waste to the establishment and the environment;

b) isolating areas within the *aquaculture establishment* where *aquatic animal products* and waste are managed from *aquatic animal* populations to minimise identified disease transmission *risks*;

c) ensuring systems are implemented for appropriate collection, treatment (inactivating *pathogenic agents*), transport, storage or disposal of *aquatic animal products* and waste to minimise the *risks* of transmitting *pathogenic agents*.

3. Water

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

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

The *risk* of transmitting *pathogenic agents* via water can be managed by:

a) Where possible, choosing water sources that are entirely free of susceptible *aquatic animal* populations and *pathogenic agents* of concern. Such water sources may include saline or fresh groundwater, de-chlorinated municipal water, and artificial seawater. These water sources may be particularly suitable for high health status *aquatic animals* such as broodstock.

b) Providing an appropriate level of screening, filtration or disinfection (in accordance with Chapter 4.3.) of water from sources that are likely to contain *susceptible species* and may present a *risk* of *pathogenic agent* transmission (e.g. oceans, streams or lakes). The level of treatment required will depend on the identified *risks*.

c) Ensuring the position of water intakes and outlets for semi-closed and closed *aquaculture establishments*, and the location of semi-open *aquaculture establishments*, minimises contamination from other farmed or wild populations or processing plants.

4. Feed

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

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

The *risk* of transmitting *pathogenic agents* via *aquatic animal feed* can be managed as described in Chapter 4.8., for example using *feed* and *feed* ingredients that:

a) have undergone sufficient processing to inactivate *pathogenic agents* of concern;

b) are from sources that are declared free from the *pathogenic agents* of concern or have been confirmed (e.g. by testing) that *pathogenic agents* are not present in the commodity;

c) have been processed, manufactured, stored and transported in a manner to prevent contamination by *pathogenic agents*.

5. Fomites

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

The level of *risk* of transferring *pathogenic agents* will depend on the presence and nature of organic matter on the fomite surface, as well as the type of surface and its ability to hold water. The *risk* of transferring *pathogenic agents* may be higher for fomites which are difficult to clean and disinfect. Equipment that is shared between *aquaculture establishments,* between *aquaculture establishments* and processing facilities or between different production units within an *aquaculture establishment* with unequal health status, may present a higher *risk* compared to new or dedicated equipment. The *risk* of transmitting *pathogenic agents* via fomites can be managed by:

a) Assessing any fomites brought into the *aquaculture establishment* for their *disease risk*.

b) Ensuring procedures and infrastructure are in place to clean and disinfect fomites, including at designated delivery and loading areas. Recommendations for the cleaning and disinfection of fomites are described in Chapter 4.3.

c) Assigning dedicated equipment for use in production units of different health status. Where equipment must be used in multiple production units it should be cleaned and disinfected prior to movement between units.

6. Vectors

Vectors can transport *pathogenic agents* to susceptible *aquatic animals* in *aquaculture establishments*. These include wild *aquatic animals* entering via the water supply, predators, wild birds, and pest animals such as rodents. *Vectors* can transfer *pathogenic agents* into, within and from an *aquaculture establishment*, either by mechanical transfer or as a developmental stage of the *pathogenic agent* within the *vector*.

Annex 12 (contd)

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

Article 4.X.7.

Risk analysis

Risk analysis is an accepted approach for evaluating *biosecurity* threats and to support the development of mitigation measures. A formal *risk analysis* has four components: *hazard* identification, *risk assessment*, *risk management* and *risk communication* (see Chapter 2.1.).

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

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

Step 1 – Hazard Identification

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

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

Step 2 – Risk Assessment

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

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

Table 1. Qualitative descriptors of likelihood

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

Table 2. Qualitative descriptors of consequences

Estimate	Descriptor
Insignificant	Impact not detectable or minimal.
Minor	Impact on *aquaculture establishment* productivity limited to some production units or short term only.
Moderate	Widespread impact on *aquaculture establishment* productivity due to increased mortality or decreased performance.
Major	Considerable impact on *aquaculture establishment* production resulting in serious supply constraints and financial impact.
Catastrophic	Complete depopulation of the *aquaculture establishment* and possibly barriers to resumption of production.

Table 3. Matrix for assessing risk

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

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

Table 4. Interpretation of risk estimates

Risk* level	**Explanation and management response**
Negligible	Acceptable level of *risk*. No action required.
Low	Acceptable level of *risk*. On-going monitoring may be required.
Medium	Unacceptable level of *risk*. Active management is required to reduce the level of *risk.*
High	Unacceptable level of *risk*. Intervention is required to mitigate the *risk.*
Extreme	Unacceptable level of *risk*. Urgent intervention is required to mitigate the level of *risk.*

**Risk* level determined by combination of likelihood and consequence score using the *risk* matrix (Table 3).

Annex 12 (contd)

Step 3 – Risk Management

Risk management is used to determine the appropriate management response for the assessed level of *risk* as described in Table 4. The *risk assessment* process identifies the steps within transmission pathways necessary for a *risk* to be realised and thus allows the most effective mitigation measures to be determined. Many of the *hazards* will share the same pathways and thus mitigation measures may be effective against more than one *hazard*.

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

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

Article 4.X.8.

Biosecurity plan development

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

1. Development of a biosecurity plan

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

a) objectives and regulatory requirements for the *biosecurity plan*;

b) information about the *aquaculture establishment* including the layout of buildings and production units, and maps showing major movements of *aquatic animals*, *aquatic animal products* and waste, water, *feed* and fomites (including staff, equipment and *vehicles*);

c) the potential pathways for entry of *pathogenic agents* into, spread within or release from the *aquaculture establishment* (refer to Article X.X.6. above);

d) a *risk analysis*, including identification of the major *disease hazards* to the *aquaculture establishment* (refer to Article X.X.7. above);

e) the mitigation measures that have been determined to address identified *risks*;

f) emergency procedures in the event of a *biosecurity* failure;

g) standard operating procedures required to support implementation of the mitigation measures, emergency procedures and the training requirements of personnel;

h) internal and external communication procedures, and roles and responsibilities of personnel;

i) monitoring and audit schedule;

j) performance evaluation.

2. Key components of a biosecurity plan

a) Standard operating procedures (SOPs)

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

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

b) Documentation and record keeping

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

Examples of documentation required may include: *aquaculture establishment* layout, movements of *aquatic animals*, escapees, origin and health status of the *aquatic animals* introduced to the *aquaculture establishment*, stocking densities, feeding and growth rates, records of staff training, treatments/vaccination, water quality, morbidity and mortality, *surveillance* and laboratory records.

c) Emergency procedures

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

d) Health monitoring

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

e) Routine review and auditing

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

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

Annex 13

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

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

Summary

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

The paper presents an evaluation of each of four existing pathways in the *Aquatic Code* for declaration of freedom at the level of country, zone and compartment. Some key findings of the evaluation include:

1. The utility of Pathway 1 (absence of susceptible species) should be reconsidered.

2. The appropriateness of each pathway for declaration of freedom at each of the levels of country, zone or compartment should be evaluated.

3. Criteria are proposed for determining the periods stated in Articles X.X.4. and X.X.5. of each disease-specific chapter (for which a country must have *basic biosecurity conditions* in place or for which surveillance must be conducted).

4. That requirements for declaration of freedom should be flexible and focus on surveillance outcomes (i.e. appropriate evidence to substantiate claims of freedom) rather than rigid requirements of surveillance inputs (e.g. either active or passive surveillance data).

5. Similar confidence in the evidence of freedom should be required regardless of the principal type of surveillance data (e.g. passive or active surveillance).

6. Possible revisions to Chapter 1.4. are suggested.

It is the intention of the Commission to use this discussion paper to engage Member Countries in exploring improvements to the standards of the *Aquatic Code* for demonstration of freedom. Any recommendations provided with the paper are for the purposes of stimulating discussion only and should not be considered the intended approaches of the Commission for revision of the *Aquatic Code*.

Member Countries are invited to comment on the paper and, for convenience, several discussion points have been provided throughout the text as a basis for Member Country responses. These discussion points are summarised in Table 3 in Section 7 of this document.

1. Background

Chapter 1.4. of the *OIE Aquatic Animal Health Code (Aquatic Code)* describes four pathways through which Member Countries can make self-declarations of freedom from a disease. These four pathways are also reflected in each of the disease-specific chapters of the *Aquatic Code* in Article X.X.4. (Country free from disease X), and Article X.X.5. (Zone or compartment free from disease X). An example of these articles is provided at Appendix 1.

Within Articles X.X.4. and X.X.5., several periods of time are specified for which a country must have *basic biosecurity conditions*[1] in place or for which surveillance must be conducted. These periods are applied differently across the four pathways for claiming freedom at the country, zone or compartment level and among the various listed diseases. Appendix 2 provides a summary of the various periods included in the *Aquatic Code* for declaration of country freedom; however, there is no documented rationale on the considerations or criteria for determining these periods.

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

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

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

2. Objectives of this paper

The primary objectives of this paper are to:

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

2. Explore possible improvements to Articles X.X.4. and X.X.5.

Secondary objectives of the paper include:

3. Identifying the nature of revisions that may be required to Chapter 1.4. (consistent with objectives 1 and 2).

4. Determine whether guidance is required within the *Aquatic Code* for Member Countries on the approach to designing surveillance for and making a self-declaration of freedom.

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[1] Basic Biosecurity Conditions is a defined term in the OIE *Aquatic Code* which means:
"*a set of conditions applying to a particular disease, and a particular zone or country, required to ensure adequate disease security, such as:*
– *the disease, including suspicion of the disease, is compulsorily notifiable to the Competent Authority; and*
– *an early detection system is in place within the zone or country; and*
– *import requirements to prevent the introduction of disease into the country or zone, as outlined in the Aquatic Code, are in place.*"

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

A. provide confidence among Member Countries in the strength of self-declarations of freedom that are made in accordance with any proposed approaches in the *Aquatic Code;*

B. should be fit for the intended purpose at the level of either country, zone or compartment;

C. be as uncomplicated as possible and readily understandable by Member Countries;

D. be practical and developed considering the resource constraints of Member Countries;

E. be sufficiently flexible to allow efficient approaches that meet principle A.

3. Analysis of existing pathways for claiming freedom

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

1. Absence of susceptible species.

2. Had no occurrence for at least the last ten years (historical freedom).

3. Unknown disease status.

4. Previously made a self-declaration of freedom free but lost their free status due to a detection.

In all cases, *basic biosecurity conditions* (compulsory notification of the disease or suspicion of the disease to the Competent Authority, an *early detection system*[2] and measures to prevent disease introduction) need to be in place to claim freedom and, for Pathways 3 and 4, targeted surveillance is also required.

Sections 3.1. to 3.4. below analyse the four existing pathways. Each section describes the current approach in the *Aquatic Code*, evaluates that approach, and provides recommendations for improvement. Discussion points are also provided for consideration by Member Countries.

[2] *Early detection system* is a defined term in the OIE *Aquatic Code* which means: an efficient system for ensuring the rapid recognition of signs that are suspicious of a listed disease, or an emerging disease situation, or unexplained mortality, in aquatic animals in an aquaculture establishment or in the wild, and the rapid communication of the event to the Competent Authority, with the aim of activating diagnostic investigation by the Aquatic Animal Health Services with minimal delay. Such a system will include the following characteristics:
- broad awareness, e.g. among the personnel employed at aquaculture establishments or involved in processing, of the characteristic signs of the listed diseases and emerging diseases;
- veterinarians or aquatic animal health professionals trained in recognising and reporting suspicions of disease occurrence;
- ability of the Aquatic Animal Health Services to undertake rapid and effective disease investigation based on a national chain of command;
- access by the Aquatic Animal Health Services to laboratories with the facilities for diagnosing and differentiating listed diseases and emerging diseases;
- the legal obligation of private veterinarians or aquatic animal health professionals to report suspicions of disease occurrence to the Competent Authority.

Annex 13 (contd)

3.1. PATHWAY 1 - ABSENCE OF SUSCEPTIBLE SPECIES

Current situation in the Aquatic Code:

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

This pathway is not currently available for some species that have a broad host range (e.g. infection with viral haemorrhagic septicaemia virus, infection with *Aphanomyces invadans*; refer Appendix 1).

Evaluation:

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

The only circumstance where this pathway may be of practical application is where a country may wish to self-declare freedom prior to introducing a new species (susceptible to the disease in question) for aquaculture purposes in accordance with Article X.X.8. of each disease-specific chapter. Under these circumstances, the *basic biosecurity conditions* would need to be in place for a period of time prior to the introduction of the susceptible species, sufficient to ensure that i) no pathogenic agent introduced via aquatic animal commodities remained present in the environment, ii) the pathogenic agent *early detection system* was properly established.

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

Recommended approach:

Consideration should be given to whether this pathway should be retained within the *Aquatic Code* for the only apparent application: where new species may be introduced into a country for the first time.

If Pathway 1 were to be retained in the *Aquatic Code*, criteria would be required to determine:

– when the pathway may not be suitable for a particular disease (e.g. due to uncertainty about host range); and

– the required period for *basic biosecurity conditions* for a country, zone or compartment.

Guidance would also be necessary on the strength of evidence required to determine that susceptible species are absent from a country, zone or compartment, as relevant.

Discussion points:
1. Is Pathway 1 likely to be used by Member Countries?
2. What is the appropriate standard of evidence that susceptible species are absent from a country?

3.2. PATHWAY 2 - HISTORICAL FREEDOM

Current situation in the Aquatic Code:

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

– there has not been an observed occurrence of the disease for at least the last ten years (the period may be longer for some diseases; refer to Appendix 1); and

– *basic biosecurity conditions* have been in place for a specified period.

Evaluation:

This pathway should only be available for diseases where sufficient confidence can be demonstrated that passive surveillance, as a part of a country's early detection system, would detect the disease if it were to occur. Importantly, the disease would need to manifest clinically, be observed, reported and investigated as part of the country's *early detection system*. If diseases are not expected to manifest clinically (e.g. ISAV HPR0), this pathway would not be appropriate.

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

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

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

Recommended approach:

Requirements for passive surveillance:

It is proposed that, in accordance with the provisions of the relevant disease-specific chapter of the *Aquatic Code*, a country or zone could be declared free from a disease on the basis of historical freedom. The evidence for historical freedom is passive surveillance data generated by a country's *early detection system* that should meet the following conditions.

- the disease has never been reported in the country, including in wild aquatic animal populations;

- the country has *basic biosecurity conditions* in place including an *early detection system* that is sufficiently sensitive to detect the disease should it occur and the following conditions (additional to the requirements of an *early detection system*) are met:

- conditions within the country (biotic and abiotic) are conducive to clinical expression of the disease such that if the pathogenic agent were present it would produce identifiable clinical signs in populations of susceptible animals;
- there must be an awareness of the clinical signs of the disease by potential observers;
- for populations of susceptible farmed aquatic animals they must be under sufficient observation such that, if clinical signs of the disease were to occur, they would be observed;
- for populations of susceptible wild aquatic animals, they must:
  - be under sufficient observation such that if clinical signs of the disease were to occur they would be observed, or
  - be epidemiologically linked to farmed populations such that the disease would occur and be observed in farmed populations if it were to occur in wild aquatic animal populations;
- there must be access to sufficient diagnostic capability to confirm or exclude cases of the disease.

Need for active surveillance:

If passive surveillance for some susceptible aquatic animal populations would not meet the requirements for passive surveillance specified above (e.g. for wild populations), it is proposed that active surveillance could be used to provide additional evidence of freedom for those identified populations.

Requirements for basic biosecurity conditions:

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

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

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

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

The default period of passive surveillance required to make a self-declaration of freedom for all diseases in the *Aquatic Code* will be ten years. This period is the minimum required to achieve 95% likelihood of detection if the annual likelihood of detection is 30% (see Box 1 for details of the method used). The minimum period of passive surveillance required to make a self-declaration of freedom for all diseases in the *Aquatic Code* will be five years. This period is the minimum required to achieve 95% likelihood of detection if the annual likelihood of detection is 50%. If, following consideration of the factors affecting the sensitivity of passive surveillance (provided above), the annual likelihood of detection is considered to be greater than 30%, the minimum period required for *basic biosecurity conditions* (including passive surveillance) defined in the relevant disease-specific chapter of the *Aquatic Code* will be set to a period between five and ten years, as appropriate (see Table 1 below).

A country making a self-declaration of freedom on the basis of historical freedom will need to provide an explanation of how the criteria (i.e. the basic biosecurity conditions) presented for this pathway have been met. Further, if the likelihood of detection is considered to be lower than 30% due to a country's circumstances (e.g. nature of the early detection system, environmental conditions, nature of the aquaculture industry), this pathway will not be available and an alternative pathway that also utilises active surveillance data (in whole or in part) will be required.

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

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

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

Discussion points:
3. Are the requirements for passive surveillance in farmed and wild aquatic animals appropriate?
4. Should historic freedom require that the disease has never been detected (as proposed) or is a period of freedom (e.g. ten years) sufficient?
5. Are the factors for determining the required period of basic biosecurity conditions for listed diseases appropriate?

3.3. PATHWAY 3 – UNKNOWN DISEASE STATUS

Current situation in the Aquatic Code:

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

– *basic biosecurity conditions* have been continuously met for certain period; and

– targeted surveillance, as described in Chapter 1.4., has been in place for a certain period without detection of *infection* with the relevant *pathogenic agent*.

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

a) *basic biosecurity conditions* are in place and effectively enforced;

b) no vaccination against the disease has been carried out unless otherwise provided in the *Aquatic Code*;

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

Evaluation:

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

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

The period required for *basic biosecurity conditions* to be in place should be at least as long as that required for surveillance so that effective controls are in place to prevent disease introduction from the time surveillance commences. However, *basic biosecurity conditions* may be necessary for a period prior to the commencement of surveillance sufficient to ensure that, if the disease had been introduced immediately prior to the implementation of measures to prevent introduction, that it would be detected.

Recommended approach:

Requirements for basic biosecurity conditions:

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

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

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

Requirements for active surveillance:

Active surveillance surveys should commence after a period of time following the implementation of *basic biosecurity conditions* that ensures the infection has reached design prevalence should it have been previously introduced (see section above).

The requirements for active surveillance will depend on epidemiology of the disease, the biology of susceptible species and the nature of production practices and systems. In general, the same criteria proposed above for *basic biosecurity conditions* should also be considered for setting the period required for active surveillance.

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

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

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

In the absence of disease-specific information to aid the development of a surveillance system, declaration of disease freedom should follow at least two surveys per year (for at least two consecutive years) to be conducted three or more months apart, on the appropriate species, at the appropriate life stage and at times of the year when temperature and season offer the best opportunity to detect the pathogenic agent. Surveys should be designed to provide an overall 95% confidence or greater and with a design prevalence at the animal and higher levels of aggregation (i.e. pond, farm, village, etc.) of 2% or lower (this value may be different for different diseases and may be provided in the disease-specific chapter in the *Aquatic Manual*).

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

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

Discussion points:
6. Are the proposed criteria for determining the periods for basic biosecurity conditions for this pathway appropriate?
7. Is one year an appropriate minimum period for *basic biosecurity conditions* to be in place prior to the commencement of active surveillance for declaring freedom for countries or zones?
8. Is one survey per year (at least three months apart) for two years an appropriate default requirement?

3.4. PATHWAY 4 – RETURNING TO FREEDOM

Current situation in the Aquatic Code:

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

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

b) infected populations have been destroyed or removed from the *infected zone* by means that minimise the *risk* of further spread of the *disease*, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; and

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

d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of the relevant *pathogenic agent.*

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

Evaluation:

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

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

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

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

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

INFECTED ZONE means a zone in which a disease has been diagnosed.

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

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

Recommended approach:

Requirements for active surveillance:

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

– Farms which had been infected.

– Farms and wild populations at greatest risk of exposure to infection during the outbreak, i.e. in close proximity, with other epidemiological contacts such as equipment or aquatic animals.

– Wild populations of susceptible species downstream or in the immediate vicinity of previously infected farms must be included.

The criteria used in Section 3.3. above would be used to determine the frequency and duration of surveillance. Thus, the minimum duration of a survey should reflect the stability of the parameters being assessed. For many pathogenic agents the likelihood of detection is greatest for a particular life stage or during periods of the year when water temperature is permissive for clinical expression. Surveys should be tailored to fit these temporal windows and this means that suitable sampling periods might occur only once or twice a year.

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

Discussion points:
9. Should countries and zones be able to return to freedom more quickly following an eradication programme than in an initial *self-declaration of freedom* for a country or zone (if appropriate criteria are met)?
10. Should compartments be able to regain freedom immediately after destocking and successful decontamination (i.e. with surveillance at the level required to maintain freedom) if *basic biosecurity conditions* have been reviewed and modified and restocking is with disease free animals (e.g. from a free country zone or compartment)?
11. When should the starting time point be for surveillance – e.g. commencement of sampling or at the conclusion of sampling for the first survey with negative results?
12. Should Chapter 1.4. provide clearer guidance on establishing infected and protection zones (perhaps in the proposed new chapter on emergency response) and sampling within them (for farmed and wild animals)?

4. Maintaining freedom

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

Current situation in the Aquatic Code:

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

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

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

Evaluation:

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

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

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

Recommended approach:

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

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

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

Discussion points:
13. Do Member Countries require additional guidance on what constitute 'conditions conducive to clinical expression'?
14. Do Member Countries require additional guidance on how to evaluate or test their 'early detection system'?

5. Revisions that may be required to Chapter 1.4.

Any revisions to Chapter 1.4. of the *Aquatic Code* would be dependent on consideration of this discussion paper by Member Countries and further development of the proposals within it. However, the following items are provided as indications of the types of revisions to Chapter 1.4. that may be necessary should the recommendations of this discussion paper be supported.

- Include the proposed criteria (included in this document) relevant to setting periods in the disease-specific chapters required for *basic biosecurity conditions* and for periods of surveillance.
- Revise the proposed pathways for claiming or reclaiming freedom.
- Include provision for flexibility to use different forms of surveillance data under each pathway rather than having a rigid requirement for one data type (e.g. active or passive surveillance).
- Include guidance on regaining freedom following eradication of a disease at the level of country, zone or compartment (not currently included); including guidance on a risk-based approach to survey design.
- Provide guidance on defining the boundaries of infection and protection zones.

- Include guidance on what constitutes an 'early detection system': which may include i) awareness by farmers of the obligations to report (disease, unexplained mortality), ii) field service capable of investigating outbreaks, iii) lab capacity, iv) register of farms/information on distribution and type of aquaculture
- Include guidance on how an 'early detection system' may be evaluated.
- Include guidance on what constitutes 'conditions conducive to clinical expression'.

6. Requirements for making a self-declaration of freedom

Current situation in the Aquatic Code:

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

There is currently no guidance within the *Aquatic Code* on the structure and contents of a self-declaration of freedom; however, the OIE has developed a procedure for the publication by the OIE of a self-declaration of freedom[3]. This document also includes information on the evidence that should be included in a self-declaration of freedom.

Evaluation:

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

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

Recommended approach:

More explicit guidance on self-declarations of freedom could be included either in Chapter 1.4. or in a separate new chapter of the *Aquatic Code*.

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

7. Discussion

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

[3] http://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/Self-declarations/EN_Procedure_self_declaration.pdf

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

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

In this paper we question whether a separate pathway based on the absence of susceptible species is justified. Firstly, this pathway is likely to be very little used. Secondly, Member Countries without susceptible species but wishing to import them (e.g. to establish a new aquaculture industry), could make a case for freedom through the historical freedom pathway.

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

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

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

Member Countries are invited to comment on this paper and, for convenience, several discussion points have been provided throughout the text to assist Member Countries to frame their responses. The discussion points are summarised in Table 3 below. In formulating responses to these discussion points it is advisable that Member Countries refer to the relevant sections of the document for additional context.

Annex 13 (contd)

Table 3. Summary of discussion points for comment by Member Countries. Refer to relevant sections of the document for additional context.

Section 3.1. Pathway 1. Absence of susceptible species
1. Is Pathway 1 likely to be used by Member Countries?
2. What is an appropriate standard of evidence that susceptible species are absent from a country?
Section 3.2. Pathway 2. Historical freedom
3. Are the proposed requirements for passive surveillance in farmed and wild aquatic animals appropriate?
4. Should historic freedom require that the disease has never been detected (as proposed) or is a period of freedom (e.g. ten years) sufficient?
5. Are the factors for determining the required period of basic biosecurity conditions for listed diseases appropriate?
Section 3.3. Pathway 3. Unknown disease status
6. Are the proposed criteria for determining the periods for basic biosecurity conditions for this pathway appropriate?
7. Is one year an appropriate minimum period for *basic biosecurity conditions* to be in place prior to the commencement of active surveillance for declaring freedom for countries or zones?
8. Is one survey per year (at least three months apart) for two years an appropriate default requirement?
Section 3.4. Pathway 4. Returning to freedom
9. Should countries and zones be able to return to freedom more quickly following an eradication programme than in an initial *self-declaration of freedom* for a country or zone (if appropriate criteria are met)?
10. Should compartments be able to regain freedom immediately after destocking and successful decontamination (i.e. with surveillance at the level required to maintain freedom) if *basic biosecurity conditions* have been reviewed and modified and restocking is with disease free animals (e.g. from a free country, zone or compartment)?
11. When should the starting time point be for surveillance – e.g. commencement of sampling or at the conclusion of sampling for the first survey with negative results?
12. Should Chapter 1.4. provide clearer guidance on establishing infected and protection zones (perhaps in the proposed new chapter on emergency response) and sampling within them (for farmed and wild animals)?
Section 4. Maintaining freedom
13. Do Member Countries require additional guidance on what constitute 'conditions conducive to clinical expression'?
14. Do Member Countries require additional guidance on how to evaluate or test their 'early detection system'?
Section 6. Requirements for making a self-declaration of freedom
15. Is the OIE procedure for the publication of a self-declaration of freedom sufficient guidance for Member Countries for making self-declarations of freedom? If not, should a separate chapter be provided within the *Aquatic Code*?

APPENDIX 1.

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

Article 9.1.4.

Country free from crayfish plague

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

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

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

OR

2) any of the *susceptible species* referred to in Article 9.1.2. are present and the following conditions have been met:

   a) there has been no observed occurrence of the *disease* for at least the last 25 years despite conditions that are conducive to its clinical expression (as described in the corresponding chapter of the *Aquatic Manual*); and

   b) *basic biosecurity conditions* have been continuously met for at least the last 10 years;

OR

3) the disease status prior to *targeted surveillance* is unknown but the following conditions have been met:

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

   b) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last five years without detection of crayfish plague;

OR

4) it previously made a *self-declaration of freedom* from crayfish plague and subsequently lost its *disease* free status due to the detection of crayfish plague but the following conditions have been met:

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

   b) infected populations have been destroyed or removed from the *infected zone* by means that minimise the *risk* of further spread of the *disease*, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; and

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

   d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last five years without detection of crayfish plague.

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

Article 9.1.5.

Zone or compartment free from crayfish plague

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

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

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

OR

2) any of the *susceptible species* referred to in Article 9.1.2. are present in the *zone* or *compartment* and the following conditions have been met:

   a) there has not been any observed occurrence of the *disease* for at least the last 25 years despite conditions that are conducive to its clinical expression (as described in the corresponding chapter of the *Aquatic Manual*); and

   b) *basic biosecurity conditions* have been continuously met for at least the last 10 years;

OR

3) the disease status prior to *targeted surveillance* is unknown but the following conditions have been met:

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

   b) *targeted surveillance*, as described in Chapter 1.4., has been in place, in the *zone* or *compartment*, for at least the last five years without detection of crayfish plague;

OR

4) it previously made a *self-declaration of freedom* for a *zone* from crayfish plague and subsequently lost its *disease* free status due to the detection of crayfish plague in the *zone* but the following conditions have been met:

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

   b) infected populations have been destroyed or removed from the *infected zone* by means that minimise the *risk* of further spread of the *disease*, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; and

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

   d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last five years without detection of crayfish plague.

Annex 13 (contd)

APPENDIX 2. Summary of the periods required for basic biosecurity conditions and active surveillance in Article X.X.4. in each disease-specific chapter of the *Aquatic Code*.

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

CHAPTER 2.2.9.

INFECTION WITH YELLOW HEAD VIRUS GENOTYPE 1

1. Scope

Infection with yellow head virus genotype 1 means infection with the pathogenic agent yellow head virus genotype 1 (YHV1) of the Genus *Okavirus*, Family *Roniviridae* and Order *Nidovirales*.

[…]

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing a species as susceptible to infection with YHV1 according to Chapter 1.5 of *Aquatic Animal Health Code* (*Aquatic Code*) include: Blue shrimp (*Penaeus stylirostris*), dagger blade grass shrimp (*Palaemonetes pugio*), giant tiger prawn (*Penaeus monodon*), grass shrimp (*Palaemonetes pugio*), jinga shrimp (*Metapenaeus affinis*) and whiteleg shrimp (*Penaeus vannamei*).~~giant tiger prawn (*P. monodon*), white leg shrimp (*P. vannamei*), blue shrimp (*P. stylirostris*), daggerblade grass shrimp (*Palaemonetes pugio*), and Jinga shrimp (*Metapenaeus affinis*).~~

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence ~~to fulfil the criteria for listing a species as~~ for susceptibility ~~susceptible to infection with YHV1~~ according to Chapter 1.5 of the *Aquatic Code* include: Banana prawn (*Penaeus merguiensis*), Carpenter prawn (*Palaemon serrifer*), kuruma prawn (*Penaeus japonicus*), northern brown shrimp (*Penaeus aztecus*), northern pink shrimp (*Penaeus duorarum*), northern white shrimp (*Penaeus setiferus*), Pacific blue prawn (*Palaemon styliferus*), red claw crayfish (*Cherax quadricarinatus*), Sunda river prawn (*Macrobrachium sintangense*) and yellow shrimp (*Metapenaeus brevicornis*).~~ Sunda river prawn (*Macrobrachium sintangense*), yellow shrimp (*Metapenaeus brevicornis*), Carpenter prawn (*Palaemon serrifer*), Pacific blue prawn (*Palaemon styliferus*), northern brown shrimp (*Penaeus aztecus*), northern pink shrimp (*Penaeus duorarum*), kuruma prawn (*Penaeus japonicus*), banana prawn (*Penaeus merguiensis*), northern white shrimp (*Penaeus setiferus*) and red claw crayfish (*Cherax quadricarinatus*). Evidence is lacking for these species to either confirm that the identity of the pathogenic agent is YHV1, transmission mimics natural pathways of infection, or presence of the pathogenic agent constitutes an infection.~~

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following species, but an active infection has not been demonstrated: Acorn barnacle (*Chelonibia patula*), blue crab (*Callinectes sapidus*), cyclopoid copepod (*Ergasilus manicatus*), gooseneck barnacle (*Octolasmis muelleri*), Gulf killifish (*Fundulus grandis*) and paste shrimp (*Acetes* sp.).

[…]

CHAPTER 2.3.6.

INFECTION WITH SALMONID ALPHAVIRUS

1. Scope

~~For the purpose of this chapter, i~~Infection with salmonid alphavirus (SAV) means infection with any ~~subtype~~ genotype of the pathogenic agent SAV, ~~of the~~ Genus *Alphavirus*, and Family *Togaviridae.*

~~Infection with SAV may cause pancreas disease (PD) or sleeping disease (SD) in Atlantic salmon (*Salmo salar* L.), rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta* L.) (Boucher *et al.*, 1995; McLoughlin & Graham, 2007). The virus is horizontally transmitted, and the main reservoirs of SAV are clinically diseased or covertly infected fish (Viljugrein *et al.*, 2009). The disease is a systemic disease characterised microscopically by necrosis and loss of exocrine pancreatic tissue, and heart and skeletal muscle changes. The mortality varies significantly, from negligible to over 50% in severe cases, and up to 15% of surviving fish will develop into long, slender fish ('runts') (McLoughlin & Graham, 2007).~~

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent, agent strains

SAV is an enveloped, spherical, single-stranded, positive-sense RNA virus, approximately 60-70 nm in diameter, with a genome of ~12 kb. The genome codes for eight proteins: four capsid glycoproteins (E1, E2, E3 and 6K) and four nonstructural proteins (nsP1–4). Glycoprotein E2 is considered to be the site of most neutralising epitopes, while E1 contains more conserved, cross-reactive epitopes (McLoughlin & Graham, 2007). SAV is considered to belong to the genus *Alphavirus* of the family *Togaviridae*. This is based on nucleotide sequence studies of SAV isolates, and is also supported by biological properties of the virus, including cross-infection and neutralisation trials. In addition, four conserved nucleotide sequence elements (CSEs) and a conserved motif (GDD), characteristic of alphaviruses, are present in the SAV genome (McLoughlin & Graham, 2007).

SAV has been divided into six genotypes (SAV1–SAV6) based solely on nucleic acid sequences for the proteins E2 and nsP3 (Fringuelli *et al.*, 2008). The level of antigenic variation among genotypes is considered low as monoclonal antibodies (MAbs) raised against a specific SAV genotype are likely to cross react with other SAV isolates (Graham *et al.*, 2014; Jewhurst *et al.*, 2004).

Infection with SAV may cause pancreas disease (PD) or sleeping disease (SD) in Atlantic salmon (*Salmo salar* L.), common dab (*Limanda limanda*) and rainbow trout (*Oncorhynchus mykiss*) McLoughlin & Graham, 2007). The disease is a systemic disease characterised microscopically by necrosis and loss of exocrine pancreatic tissue, and heart and skeletal muscle changes.

The genotype groups and their geographical distributions are presented in the table below (abbreviations: SW = sea water, FW = fresh water, PD = pancreas disease, SD = sleeping disease):

Table 2.1 SAV genotypes by host, environment and geographic distribution

SAV ~~subtype~~ genotype	Host and environment	Country
SAV 1 (PD)	Atlantic salmon (SW) Rainbow trout (FW)	Ireland, UK (Northern Ireland, Scotland)
SAV 2 FW (SD)	Rainbow trout (FW) ~~Atlantic salmon (SW)~~ Atlantic salmon (FW) Arctic charr (FW)	France, Germany, Italy, Spain, Switzerland, Poland, UK (England, Scotland) Scotland Austria
SAV 2 Marine (PD)	Atlantic salmon (SW)	Norway, UK (Scotland)
SAV 3 (PD)	Rainbow trout (SW) Atlantic salmon (SW)	Norway

SAV ~~subtype~~ genotype	Host and environment	Country
SAV 4 (PD)	Atlantic salmon (SW)	Ireland, UK (Northern Ireland, Scotland)
SAV 5 (PD)	Atlantic salmon (SW) Common dab (SW)	UK (Scotland) UK (Scotland), Ireland
SAV 6 (PD)	Atlantic salmon (SW)	Ireland

2.1.2. Survival outside the host

Laboratory tests suggest that SAV would survive for extended periods in the aquatic environment. In these tests, virus survival was inversely related to temperature. In the presence of organic matter, marked longer survival times were observed in sea water compared with fresh water (Graham *et al.,* 2007c). SAV has been detected in fat leaking from dead fish, indicating that this may be a route for transmission. Fat droplets may accumulate at the sea water surface, contributing to long distance spread (Stene *et al.,* submitted).

The half-life of SAV in serum has been found to be inversely related to temperature, emphasising the need for rapid shipment of samples at 4°C to laboratories for virus isolation. For long-term conservation of SAV-positive samples and cultured virus, storage at –80°C is recommended (Graham *et al.,* 2007c).

2.1.3. Stability of the agent

SAV is rapidly inactivated in the presence of high levels of organic matter at 60°C, at pH 7.2, and at pH 4 and pH 12 at 4°C, suggesting that composting, ensiling and alkaline hydrolysis would all be effective at inactivating virus in fish waste (Graham *et al.,* 2007a).

2.1.4. Life cycle

Probable infection routes are through the gills or via the intestine. In the acute stages of the disease, large amounts of SAV can be detected and live virus can be isolated from the heart, kidney, blood and several other organs, but the actual target cells for the virus has not yet been identified.

Viraemia precedes both the onset of histological changes and clinical signs (McLoughlin & Graham, 2007). The route of shedding may be through natural excretions/secretions, supported by the detection of SAV by reverse-transcriptase polymerase chain reaction (RT-PCR) in the faeces and mucous of experimentally infected Atlantic salmon. These matrices may therefore play a role in the horizontal transmission of SAV through water (Graham *et al.,* 2012). Virus has been detected in water 4–13 days ~~after~~ post-infection, indicating that virus shedding coincides with the viraemic stage (Andersen *et al.,* 2010). An incubation period of 7–10 days at sea water temperatures of 12–15°C has been estimated based on analysis of antibody production in intraperitoneally infected fish and cohabitants in an experimental trial (McLoughlin & Graham, 2007). Several studies have shown that SAV RNA can be detected in fish for an extended period post-infection (Jansen *et al.,* 2010a; McLoughlin & Graham, 2007). Subclinical infection has been reported, suggesting that the severity of an outbreak may be influenced by several environmental factors (McLoughlin & Graham, 2007), and ~~recent data show that~~ seasonal increases in water temperature may trigger disease outbreaks ~~in SAV infected farms~~ (Stene *et al.,* 2014).

2.2. Host factors

2.2.1. Susceptible host species

~~Disease outbreaks and infection experiments have shown that Atlantic salmon, rainbow trout and brown trout are susceptible (Boucher *et al.,* 1995; McLoughlin & Graham, 2007).~~

Species that fulfil the criteria for listing a species as susceptible to infection with SAV according to Chapter 1.5. of the Aquatic Animal Health Code (Aquatic Code) include: Arctic charr (*Salvelinus alpinus*), Atlantic salmon (*Salmo salar*), common dab (*Limanda limanda*) and rainbow trout (*Oncorhynchus mykiss*).

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence for susceptibility according to Chapter 1.5. of the *Aquatic Code* include: long rough dab (*Hippoglossoides platessoides*) and plaice (*Pleuronectes platessa*).

In addition, pathogen-specific positive PCR results have been reported in the following ~~organisms~~ species, but an active infection has not been demonstrated: Argentine hake (*Merluccius hubbsi*), ~~b~~Ballan wrasse (*Labrus bergylta*), brown trout (*Salmo trutta*), cod (*Gadus morhua*), European flounder (*Platichthys flesus*), haddock (*Melanogrammus aeglefinus*), herring (*Glupea harengus*), Norway pout (*Trisopterus esmarkii)*, saithe (*Pollachius virens*), ~~salmon louse (*Lepeophtheirus salmonis*),~~ sculpin sp. (*Myoxocephalus octodecemspinosus*) and whiting (*Merlangius merlangus*).

2.2.~~2~~3. Susceptible stages of the host

All life stages should be considered as susceptible to infection with SAV.

Farmed rainbow trout in fresh water are affected at all stages of production (Kerbart Boscher *et al.,* 2006). Experience from Norway shows that farmed rainbow trout and Atlantic salmon are susceptible at all stages in sea water, probably reflecting a sea water reservoir of SAV. Experimental infection by injection indicates susceptibility of Atlantic salmon parr in fresh water (McVicar, 1990).

2.2.~~3~~4. Species or subpopulation predilection (probability of detection)

There is no known species or subpopulation predilection.

2.2.~~4~~5. Target organs and infected tissue

Infection with SAV is a systemic disease with an early viraemic phase. After infection, SAV has been detected in all organs that have been examined: brain, gill, pseudobranch, heart, pancreas, kidney and skeletal muscle (Andersen *et al.,* 2007; McLoughlin & Graham, 2007) as well as in mucous and faeces (Graham *et al.,* 2012).

2.2.~~5~~6. Persistent infection with lifelong carriers

SAV has been detected in surviving fish 6 months after experimental infection (Andersen *et al.,* 2007). At the farm level, an infected population will harbour SAV until slaughter (Jansen *et al.,* 2010a; 2010b). On an individual level, however, lifelong persistent infection has not been documented.

2.2.~~6~~7. Vectors

SAV has been detected by RT-PCR in salmon lice (*Lepeophtheirus salmonis*) collected during acute disease outbreaks in Atlantic salmon, but transfer to susceptible fish species has not been studied (Petterson *et al.,* 2009). Vectors are not needed for transmission of SAV.

2.2.~~7~~8. Known or ~~S~~suspected wild aquatic animal carriers

In surveys of wild marine fish, SAV RNA has been detected in the flatfish species common dab (*Limanda limanda*), long rough dab (*Hippoglossoides platessoides*) and plaice (*Pleuronectes platessa*) (McCleary *et al.,* 2014; Snow *et al.,* 2010). The importance of wild marine or fresh water species as ~~virus~~ carriers needs to be determined ~~clarified~~.

2.3. Disease pattern

2.3.1. Transmission mechanisms

Transmission of SAV occurs horizontally. This is supported by phylogenetic studies, successful transmission among fish in cohabitant studies, proven transmission between farming sites, studies on survival of SAV in sea water and the spread via water currents (Graham *et al.,* 2007c; 2011; Jansen *et al.,* 2010a; Kristoffersen *et al.,* 2009; Viljugrein *et al.,* 2009).

Long-distance transmission and thus introduction of SAV in a previously uninfected area is most likely assigned to movement of infected live fish (Kristoffersen *et al.,* 2009; Rodger & Mitchell, 2007). Once SAV has been introduced into an area, ~~shared ownership and close site~~ farm proximity and water currents are factors involved in local transmission (Aldrin *et al.,* 2010; Kristoffersen *et al.,* 2009; Viljugrein *et al.,* 2009). Risk factors for outbreaks on a farming site include a previous history of infection with SAV, high feeding rate, high sea lice burden, the use of autumn smolts and previous outbreak of infectious pancreatic necrosis (IPN) (Bang Jensen *et al.,* 2012; Kristoffersen *et al.,* 2009; Rodger & Mitchell, 2007).

Vertical transmission of SAV has been suggested (Bratland & Nylund, 2009), but the evidence is not convincing (Kongtorp *et al.,* 2010; McLoughlin & Graham, 2007). The Norwegian Scientific Committee for Food Safety ~~has recently~~ carried out a risk assessment ~~on brood fish surveillance~~ and ~~vertical transmission of infection,~~ concluded that the risk of vertical transmission of SAV is negligible.

2.3.2. Prevalence

The prevalence of ~~infected fish within an~~ infection with SAV ~~infected fish farm~~ may vary. During disease outbreaks, the prevalence is usually high; prevalences of 70–100% have been reported in Atlantic salmon farming sites (Graham *et al.,* 2010). If moribund or thin fish or runts are sampled, the probability of detecting SAV ~~infected fish~~ is higher than if randomly selected, apparently healthy fish are sampled (Jansen *et al.,* 2010b). Prevalence estimates will also vary with the diagnostic method used.

Prevalence in wild fish is largely unknown. SAV RNA has been detected in some flatfish species in sea water in Scotland (Snow *et al.,* 2010). A serological survey of wild salmonids in fresh water river systems in Northern Ireland did not detect virus neutralisation antibodies against SAV in any of 188 sera tested, whereas the majority of sera from farmed salmon in sea water in the same area tested positive (Graham *et al.,* 2003).

2.3.3. Geographical distribution

Infection with SAV is known to be present in farmed salmonid fish in Croatia, France, Germany, Ireland, Italy, Norway, Poland, Spain, Switzerland and the United Kingdom (England, Scotland and Northern Ireland).

2.3.4. Mortality and morbidity

Mortality rates due to infection with SAV may vary with genotype ~~subtype~~, season, year, use of biosecurity measures and species of fish (Bang Jensen *et al.,* 2012; Graham *et al.,* 2011; Rodger & Mitchell, 2007; Stormoen *et al.,* 2013). The cumulative mortality at the farm level ranges from negligible to over 50% in severe cases (Bang Jensen *et al.,* 2012; Graham *et al.,* 2003; Rodger & Mitchell, 2007; Ruane *et al.,* 2008; Stene *et al.,* 2014).

Duration of disease outbreaks, defined as the period with increased mortality, varies from 1 to 32 weeks (Jansen *et al.* 2010a; 2014; Ruane *et al.,* 2008).

2.3.5. Environmental factors

Clinical outbreaks and mortality are influenced by water temperature and season (McLoughlin & Graham, 2007; Rodger & Mitchell, 2007; Stene *et al.,* 2014; Stormoen *et al.,* 2013). Stressing the fish by movement, crowding or treatment may initiate disease outbreaks on infected farms.

2.4. Control and prevention

2.4.1. Vaccination

At present, one vaccine is commercially available. This vaccine was introduced in 2007 and is widely used in Atlantic salmon farms in endemic areas in Norway, Ireland and Scotland. This vaccine is based on inactivated SAV genotype ~~subtype~~ 1, and claims a reduction in mortality of at least 50% in comparisons of vaccinated fish against unvaccinated fish at the same farm. The vaccine does not seem to offer complete protection, but a field evaluation carried out in Norway demonstrated that the mortality in farms with vaccinated fish is comparable with mortality in farms without infection with SAV. Furthermore, a small reduction in the number of outbreaks was seen (Bang Jensen *et al.,* 2012).

A vaccine based on inactivated SAV of another genotype ~~subtype~~ is under development. Furthermore, a DNA-based vaccine is showing promising results. To date, only Canada has allowed the use of DNA-based vaccines for control of fish diseases; it is not certain whether this vaccine will be licensed for use in other markets.

2.4.2. Chemotherapy

No chemotherapy is available.

2.4.3. Immunostimulation

No immunostimulation is available.

2.4.4. Resistance breeding

Differences in susceptibility among different family groups of Atlantic salmon have been observed in challenge experiments and in the field, indicating the potential for resistance breeding. Both in Ireland and Norway, efforts are being made to breed fish that are more resistant to infection with SAV (McLoughlin & Graham, 2007). Selection of brood fish by using gene markers for resistance is in an early phase.

2.4.5. Restocking with resistant species

Not relevant.

2.4.6. Blocking agents

Not relevant.

2.4.7. Disinfection of eggs and larvae

Disinfection procedures were evaluated in fertilised ova from SAV3 positive broodstock (Kongtorp *et al.,* 2010). Nevertheless, further investigation is needed. ~~(See Graham *et al.,* 2007b; Kongtorp *et al.,* 2010.)~~

2.4.8. General husbandry practices

To avoid infection with SAV, general good hygiene practices should be applied: use of appropriate sites for farming, segregation of generations, stocking with good quality fish, removal of dead fish, regular cleaning of tanks and pens, controlling parasites and other pathogens as well as careful handling of fish. Once a site has been infected, mortality may be reduced by imposing a general stop on handling of the fish as well as a general stop on feeding the fish.

3. Sampling

3.1. Selection of individual specimens

All production units (ponds, tanks, net-cages, etc.) should be inspected for the presence of dead, weak or abnormally behaving fish. Extremely weak ('sleeping') fish may be found at the bottom of a tank or in the net-cages. If the number of clinically diseased fish is low, samples from long, thin fish ('runts') may be added (Jansen *et al.,* 2010b).

3.2. Preservation of samples for submission

Table 3.1. Preservative used for each method

Method	**Preservative**
Histology and immunohistochemistry	Fixation in neutral phosphate-buffered 10% formalin
Molecular biology (RT-PCR and sequencing)	Appropriate medium for preservation of RNA
Cell culture	Virus transport medium
Serology	Blood plasma or serum

3.3. Pooling of samples

~~For diagnostic purposes, pooling of samples from different individuals is not considered necessary or recommended as detection of SAV and characteristic histopathological changes in the same individual will strengthen the connection between the virus and the observed disease. For surveillance purposes, pooling of samples for virological examination (PCR or cell culture) may be accepted, but may decrease the sensitivity of the tests.~~

Pooling of samples may be acceptable, however, the impact on sensitivity and design prevalence must be considered.

3.4. Best organs or tissues

Heart and mid-kidney are the recommended organs for detection of SAV either by molecular biological methods or by cell culture. During the course of the disease, the heart usually contains more SAV than other tissues and should always be sampled. After disease outbreaks, gills and heart (Graham *et al.,* 2010) and pools of heart and mid-kidney (Jansen *et al.,* 2010a; 2010b) remained PCR positive for months after initial detection.

During the initial viraemic phase, serum samples are also suitable for detection of SAV either by molecular biological methods or by cell culture. Serum sampling may therefore be used for early warning screening tests (Graham *et al*., 2010). From approximately 3 weeks after SAV infection, blood serum or plasma is suitable for a virus neutralisation test that identifies neutralising antibodies against SAV in fish exposed to SAV (Graham *et al.,* 2003).

Tissues for histological examinations should include gill, heart, pyloric caeca with attached pancreatic tissue, liver, kidney, spleen and skeletal muscle containing both red (aerobe) and white (anaerobe) muscle. Skin with associated skeletal muscle sample should be taken at the lateral line level and deep enough to include both red and white muscle.

4. Diagnostic methods

4.1. Field diagnostic methods

4.1.1. Clinical signs

A sudden drop in appetite may be observed 1–2 weeks before the detection of ~~enhanced~~ elevated mortality. Clinically diseased fish may be observed swimming slowly at the water surface. In some cases, extremely weak (“sleeping”) fish can be found at the bottom of tanks or in net-cages. An increased number of faecal casts may also be observed ~~in the water~~. However, it is important to ~~notice~~ note that clinical signs are not pathognomonic, ~~and that careful observation and examinations~~ Careful investigation of any dead, ~~weak~~ moribund or abnormally behaving fish is necessary to determine involvement of SAV and rule out other *pathogenic agents*.

Initially, nutritional status is usually normal, but in the months after an outbreak or in the later stages of disease, long slender fish (‘runts’) with ~~low~~ poor body condition are typically observed. The ~~development~~ presentation of long, slender fish can be caused by factors other than SAV.

4.2. Clinical methods

4.2.1. Gross pathology

Yellow mucoid gut contents are a usual post-mortem finding, as is typically seen in fish that are not eating. Occasionally signs of circulatory disturbances, such as petechial haemorrhages, small ascites or reddening of the pancreatic region between the pyloric caeca, may be seen. Some diseased fish may show pale hearts or heart ruptures. It is important to note that post-mortem findings are not pathognomonic.

4.2.2. Clinical chemistry

Not documented for diagnostic use.

4.2.3. Microscopic pathology

The changes most commonly found in clinically diseased fish are severe loss of exocrine pancreatic tissue, cardiomyocytic necrosis and inflammation, red (aerobe) skeletal muscle inflammation and white (anaerobe) skeletal muscle degeneration or inflammation. A less frequent but supporting finding is the detection of cells with many cytoplasmic eosinophilic granules along kidney sinusoids.

As the disease progresses, the development of these changes is not simultaneous in all organs: In a very short, early phase, the only lesion present can be necrosis of exocrine pancreatic tissue and a variable inflammatory reaction in the peripancreatic fat. Shortly thereafter, heart muscle cell degeneration and necrosis develop~~s~~ before the inflammation response in the heart becomes more pronounced. The pancreatic necrotic debris will seemingly disappear and the typical picture of severe loss of exocrine pancreatic tissue will soon appear simultaneously with the increasing inflammation in the heart. Somewhat later, skeletal muscle degeneration, inflammation and fibrosis develop. In a proportion of fish, severe fibrosis of the peri-acinar tissue may occur, and in this case the pancreas does not recover (runts) (Christie *et al.,* 2007; Kerbart Boscher *et al.,* 2006; McLoughlin & Graham, 2007; Taksdal *et al.,* 2007).

4.2.4. Wet mounts

Not relevant.

4.2.5. Smears

Not relevant.

4.2.6. Fixed sections, immunohistochemistry

~~The single~~ Immunohistochemical ~~method published~~ testing (Taksdal *et al.,* 2007) is only recommended for samples from fish with acute necrosis of exocrine pancreatic tissue.

4.2.6.1. Preparation of tissue sections

The tissues are fixed in neutral phosphate-buffered 10% formalin for at least 1 day, dehydrated in graded ethanol, cleared in xylene and embedded in paraffin, according to standard protocols. Approximately 3 µm thick sections (for immunohistochemistry sampled on poly-L-lysine-coated slides) are heated at 56–58°C (maximum 60°C) for 20 minutes, dewaxed in xylene, rehydrated through graded ethanol, and stained with haematoxylin and eosin for histopathology and immunohistochemistry as described below.

4.2.6.2. Staining procedure for immunohistochemistry

All incubations are carried out at room temperature and all washing steps are done with Tris-buffered saline (TBS).

i) Nonspecific antibody binding sites are first blocked in 5% bovine serum albumin (BSA) in TBS for 20 minutes. The solution is then poured off without washing.

ii) Sections are incubated with primary antibody (monoclonal mouse antibody 4H1 against E1 SAV glycoprotein [Todd *et al.,* 2001]), diluted 1/3000 in 2.5% BSA in TBS and then incubated overnight, followed by two wash out baths lasting a minimum of 5 minutes.

iii) Sections are incubated with secondary antibody (biotinylated rabbit anti-mouse Ig) diluted 1/300 for 30 minutes, followed by wash out baths as in step ii above.

iv) Sections are incubated with streptavidin with alkaline phosphatase 1/500 for 30 minutes followed by wash out baths as in step ii above.

v) For detection of bound antibodies, sections are incubated with Fast Red[4] (1 mg ml^{-1}) and Naphthol AS-MX phosphate (0.2 mg ml^{-1}) with 1 mM Levamisole in 0.1 M TBS (pH 8.2) and allowed to develop for 20 minutes followed by one wash in tap water before counterstaining with Mayer's haematoxylin and mounting in aqueous mounting medium.

SAV-positive and SAV-negative tissue sections are included as controls in every setup (Taksdal *et al.,* 2007).

4.2.7. Electron microscopy/cytopathology

Not relevant for diagnostic use.

4.2.8. Differential diagnoses

4.2.8.1. Differential diagnoses relevant for microscopic pathology (Section 4.2.3)

Tissues that are changed by infection with SAV are also changed by heart and skeletal muscle inflammation (HSMI), cardiomyopathy syndrome (CMS) and IPN. However, if all the main organs are examined by histopathology, the pattern of affected organs will usually appear different.

Table 4.1. Tissue changes associated with infection with SAV, HSMI, CMS and IPN

	Infection with SAV	HSMI	CMS	IPN
Heart*	+	+	+	–
Pancreas	+	–	–	+
Skeletal muscle	+	+	–	–

*Heart changes in CMS affects mainly the inner spongy layer of the ventricle and the atrium, whereas in Infection with SAV and HSMI, the compact layer of the ventricle is more severely affected. Although these three diseases induce epicarditis, HSMI causes the most severely inflamed epicardium.

In a very short, early acute stage of infection, when only necrosis of exocrine pancreas has developed, infection with SAV might be mistaken for IPN caused by infection with IPN virus (IPNV). In such cases, virological examination will clarify the causal agent.

Virological and serological examinations combined with histopathological examination of 5–10 clinically diseased fish will usually clarify the situation. HSMI and CMS have only been detected in Atlantic salmon.

4.3. Agent detection and identification methods

4.3.1. Direct detection methods

4.3.1.1. Agent isolation and identification

4.3.1.1.1. Cell culture

Isolation of field isolates of SAV in cell culture may be challenging (Christie, 1998; Graham, 2007c; Petterson *et al.,* 2013). CHSE-214 are commonly used for primary SAV isolation, but susceptible cell lines such as BF-2, FHM, SHK-1, EPC, CHH-1 or others, may be used. Variation in cell line susceptibility among different SAV field isolates has been reported (Graham *et al.,* 2008; Herath *et al.,* 2009), and it is therefore recommended that several cell lines are tested for initial cell culture isolation of SAV in a new laboratory or for a new virus strain.

4 Reference to specific commercial products as examples does not imply their endorsement by the OIE. This applies to all commercial products referred to in this *Aquatic Manual*.

The CHSE-214 cells are grown at 20°C in Eagle's minimal essential medium (EMEM) with non-essential amino acids and 0.01 M HEPES (N-2-hydroxyethyl-piperazine-N-2-ethanesulfonic acid) buffer, or Leibovitz's L-15 cell culture medium, both supplemented with fetal bovine serum (FBS) (5% or 10%) and L-glutamine (4 mM).

For virus isolation, cells are grown in tissue culture flasks or multi-well cell culture plates. SAV-positive controls may be inoculated in parallel with the tissue samples as a test for cell susceptibility to SAV. When positive controls are included, measures must be taken to avoid contamination.

i) Inoculation of cell monolayers

Prepare a 2% suspension of tissue homogenate or a 10% suspension of serum using L-15 medium or EMEM without serum or other medium with documented suitability. Remove growth medium from actively growing monolayers (1- to 2-day-old cultures or cultures of 70–80% confluency) grown in tissue culture flasks or multi-well cell culture plates (see above). Inoculate monolayers with a low volume of the 2% tissue homogenate or 10% serum dilution (for 25 cm^2 flasks: 1.5 ml). Adjust volume to the respective surface area in use. Allow 2–3 hours' incubation at 15°C followed by removal of the inoculum, and addition of fresh L-15 or EMEM medium supplemented with 2–5% fetal bovine serum (for 25 cm^2 flasks: 5 ml).

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

ii) Monitoring incubation

Inoculated cell cultures (kept at 15°C) are examined at regular intervals (at least every 7 days) for the occurrence of cytopathic effect (CPE). Typical CPE due to SAV appears as plaques of pyknotic, vacuolated cells. However, Norwegian SAV field isolates (both SAV3 and marine SAV2) usually do not produce CPE in low passages, and this is also reported for other SAV ~~subtypes~~ genotypes (Graham *et al.*, 2008; Petterson *et al.*, 2013). If no CPE has developed after 14 days, subculture to fresh cell cultures.

iii) Subcultivation procedure

14 days (or earlier when obvious CPE appears) after inoculation, the cultures are freeze–thawed at –80°C (the procedure can be repeated 1–2 times) to release virus from the infected cells.

Following centrifugation at 3000 ***g*** for 5 minutes, the supernatants are inoculated into fresh cell cultures as described for the primary inoculation: remove growth medium, inoculate monolayers with a small volume of diluted supernatant (1/5 and higher dilutions) for 2–3 hours before addition of fresh medium.

Inoculated cell cultures are incubated for at least 14 days and examined at regular intervals, as described for the primary inoculation. At the end of the incubation period, or earlier if obvious CPE appears, the medium is collected for virus identification, as described below. Cell cultures should always be examined for the presence of SAV by immunofluorescence (indirect fluorescent antibody test [IFAT]), as virus replication may occur without development of apparent CPE.

iv) Antibody-based verification of SAV growth in cell culture

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

a) Prepare monolayers of cells in appropriate tissue culture plates (e.g. 96-well plates), or on cover-slips, depending on the type of microscope available (an inverted microscope equipped with UV light is necessary for monolayers grown on tissue culture plates). The necessary monolayers for negative and positive controls must be included.

b) Inoculate the monolayers with the virus suspensions to be identified in tenfold dilutions, two monolayers for each dilution. Add positive virus control in dilutions known to give a good staining reaction. Incubate inoculated cell cultures at 15°C for 9–11 days.

c) Fix in 80% acetone for 20 minutes after removing cell culture medium and rinsing once with 80% acetone. Remove the fixative and air dry for 1 hour. If necessary, the fixed cell cultures may be stored dry for 14 days at 4°C until staining.

d) Incubate the cell monolayers with anti-SAV MAb in an appropriate dilution in phosphate-buffered saline (PBS) for 1 hour and rinse three times with PBS with 0.05% Tween 20.

e) Incubate with fluorescein isothiocyanate (FITC)-conjugated anti-mouse immunoglobulin for 1 hour (or if the primary Ab is polyclonal from rabbits, use FITC-conjugated antibody against rabbit immunoglobulin), according to the instructions of the supplier. To increase the sensitivity of the test, FITC-conjugated anti-mouse Ig may be replaced with biotin-labelled anti-mouse Ig and FITC-labelled streptavidin with rinsing as in step d in between the steps. The nuclei can be stained with propidium iodide (100 µg ml^{-1} in sterile distilled water). Add PBS (without Tween 20) and examine under UV light. To avoid fading, the stained plates should be kept in the dark until examination. For long periods of storage (more than 2–3 weeks) a solution of 1,4-diazabicyclooctane (DABCO 2.5% in PBS, pH 8.2) or similar reagent may be added as an anti-fade solution.

4.3.1.1.2. Reverse-transcription polymerase chain reaction (RT-PCR), real-time RT-PCR, and genotyping by sequencing

The primers described below for real-time RT-PCR and RT-PCR with sequencing will detect all known ~~subtypes~~ genotypes of SAV.

RT-PCR may be used for detection of SAV from total RNA (or total nucleic acids) extracted from recommended organs or tissues (see Section 3.4). Real-time RT-PCR for the detection of SAV is recommended as it increases the specificity and also the sensitivity of the test.

For genotyping, RT-PCR with subsequent sequencing of fragments from the E2 and nsP3 genes is recommended.

The primers and probe for real-time RT-PCR from the nsP1 gene, as well as primers for genotyping, are listed below. The E2-primers may also be used for conventional RT-PCR detection of SAV, if necessary. A variety of kits designed for RNA extraction/RT-PCR and qPCR machines can be used. The PCR programme depends on the kit and real-time PCR equipment used in the laboratory. The conditions for performing the real-time RT-PCR in the OIE Reference Laboratory is as follows: 50°C for 10 minutes, 95°C for 3 minutes, and 40 cycles of (95°C for 10 seconds, 60°C for 20 seconds). For the conventional RT-PCRs (sequencing), the following programme is used: 50°C for 30 minutes, 95°C for 15 minutes, and 45 cycles of (94°C for 60 seconds, 55°C for 45 seconds, 72°C for 60 seconds).

Table 3.1. Characteristic of primers and probe sequences

RT-PCR: Primer and probe sequences	Named	Genomic segment	Product size	Reference
QnsP1F: 5'-CCG-GCC-CTG-AAC-CAG-TT-3' QnsP1R: 5'-GTA-GCC-AAG-TGG-GAG-AAA-GCT-3' QnsP1probe: 5'FAM-CTG-GCC-ACC-ACT-TCG-A-MGB3'	forward primer reverse primer Taqman®probe	QnsP1	107 nt	Hodneland *et al.*, 2006
E2F: 5'-CCG-TTG-CGG-CCA-CAC-TGG-ATG-3' E2R: 5'-CCT-CAT-AGG-TGA-TCG-ACG-GCA-G-3'	forward primer reverse primer	E2	516 nt	Fringuelli *et al.*, 2008
nsP3F: 5'-CGC-AGT-CCA-GCG-TCA-CCT-CAT-C-3' nsP3R: 5'-TCA-CGT-TGC-CCT-CTG-CGC-CG-3'	forward primer reverse primer	nsP3	490 nt	Fringuelli *et al.*, 2008

4.3.2. Serological methods

4.3.2.1 Immunoperoxidase-based serum neutralisation assay (Graham *et al.,* 2003)

Experimental studies have shown that neutralising antibodies can first be detected 10–16 days post-infection (Graham *et al.,* 2003), and serum neutralisation (SN) assays can be used as a diagnostic tool for the detection of SAV antibodies. SN assays are based on the presence or absence of detectable virus growth in cultured cells following incubation with serum that may contain neutralising antibodies. In addition, the assay allows detection of virus in serum or plasma, if present.

CHSE-214 cells are grown as described in Section 4.3.1.1.1 Cell culture. A suspension of trypsinised cells, diluted 1/3 in growth medium (10% FBS) is prepared for the SN assay.

i) 1/20 and 1/40 dilutions of each test serum are prepared in maintenance medium (2% FBS), and transferred to two duplicate wells (15 µl per well) on a flat-bottomed tissue culture grade microtitre plate. An equal volume of virus (100 $TCID_{50}$ [median tissue culture infective dose]) is added and the plate is incubated for 2 hours at room temperature.

ii) 70 µl of maintenance medium, and 50 µl of the CHSE-214 cell suspension is added to each well, and the plates are incubated for 3 days at 15°C.

iii) The cell monolayer is then fixed and stained as described in Section 4.3.1.1.1, step iv *Antibody-based verification of SAV growth in cell culture*, or using the following procedure: monolayers of CHSE-214 cells are fixed for 30 minutes at room temperature in 10% neutral buffered formalin. Following two washes with 0.01 M PBS, a MAb against SAV is added to the monolayers in an appropriate dilution. Bound MAb is visualised using a labelled streptavidin–biotin system according to the manufacturer's instructions.

iv) SN titres (ND_{50}) are then calculated according to the method of Karber (1931), with titres ≥ 1:20 being considered positive. Both serum controls (without virus added) and a virus control (without serum added) must always be included in the assay, to ensure valid results.

5. Rating of tests against purpose of use

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

Table 5.1. *Methods for targeted surveillance and diagnosis*

Method	Targeted surveillance			Presumptive diagnosis	Confirmatory diagnosis
	Fry	Juveniles	Adults		
Gross signs	d	d	d	c	d
Histopathology	c	c	c	~~a~~ b	~~a~~ d
Immunohistochemistry	d	d	d	b	b
Isolation in cell culture	d	d	d	c	c
Serum neutralisation assay	d	c	b	a	b
Real-time RT-PCR	b	b	b	b	b
RT-PCR with sequencing	d	b	b	b	a

RT-PCR = Reverse-transcriptase polymerase chain reaction.

6. Test(s) recommended for targeted surveillance to declare freedom from infection with SAV

The recommended test to be used in surveillance of susceptible fish populations for declaration of freedom from SAV is RT-PCR as described in Section 4.3.1.1.2 in this chapter.

Annex 15 (contd)

7. Corroborative diagnostic criteria

7.1. Definition of suspect case

A suspected case of infection with SAV is defined as:

i) Clinical signs consistent with infection with SAV (Section 4.1.1)

or

ii) Gross and microscopic~~ally~~ pathology consistent with the disease (Sections 4.2.1 and 4.2.3)

or

iii) Detection of antibodies against SAV (Section 4.3.2.1) or detection of SAV (Section 4.3.1.1.)

or

iv) If epidemiological information of infectious contact with suspected or confirmed case(s) appears.

7.2. Definition of confirmed case

Evidence for the presence of SAV from two independent laboratory tests as microscopic pathology (Section 4.2.3), cell culture (Section 4.3.1.1.1), RT-PCR (Section 4.3.1.1.2) or serology (Section 4.3.2).

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* *

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

NB: FIRST ADOPTED IN 2014.

CHAPTER 2.3.7.

INFECTION WITH KOI HERPESVIRUS ~~DISEASE~~

1. Scope

Infection with koi herpesvirus ~~disease (KHVD)~~ means infection with the pathogenic agent koi herpesvirus (KHV) of the Genus *Cyprinivirus* and Family *Alloherpesviridae* ~~a herpesvirus infection (Hedrick *et al.*, 2000) capable of inducing a contagious and acute viraemia in common carp (*Cyprinus carpio*) and varieties such as koi carp and ghost carp (Haenen *et al.*, 2004)~~.

[…]

2.2. Host factors

2.2.1. Susceptible host species

~~Naturally occurring KHV infections have only been recorded from common carp (Cyprinus carpio) and varieties of this species (e.g. koi carp). Goldfish × common carp hybrids, produced by hybridising male goldfish with female carp, have been reported to show some susceptibility to KHV infections. Although mortality rate was low (5%), approximately 50% of these hybrids examined 25 days after intraperitoneal injection with a high dose of KHV possessed viral genomic DNA, as detected by polymerase chain reaction (PCR) (Hedrick et al., 2006). In a more recent study, infection by bath immersion with different KHV strains caused mortality of 35–42% in goldfish × koi carp hybrids and 91–100% in crucian carp × koi carp hybrids. The most marked clinical signs were large skin ulcers, excess mucus production and haemorrhages in the fins with the most extensive signs noted in the crucian carp × koi carp hybrids. Viral DNA was detected in all of the hybrid mortalities by PCR assay (Bergmann et al., 2010b).~~

Species that fulfil the criteria for listing a species as susceptible to infection with KHV according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) include: All varieties and subspecies of common carp (*Cyprinus carpio* ~~*carpio*~~), and common carp hybrids (e.g. *Cyprinus carpio* × *Carassius auratus*).

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence for susceptibility according to Chapter 1.5. of the *Aquatic Code* include: Goldfish (*Carassius auratus*), grass carp (*Ctenopharyngodon idella*) and Syberian crucian carp (*Carrassius auratus*).

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following ~~organisms~~ species, but an ~~active~~ infection has not been demonstrated: Atlantic sturgeon (*Acipenser gueldenstaedtii*), blue back ide (*Leuciscus idus*), common roach (*Rutilus rutilus*), Euraseas ruffe (*Gymnocephalus cernuus*), European perch (*Perca fluviatilis*), hybrid sterlet × beluga (*Acipenser ruthenus* × *Huso huso*), rainbow trout (*Oncorhynchus mykiss*), Russian sturgeon (*Acipencer oxyrinchus*), scud (crustacean) (*Gammarus pulex*), silver carp (*Hypophthalmichthys militrix*), stone loach (*Barbatula barbatula*), swan mussel (*Anodonta cygnea*) and tench (*Tinca tinca*).

[…]

CHAPTER 2.3.4

INFECTION WITH INFECTIOUS HAEMATOPOIETIC NECROSIS VIRUS

1. Scope

~~Infectious haematopoietic necrosis (IHN)~~ Infection with infectious haematopoietic necrosis virus means infection with the *pathogenic agent* salmonid *Novirhabdovirus* (also known as infectious haematopoietic necrosis virus [IHNV]) of the Genus *Novirhabdovirus* and Family *Rhabdoviridae*. ~~is a viral disease affecting most species of salmonid fish reared in fresh water or sea water. Caused by the rhabdovirus, infectious haematopoietic necrosis virus (IHNV), the principal clinical and economic consequences of IHN occur on farms rearing rainbow trout where acute outbreaks can result in very high mortality. However, both Pacific and Atlantic salmon can be severely affected. For the purpose of this chapter, IHN is considered to be infection with IHNV~~.

2. Disease information

For detailed reviews of the disease, see Bootland & Leong (1999) or Wolf (1988).

2.1. Agent factors

2.1.1. Aetiological agent, agent strains

The fish rhabdovirus, IHNV, has a bullet-shaped virion containing a non-segmented, negative-sense, single-stranded RNA genome of approximately 11,000 nucleotides that encodes six proteins in the following order: a nucleoprotein (N), a phosphoprotein (P), a matrix protein (M), a glycoprotein (G), a non-virion protein (NV), and a polymerase (L). The presence of the unique NV gene and sequence similarity with certain other fish rhabdoviruses, such as viral haemorrhagic septicaemia virus, has resulted in the creation of the *Novirhabdovirus* genus of the family *Rhabdoviridae*, with IHNV as the type species. The type strain of IHNV is the Western Regional Aquaculture Center (WRAC) strain available from the American Type Culture Collection (ATCC VR-1392). The GenBank accession number of the genomic sequence of the WRAC strain is L40883 (Morzunov *et al*., 1995; Winton & Einer-Jensen, 2002).

Sequence analysis has been used to compare IHNV isolates from North America, Europe and Asia (Emmenegger *et al*., 2000; Enzmann *et al*., 2005; Enzmann *et al*., 2010; Johansson *et al*., 2009; Kim *et al*., 2007; Kolodziejek *et al*., 2008; Kurath *et al*., 2003; Nishizawa *et al*., 2006; Troyer & Kurath, 2003). Within the historical natural range of the virus in western North America, most isolates of IHNV from Pacific salmon form two genogroups that are related to geographical location and not to year of isolation or host species. The isolates within these two genogroups show a relatively low level of nucleotide diversity, suggesting evolutionary stasis or an older host-pathogen relationship. Conversely, isolates of IHNV from farmed rainbow trout in the USA form a third genogroup with more genetic diversity and an evolutionary pattern indicative of ongoing adaptation to a new host or rearing conditions. Isolates from farmed rainbow trout in Europe and Asia appear to have originated from North America, but show further, independent, divergence within their new geographical range (Enzmann *et al*., 2010; Kim *et al*., 2007; Nishizawa *et al*., 2006).

On the basis of antigenic studies using neutralising polyclonal rabbit antisera, IHNV isolates form a single serogroup (Engelking *et al.,* 1991), while mouse monoclonal antibodies have revealed a number of neutralising epitopes on the glycoprotein (Huang *et al.,* 1994; Ristow & Arnzen De Avila, 1991; Winton *et al.,* 1988), as well as the existence of a non-neutralising group epitope borne by the nucleoprotein (Ristow & Arnzen, 1989). However, there appears to be little or no correlation between genotypes and serotypes (Johansson *et al.,* 2009). Variations in the virulence and host preference of IHNV strains have been recorded during both natural cases of disease and in experimental infections (Garver *et al.,* 2006; LaPatra *et al.,* 1993a).

2.1.2. Survival outside the host

IHNV is heat, acid and ether labile. The virus will survive in fresh water for at least 1 month at cooler temperatures, especially if organic material is present.

2.1.3. Stability of the agent (effective inactivation methods)

IHNV is readily inactivated by common disinfectants and drying (Wolf, 1988).

2.1.4. Life cycle

Reservoirs of IHNV are clinically infected fish and covert carriers among cultured, feral or wild fish. Virus is shed via urine, sexual fluids and from external mucus, whereas kidney, spleen and other internal organs are the sites in which virus is most abundant during the course of overt infection (Bootland & Leong, 1999; Wolf, 1988).

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with IHNV according to Chapter 1.5. of the Aquatic Animal Health Code (*Aquatic Code*) include: ~~The principal hosts for IHNV are members of the family *Salmonidae*. Species reported to be naturally infected with IHNV include~~ Arctic char (*Salvelinus alpinus*), Atlantic salmon (*Salmo salar*), brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*), chinook salmon (*Oncorhynchus tshawytscha*), chum salmon (*Oncorhynchus keta*), coho salmon (*Oncorhynchus kisutch*), cutthroat trout (*Onchorynchus clarkii*), lake trout (*Salvelinus namaycush*), masou salmon (*Oncorhynchus masou*), marble trout (*Salmo marmoratus*), rainbow trout ~~or steelhead~~ (*Oncorhynchus mykiss*) ~~Chinook (*O. tshawytscha*), sockeye (*O. nerka*), chum (*O. keta*), amago (*O. rhodurus*), masou (*O. masou*), coho (*O. kisutch*),~~ and sockeye salmon (*Oncorhynchus nerka*) ~~Atlantic salmon (*Salmo salar*). Other salmonids including brown trout (*S. trutta*) and cutthroat trout (*O. clarki*), some chars (*Salvelinus namaycush*, *S. alpinus*, *S. fontinalis*, and *S. leucomaenis*), ayu (*Plecoglossus altivelis*) and non-salmonids including European eel (*Anguilla anguilla*), herring (*Clupea pallasi*), cod (*Gadus morhua*), sturgeon (*Acipenser transmontanus*), pike (*Esox lucius*), shiner perch (*Cymatogaster aggregata*) and tube snout (*Aulorhychus flavidus*) have occasionally been found to be infected in the wild or shown to be susceptible by a natural route of infection (Bootland & Leong, 1999; EFSA, 2008; Wolf, 1988).~~

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to infection with IHNV according to Chapter 1.5. of the *Aquatic Code* include: Northern pike (*Esox lucius*), Pacific herring (*Clupea pallasii*), shiner perch (*Cymatogaster aggregate*), tube-snout (*Aulorhynchus flavidus*), burbot (*Lota* lota) and white sturgeon (*Acipenser transmontanus*).

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following species, but an active infection has not been demonstrated: all varieties and subspecies of common carp (*Cyprinus carpio*) *and* American yellow perch (*Perca flavescens*).

2.2.~~2~~3. Susceptible stages of the host

Infection with IHNV occurs among several species of salmonids with fry being the most highly susceptible stage. Older fish are typically more resistant to clinical disease, but among individuals, there is a high degree of variation in susceptibility to infection with IHNV. As with viral haemorrhagic septicaemia virus, good fish health condition seems to decrease susceptibility to overt infection with IHNV, while co-infections with bacterial diseases (e.g. bacterial coldwater disease), handling and other stressors can cause subclinical infections to become overt. Fish become increasingly resistant to infection with age until spawning, when they once again become highly susceptible and may shed large amounts of virus in sexual products. Survivors of infection with IHNV demonstrate a strong protective immunity with the synthesis of circulating antibodies to the virus (LaPatra *et al*., 1993b).

2.2.3~~4~~. Species or subpopulation predilection (probability of detection)

IHNV shows a strong phylogeographic signature (Enzmann *et al*., 2010; Kurath *et al*., 2003; Nishizawa *et al*., 2006) that reflects the host species from which the virus is most commonly isolated in various geographical areas (e.g. sockeye salmon in the Northeast Pacific – U genogroup; Chinook salmon in California, USA – L genogroup; and rainbow trout in Europe, Asia and Idaho, USA – E, J and M genogroups, respectively).

2.2.4~~5~~. Target organs and infected tissue

Virus entry is thought to occur through the gills and at bases of fins while kidney, spleen and other internal organs are the sites in which virus is most abundant during the course of overt infection (Bootland & Leong, 1999; Wolf, 1988).

2.2.5~~6~~. Persistent infection with lifelong carriers

Historically, the geographic range of infection with IHNV was limited to western North America, but the disease has spread to Europe and Asia via the importation of infected fish and eggs. Once IHNV is introduced into a farmed stock, the disease may become established among susceptible species of wild fish in the watershed. The length that individual fish are infected with IHNV varies with temperature; however, unlike infectious pancreatic necrosis virus (IPNV) or channel catfish virus (CCV), a true, life-long carrier state with IHNV appears to be a rare event at normal temperatures.

2.2.6~~7~~. Vectors

Horizontal transmission of IHNV is typically by direct exposure, but invertebrate vectors have been proposed to play a role in some cases (Bootland & Leong, 1999).

Mayfly (*Callibaetis* sp.) (Shors & Winston, 1988) and salmon lice (*Lepeophtheirus salmonis*) (Jakob *et al.,* 2011) are potential vectors for IHNV.

2.2.7~~8~~. Known or suspected wild aquatic animal carriers

IHNV is endemic among many populations of free-ranging salmonids. A marine reservoir has been proposed, but not confirmed.

2.3. Disease pattern

Infection with IHNV often leads to mortality due to the impairment of osmotic balance and occurs within a clinical context of oedema and haemorrhage. Virus multiplication in endothelial cells of blood capillaries, haematopoietic tissues, and cells of the kidney underlies the clinical signs.

2.3.1. Transmission mechanisms

The transmission of IHNV between fish is primarily horizontal and high levels of virus are shed from infected juvenile fish, however, cases of vertical or egg-associated transmission have been recorded. Although egg-associated transmission is significantly reduced by the now common practice of surface disinfection of eggs with an iodophor solution, it is the only mechanism accounting for the occurrence of infection with IHNV in new geographical locations among alevins originating from eggs that were incubated and hatched in virus-free water (Winton, 1991).

2.3.2. Prevalence

Infection with IHNV is endemic and widely prevalent among populations of free-ranging salmonids throughout much of its historical range along the west coast of North America. The virus has also become established with a high prevalence of infection in major trout growing regions of North America, Europe and Asia where IHNV was introduced through the movement of infected fish or eggs.

2.3.3. Geographical distribution

Infection with IHNV has been detected in North America, Asia and Europe, but not in the Southern Hemisphere. Countries reporting confirmed or suspect cases of infection with IHNV to the OIE include: Austria, Belgium, Canada, China (People's Rep. of), Croatia, Czech Republic, France, Germany, Iran, Italy, Japan, Korea (Rep. of), Netherlands, Poland, Russia, Slovenia, Spain, Switzerland and United States of America. Infections and overt disease have been reported among fish reared in both fresh and sea water.

2.3.4. Mortality and morbidity

Depending on the species of fish, rearing conditions, temperature, and, to some extent, the virus strain, outbreaks of infection with IHNV may range from explosive to chronic. Losses in acute outbreaks will exceed several per cent of the population per day and cumulative mortality may reach 90–95% or more (Bootland & Leong, 1999). In chronic cases, losses are protracted and fish in various stages of disease can be observed in the pond.

2.3.5. Environmental factors

The most important environmental factor affecting the progress of infection with IHNV is water temperature. Experimental trials have demonstrated infection with IHNV can produce mortality from 3°C to 18°C (Bootland & Leong, 1999); however, clinical disease typically occurs between 8°C and 15°C under natural conditions.

2.4. Control and prevention

Control methods for infection with IHNV currently rely on avoidance of exposure to the virus through the implementation of strict control policies and sound hygiene practices (Winton, 1991). The thorough disinfection of fertilised eggs, the use of virus-free water supplies for incubation and rearing, and the operation of facilities under established biosecurity measures are all critical for preventing infection with IHNV at a fish production site.

2.4.1. Vaccination

Experimental vaccines to protect salmonids against infection with IHNV have been the subject of research for more than 40 years with some showing promise in both laboratory and field trials when delivered by immersion or injection (Kurath, 2008; Winton, 1991; Winton, 1997). Both autogenous, killed vaccines and a DNA vaccine have been licensed for commercial use in Atlantic salmon net-pen aquaculture on the west coast of North America where such vaccines can be delivered economically by injection. However, vaccines against infection with IHNV have not yet been licensed in other countries where the application of vaccines to millions of smaller fish will require additional research on novel mass delivery methods.

2.4.2. Chemotherapy

Although chemotherapeutic approaches for control of infection with IHNV have been studied, they have not found commercial use in aquaculture against ~~IHNV~~ the disease (Winton, 1991).

2.4.3. Immunostimulation

Immunostimulants are an active area of research, but have not found commercial use in aquaculture against infection with IHNV.

2.4.4. Resistance breeding

Experimental trials of triploid or inter-species hybrids have shown promise (Barroso *et al*., 2008; Winton, 1991) and the genetic basis of resistance to IHNV has been an active area of recent research (Miller *et al*., 2004; Purcell *et al*., 2010).

2.4.5. Restocking with resistant species

Within endemic areas, the use of less susceptible species has been used to reduce the impact of infection with IHNV in aquaculture.

2.4.6. Blocking agents

Natural compounds have been identified from aquatic microbes that have antiviral activity; however, these have not found commercial use in aquaculture against infection with IHNV (Winton, 1991).

2.4.7. Disinfection of eggs and larvae

Disinfection of eggs is a highly effective method to block egg-associated transmission of IHNV in aquaculture settings (Bovo *et al*., 2005). The method is widely practiced in areas where the virus is endemic.

2.4.8. General husbandry practices

In addition to disinfection of eggs, use of a virus-free water supply has been shown to be a critical factor in the management of infection with IHNV within endemic areas. Several approaches include use of wells or springs that are free of fish or other sources of IHNV and disinfection of surface water sources using UV light or ozone (Winton, 1991).

3. Sampling

3.1. Selection of individual specimens

Clinical inspections are best carried out during a period whenever the water temperature is below 14°C. All production units (ponds, tanks, net-cages, etc.) must be inspected for the presence of dead, weak or abnormally behaving fish. Particular attention must be paid to the water outlet area where weak fish tend to accumulate.

In farms with salmonids, if rainbow trout are present, only fish of that species are selected for sampling. If rainbow trout are not present, the sample has to be obtained from fish of all other infection with IHNV susceptible species present, as listed in Section 2.2.1. Susceptible species should be sampled proportionally, or following risk-based criteria for targeted selection of 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 risk status).

If more than one water source is used for fish production, fish from all water sources must be included in the sample. If weak, abnormally behaving or freshly dead (not decomposed) fish are present, such fish are selected. If such fish are not present, the fish selected must 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.

3.2. Preservation of samples for submission

Before shipment or transfer to the laboratory, parts of the organs to be examined must be removed from the fish with sterile dissection instruments and transferred to sterile plastic tubes containing transport medium, i.e. cell culture medium with 10% foetal calf serum (FCS) and antibiotics. Addition of 200 International Units (IU) penicillin, 200 µg streptomycin, and 200 µg kanamycin per ml are recommended, although other antibiotics of proven efficiency may also be used.

3.3. Pooling of samples

Ovarian fluid or organ pieces from a maximum of ten fish may be collected in one sterile tube containing at least 4 ml transport medium and this represents one pooled sample. The tissue in each sample should weigh a minimum of 0.5 g. The tubes should be placed in insulated containers (for instance, thick-walled polystyrene boxes) together with sufficient ice or ‘freezer blocks’ to ensure chilling of the samples during transportation to the laboratory. Freezing must be avoided. The temperature of a sample during transit

should never exceed 10°C and ice should still be present in the transport box at receipt or one or more freeze blocks must still be partly or completely frozen. Virological examination must be started as soon as possible and not later than 48 hours after collection of the samples. In exceptional cases, the virological examination may be started at the latest within 72 hours after collection of the material, provided that the material to be examined is protected by transport medium and that the temperature requirements during transportation are fulfilled.

Whole fish may be sent to the laboratory if the temperature requirements during transportation can be fulfilled. Whole fish may be wrapped in paper with absorptive capacity and must be shipped in a plastic bag, chilled as mentioned above. Live fish can also be shipped. All packaging and labelling must be performed in accordance with present national and international transport regulations, as appropriate.

3.4. Best organs or tissues

The optimal tissue material to be examined is spleen, anterior kidney, and either heart or encephalon. In some cases, ovarian fluid and milt must be examined.

In case of small fry, whole fish less than 4 cm long can be minced with sterile scissors or a scalpel after removal of the body behind the gut opening. If a sample consists of whole fish with a body length between 4 cm and 6 cm, the viscera including kidney should be collected. If a sample consisted of whole fish less than 4 cm long, these should be minced with sterile scissors or a scalpel, after removal of the body behind the gut opening. If a sample consisted of whole fish with a body length between 4 cm and 6 cm, the viscera, including kidney, should be collected. If a sample consisted of whole fish more than 6 cm long, tissue specimens should be collected as described above. The tissue specimens should be minced with sterile scissors or a scalpel, homogenised and suspended in transport medium.

3.5. Samples/tissues that are not suitable

IHNV is very sensitive to degradation, therefore sampling tissues with high enzymatic activities or large numbers of contaminating bacteria such as the intestine or skin should be avoided when possible. Muscle tissue is also less useful as it typically contains a lower virus load.

4. Diagnostic methods

The “Gold Standard” for detection of IHNV is the isolation of the virus in cell culture followed by its immunological or molecular identification. While the other diagnostic methods listed below can be used for confirmation of the identity of virus isolated in cell culture or for confirmation of overt infections in fish, they are not approved for use as primary surveillance methods for obtaining or maintaining approved infection with IHNV-free status.

Due to substantial variation in the strength and duration of the serological responses of fish to virus infections, the detection of fish antibodies to viruses has not thus far been accepted as a routine diagnostic method for assessing the viral status of fish populations. In the future, validation of serological techniques for diagnosis of fish virus infections could render the use of fish serology more widely acceptable for diagnostic purposes. However, when present, a positive serological response is considered presumptive evidence of past exposure to infection with IHNV (Jorgensen *et al*., 1991).

4.1. Field diagnostic methods

4.1.1. Clinical signs

The disease is typically characterised by gross signs that include lethargy interspersed with bouts of frenzied, abnormal activity, darkening of the skin, pale gills, ascites, distended abdomen, exophthalmia, and petechial haemorrhages internally and externally.

4.1.2. Behavioural changes

During outbreaks, fish are typically lethargic with bouts of frenzied, abnormal activity, such as spiral swimming and flashing. A trailing faecal cast is observed in some species. Spinal deformities are present among some of the surviving fish (Bootland & Leong, 1999).

4.2. Clinical methods

4.2.1. Gross pathology

Affected fish exhibit darkening of the skin, pale gills, ascites, distended abdomen, exophthalmia, and petechial haemorrhages internally and externally. Internally, fish appear anaemic and lack food in the gut. The liver, kidney and spleen are pale. Ascitic fluid is present and petechiae are observed in the organs of the body cavity.

4.2.2. Clinical chemistry

The blood of affected fry shows reduced haematocrit, leukopenia, degeneration of leucocytes and thrombocytes, and large amounts of cellular debris. As with other haemorrhagic viraemias of fish, blood chemistry is altered in severe cases (Bootland & Leong, 1999).

4.2.3. Microscopic pathology

Histopathological findings reveal degenerative necrosis in haematopoietic tissues, kidney, spleen, liver, pancreas, and digestive tract. Necrosis of eosinophilic granular cells in the intestinal wall is pathognomonic of infection with IHNV ~~infection~~ (Bootland & Leong, 1999).

4.2.4. Wet mounts

Wet mounts have limited diagnostic value.

4.2.5. Tissue imprints and smears

Necrobiotic bodies and foamy macrophages, indicative of a clinical manifestation of infection with IHNV, can be best observed using tissue imprints obtained from the kidney and spleen rather than smears.

4.2.6. Electron microscopy/cytopathology

Electron microscopy of virus-infected cells reveals bullet-shaped virions of approximately 150–190 nm in length and 65–75 nm in width (Wolf, 1988). The virions are visible at the cell surface or within vacuoles or intracellular spaces after budding through cellular membranes. The virion possesses an outer envelope containing host lipids and the viral glycoprotein spikes that react with immunogold staining to decorate the virion surface.

4.3. Agent detection and identification methods

The traditional procedure for detection of IHNV is based on virus isolation in cell culture. Confirmatory identification may be achieved by use of immunological (neutralisation, indirect fluorescent antibody test or enzyme-linked immunosorbent assay), or molecular (polymerase chain reaction, DNA probe or sequencing) methods (Arakawa *et al*., 1990; Arnzen *et al*., 1991; Deering *et al*., 1991; Dixon & Hill, 1984; Jorgensen *et al*., 1991; LaPatra *et al*., 1989; Purcell *et al*., 2006; Winton & Einer-Jensen, 2002).

4.3.1. Direct detection methods

4.3.1.1. Microscopic methods

4.3.1.1.1. Wet mounts

Wet mounts are not appropriate for detection or identification of IHNV.

4.3.1.1.2. Smears

Smears are not appropriate for detection or identification of IHNV.

4.3.1.1.3. Fixed sections

Immunohistochemistry and *in-situ* hybridisation (ISH) methods have been used in research applications, but are not appropriate for detection or identification of IHNV in a diagnostic setting.

4.3.1.2. Agent isolation and identification

4.3.1.2.1. Cell culture/artificial media

Cell lines to be used: EPC or FHM.

Detection of virus through the development of viral cytopathic effect (CPE) in cell culture would be followed by virus identification through either antibody-based tests or nucleic acid-based tests. Any antibody-based tests would require the use of antibodies validated for their sensitivity and specificity.

4.3.1.2.1.1. Virus extraction

In the laboratory the tissue in the tubes must be completely homogenised (either by stomacher, blender mortar and pestle with sterile sand or any other suitable and validated homogeniser) and subsequently suspended in the original transport medium. The final ratio between tissue material and transport medium must be adjusted in the laboratory to 1:10.

The homogenate is centrifuged in a refrigerated centrifuge at 2°C–5°C at 2000–4000 ***g*** for 15 minutes and the supernatant collected and treated for either four hours at 15°C or overnight at 4°C with antibiotics (e.g. 1 mg ml^{-1} gentamicin may be useful at this stage). If shipment of the sample has been made in a transport medium (i.e. with exposure to antibiotics) the treatment of the supernatant with antibiotics may be omitted. The antibiotic treatment aims at controlling bacterial contamination in the samples and makes filtration through membrane filters unnecessary.

Where practical difficulties arise (e.g. incubator breakdown, problems with cell cultures, etc.), which make it impossible to inoculate cells within 48 hours after the collection of the tissue samples, it is acceptable to freeze the supernatant at –80°C and carry out virological examination within 14 days. If the collected supernatant is stored at –80°C within 48 hours after the sampling it may be reused only once for virological examination.

Optional treatment of homogenate to inactivate competing virus: treatment of inocula with antiserum to IPNV (which in some parts of the world occurs in 50% of fish samples) aims at preventing CPE due to IPNV from confounding the ability to detect IHNV in cell culture. When samples come from production units, which are considered free from IPN, treatment of inocula with antiserum to IPNV should be omitted. Prior to the inoculation of the cells, the supernatant is mixed with equal parts of a suitably diluted pool of antisera to the indigenous serotypes of IPNV and incubated with this for a minimum of one hour at 15°C or a maximum of 18 hours at 4°C. The titre of the antiserum must be at least 1/2000 in a 50% plaque neutralisation test.

4.3.1.2.1.2. Inoculation of cell monolayers

EPC or FHM cells are grown at 20–30°C in suitable medium, e.g. Eagle's MEM (or modifications thereof) with a supplement of 10% fetal bovine serum (FBS) and antibiotics in standard concentrations. When the cells are cultivated in closed vials, it is recommended to buffer the medium with bicarbonate. The medium used for cultivation of cells in open units may be buffered with Tris/HCl (23 mM) and Na-bicarbonate (6 mM). The pH must be 7.6 ± 0.2. Cell cultures to be used for inoculation with tissue material should be young (4-48 hours old) and actively growing (not confluent) at inoculation.

Antibiotic-treated organ suspension is inoculated into cell cultures in at least two dilutions, i.e. the primary dilution and, in addition, a 1:10 dilution thereof, resulting in final dilutions of tissue material in cell culture medium of 1:100 and 1:1000, respectively, (in order to prevent homologous interference). The ratio between inoculum size and volume of cell culture medium should be about 1:10. For each dilution and each cell line, a minimum of about 2 cm^2 cell area, corresponding to one well in a 24-well cell culture tray, has to be used. Use of cell culture trays is recommended, but other units of similar or with larger growth area are acceptable as well.

4.3.1.2.1.3. Incubation of cell cultures

Inoculated cell cultures are incubated at 15°C for 7–10 days. If the colour of the cell culture medium changes from red to yellow, indicating medium acidification, pH adjustment with sterile bicarbonate solution or equivalent substances has to be performed to maintain cell susceptibility to virus infection.

At least every six months or if decreased cell susceptibility is suspected, titration of frozen stocks of IHNV is performed to verify the susceptibility of the cell cultures to infection.

4.3.1.2.1.4. Microscopy

Inoculated cell cultures must be inspected regularly (at least three times a week) for the occurrence of CPE at 40–150 × magnification. The use of a phase-contrast microscope is recommended. If obvious CPE is observed, virus identification procedures have to be initiated immediately.

4.3.1.2.1.5. Subcultivation

If no CPE has developed after the primary incubation for 7–10 days, subcultivation is performed to fresh cell cultures utilising a cell area similar to that of the primary culture.

Aliquots of medium (supernatant) from all cultures/wells constituting the primary culture are pooled according to the cell line 7–10 days after inoculation. The pools are then inoculated into homologous cell cultures undiluted and diluted 1:10 (resulting in final dilutions of 1:10 and 1:100, respectively, of the supernatant) as described in Section 4.3.1.2.1.2above.

Alternatively, aliquots of 10% of the medium constituting the primary culture are inoculated directly into a well with fresh cell culture (well-to-well subcultivation). In case of salmonid samples, the inoculation may be preceded by preincubation of the dilutions with the antiserum to IPNV at an appropriate dilution as described above.

The inoculated cultures are then incubated for 7–10 days at 15°C with observation as in Section 4.3.1.2.1.4If toxic CPE occurs within the first three days of incubation, subcultivation may be performed at that stage, but the cells must then be incubated for seven days and subcultivated again with a further seven days incubation. When toxic CPE develops after three days, the cells may be passed once and incubated to achieve the total of 14 days from the primary inoculation. There should be no evidence of toxicity in the final seven days of incubation.

If bacterial contamination occurs, despite treatment with antibiotics, subcultivation must be preceded by centrifugation at 2000–4000 ***g*** for 15–30 minutes at 2–5°C, and/or filtration of the supernatant through a 0.45 µm filter (low protein-binding membrane). In addition to this, subcultivation procedures are the same as for toxic CPE.

If no CPE occurs the test may be declared negative.

4.3.1.2.2. Antibody-based antigen detection methods

4.3.1.2.2.1. Neutralisation test (identification in cell culture)

i) Collect the culture medium of the cell monolayers exhibiting CPE and centrifuge an aliquot at 2000 ***g*** for 15 minutes at 4°C, or filter through a 0.45 µm (or 450 nm) pore membrane to remove cell debris.

ii) Dilute virus-containing medium from 10^2–10^4.

Iii Mix aliquots (for example 200 µl) of each dilution with equal volumes of an IHNV antibody solution.

The neutralising antibody (Nab) solution must have a 50% plaque reduction titre of at least 2000. Likewise, treat a set of aliquots of each virus dilution with cell culture medium to provide a non-neutralised control.

iv) In parallel, a neutralisation test must be performed against a homologous IHNV strain (positive neutralisation test) to confirm the reactivity of the antiserum.

v) Incubate all the mixtures at 15°C for 1 hour.

vi) Transfer aliquots of each of the above mixtures on to 24-hour-old monolayers overlaid with cell culture medium containing 10% FBS (inoculate two wells per dilution) and incubate at 15°C; 24- or 12-well cell culture plates are suitable for this purpose, using a 50 µl inoculum.

vii) Check the cell cultures for the onset of CPE and read the results for each suspect IHNV sample as soon as it CPE occurs in non-neutralised controls. Results are recorded either after a simple microscopic examination (phase contrast preferable) or after discarding the cell culture medium and staining cell monolayers with a solution of 1% crystal violet in 20% ethanol.

viii) The tested virus is identified as IHNV when CPE is prevented or noticeably delayed in the cell cultures that received the virus suspension treated with the IHNV-specific antibody, whereas CPE is evident in all other cell cultures.

Other neutralisation tests of proven efficiency may be used alternatively.

4.3.1.2.2.2. Indirect fluorescent antibody test (IFAT)

Antibody-based antigen detection methods such as IFAT, ELISA and various immunohistochemical procedures for the detection of IHNV have been developed over the years. These techniques can provide detection and identification relatively quickly compared with virus isolation in cell culture. However, various parameters such as antibody sensitivity and specificity and sample preparation can influence the results; a negative result should be viewed with caution. These techniques should not be used in attempts to detect carrier fish.

4.3.1.2.2.2.1. Indirect fluorescent antibody test in cell cultures

i) Prepare monolayers of cells in 2 cm^2 wells of cell culture plastic plates or on cover slips in order to reach around 80% confluency, which is usually achieved within 24 hours of incubation at 22°C (seed six cell monolayers per virus isolate to be identified, plus two for positive and two for negative controls). The FBS content of the cell culture medium can be reduced to 2–4%. If numerous virus isolates have to be identified, the use of black 96-well plates for immunofluorescence is recommended.

ii) When the cell monolayers are ready for infection (i.e. on the same day or on the day after seeding) inoculate the virus suspensions to be identified by making tenfold dilution steps directly in the cell culture wells or flasks.

iii) Dilute the control virus suspension of IHNV in a similar way, in order to obtain a virus titre of about 5,000–10,000 plaque-forming units (PFU) per ml in the cell culture medium.

iv) Incubate at 15°C for 24 hours.

v) Remove the cell culture medium, rinse once with 0.01 M phosphate buffered saline (PBS), pH 7.2, then three times briefly with a cold mixture of acetone 30%/ethanol 70% (v/v) (stored at –20°C).

vi) Let the fixative act for 15 minutes. A volume of 0.5 ml is adequate for 2 cm^2 of cell monolayer.

vii) Allow the cell monolayers to air-dry for at least 30 minutes and process immediately or freeze at –20°C.

viii) Prepare a solution of purified IHNV antibody or serum in 0.01 M PBS, pH 7.2, containing 0.05% Tween-80 (PBST), at the appropriate dilution (which has been established previously or is given by the reagent supplier).

ix) Rehydrate the dried cell monolayers by four rinsing steps with the PBST solution, and remove this buffer completely after the last rinsing.

x) Treat the cell monolayers with the antibody solution for 1 hour at 37°C in a humid chamber and do not allow evaporation to occur (e.g. by adding a piece of wet cotton to the humid chamber). The volume of solution to be used is 0.25 ml 2 cm^{-2} well.

xi) Rinse four times with PBST as above.

xii) Treat the cell monolayers for 1 hour at 37°C with a solution of FITC- or tetramethylrhodamine-5-(and-6-) isothiocyanate (TRITC)-conjugated antibody to the immunoglobulin used in the first layer and prepared according to the instructions of the supplier. These conjugated antibodies are most often rabbit or goat antibodies.

xiii) Rinse four times with PBST.

xiv) Examine the treated cell monolayers on plastic plates immediately, or mount the cover slips using, for example, glycerol saline, pH 8.5 prior to microscopic observation.

xv) Examine under incident UV light using a microscope with × 10 eye pieces and × 20–40 objective lens having numerical aperture >0.65 and >1.3, respectively. Positive and negative controls must be found to give the expected results prior to any other observation.

4.3.1.2.2.2.2. Indirect fluorescent antibody test on imprints

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) Store the kidney pieces together with the other organs required for virus isolation in case this becomes necessary later.

iv) Allow the imprint to air-dry for 20 minutes.

v) Fix with acetone or ethanol/acetone and dry.

vi) Rehydrate the above preparations and block with 5% skim milk or 1% bovine serum albumin, in PBST for 30 minutes at 37°C. vii) Rinse four times with PBST.

viii) Treat the imprints with the solution of antibody to IHNV and rinse.

ix) Block and rinse.

x) Reveal the reaction with suitable fluorescein isothiocyanate (FITC)-conjugated specific antibody, rinse and observe.

xi) If the test is negative, process the organ samples stored at 4°C for virus isolation in cell culture, as described above.

Other IFAT or immunocytochemical (alkaline phosphatase or peroxidase) techniques of proven efficiency may be used alternatively.

4.3.1.2.2.3. Enzyme-linked immunosorbent assay (ELISA)

i) Coat the wells of microplates designed for ELISAs with appropriate dilutions of purified immunoglobulins (Ig) or serum specific for IHNV, in 0.01 M PBS, pH 7.2 (200 µl/well).

ii) Incubate overnight at 4°C.

iii) Rinse four times with 0.01 M PBS containing 0.05% Tween-20 (PBST).

iv) Block with skim milk (5% in PBST) or other blocking solution for 1 hour at 37°C (200 µl/well).

v) Rinse four times with PBST.

vi) Add 2% Triton X-100 to the virus suspension to be identified.

vii) Dispense 100 µl/well of two- or four-step dilutions of the virus to be identified and of IHNV control virus, and a heterologous virus control (e.g. viral haemorrhagic septicaemia virus). Allow the samples to react with the coated antibody to IHNV for 1 hour at 20°C.

viii) Rinse four times with PBST.

ix) Add to the wells either biotinylated polyclonal IHNV antiserum or MAb to N protein specific for a domain different from the one of the coating MAb and previously conjugated with biotin.

x) Incubate for 1 hour at 37°C.

xi) Rinse four times with PBST.

xii) Add streptavidin-conjugated horseradish peroxidase to those wells that have received the biotin-conjugated antibody, and incubate for 1 hour at 20°C.

xiii) Rinse four times with PBST. Add the substrate and chromogen. Stop the course of the test when positive controls react, and read the results.

xiv) Interpretations of the results is according to the optical absorbencies achieved by negative and positive controls and must follow the guidelines for each test, e.g. absorbency at 450 nm of positive control must be minimum 5–10 × A450 of negative control.

The above biotin-avidin-based ELISA version is given as an example. Other ELISA versions of proven efficiency may be used instead.

4.3.1.2.3. Molecular techniques

4.3.1.2.3.1. Polymerase chain reaction

4.3.1.2.3.1.1. Viral RNA preparation

Total RNA from infected cells is extracted using a phase-separation method (e.g. phenol-chloroform or Trizol) or by use of a commercially-available RNA isolation kit used according to the manufacturer's instructions. While all of these methods work well for drained cell monolayers or cell pellets, RNA binding to affinity columns can be affected by salts present in tissue culture media and phase-separation methods should be used for extraction of RNA from cell culture fluids.

4.3.1.2.3.1.2. Reverse-transcription (RT) and standard PCR protocol

i) Prepare a master mix for the number of samples to be analysed. Work under a hood and wear gloves.

ii) The master mix for one 50 µl reverse-transcription PCR is prepared as follows: 23.75 µl ribonuclease-free (DEPC-treated) or molecular biology grade water; 5 µl 10 × buffer; 5 µl 25 mM $MgCl_2$; 5 µl 2 mM dNTP; 2.5 µl (20 pmoles $µl^{-1}$) Upstream Primer

5'-AGA-GAT-CCC-TAC-ACC-AGA-GAC-3'; 2.5 µl (20 pmoles $µl^{-1}$) Downstream Primer

5'-GGT-GGT-GTT-GTT-TCC-GTG-CAA-3'; 0.5 µl *Taq* polymerase (5 U $µl^{-1}$); 0.5 µl AMV reverse transcriptase (9 U $µl^{-1}$); 0.25 µl RNasin (39 U $µl^{-1}$).

iii) Centrifuge the tubes briefly (10 seconds) to make sure the contents are at the bottom.

iv) Place the tubes in the thermal cycler and start the following cycles – 1 cycle: 50°C for 30 minutes; 1 cycle: 95°C for 2 minutes; 30 cycles: 95°C for 30 seconds, 50°C for 30 seconds, 72°C for 60 seconds; 1 cycle: 72°C for 7 minutes and soak at 4°C.

v) Visualise the 693 bp PCR amplicon by electrophoresis of the product in 1.5% agarose gel with ethidium bromide and observe using UV transillumination.

NOTE: These PCR primers target a central region of the IHNV G gene (Emmenegger *et al*., 2000). While other primer sets can be used for amplification of portions of the N or G genes of IHNV (Winton & Einer-Jensen, 2002), the primer sequences listed above have been shown to be conserved among a broad range of IHNV isolates and are not present in the G gene of the related fish rhabdoviruses, viral haemorrhagic septicaemia virus or hirame rhabdovirus. Additionally, the new primers produce an amplicon that can be used as a template for sequence analysis of the 'mid-G' region of the IHNV genome for epidemiological purposes (Emmenegger *et al*., 2000; Kurath *et al*., 2003).

4.3.1.2.3.2. Other amplification-based assays

Other methods to detect IHNV based on amplification of target sequences of genomic or messenger RNA have been developed that use a loop-mediated isothermal amplification (LAMP) method (Gunimaladevi *et al*., 2005) or a highly sensitive quantitative reverse-transcriptase PCR assay (Overturf *et al*., 2001). However, these assays have not yet undergone sufficient laboratory validation using a panel of isolates representing the various IHNV genotypes to make them suitable for listing as a confirmatory method.

4.3.1.2.3.3. Sequencing

Sequence analysis of PCR amplicons has become much more rapid and less costly in recent years and is a good method for confirmation of IHNV (Winton & Einer-Jensen, 2002). In addition, sequence analysis provides one of the best approaches for identification of genetic strains and for epidemiological tracing of virus movement (Emmenegger *et al*., 2000; Kim *et al*., 2007; Kurath *et al*., 2003; Nishizawa *et al*., 2006).

5. Rating of tests against purpose of use

The methods currently available for surveillance, detection, and diagnosis of infection with IHNV are listed in Table 5.1.The designations used in the Table indicate: a = the method is the recommended method for reasons of availability, utility, and diagnostic specificity and sensitivity; b = the method is a standard method with good diagnostic sensitivity and specificity; c = the method has application in some situations, but cost, accuracy, or other factors severely limits its application; and d = the method is presently not recommended for this purpose. These are somewhat subjective as suitability involves issues of reliability, sensitivity, specificity and utility. Although not all of the tests listed as category a or b have undergone formal standardisation and validation, their routine nature and the fact that they have been used widely without dubious results, makes them acceptable.

Table 5.1. *Methods for targeted surveillance and diagnosis*

Method	Targeted surveillance				Presumptive diagnosis	Confirmatory diagnosis
	Gametes	Fry	Juveniles	Adults		
Gross signs	d	c	c	d	b	d
Virus isolation	a	a	a	a	a	c
Direct LM	d	c	d	d	b	c
Histopathology	d	c	d	d	b	c
Transmission EM	d	d	d	d	b	c
Antibody-based assays	d	c	c	c	a	b
PCR assays	c	c	c	c	a	a
Sequencing	d	d	d	d	c	a

LM = light microscopy; EM = electron microscopy; PCR = polymerase chain reaction.

6. Test(s) recommended for targeted surveillance to declare freedom from infectious haematopoietic necrosis

The method for targeted surveillance to declare freedom from infection with IHNV is isolation of virus in cell culture. For this purpose, the most susceptible stages of the most susceptible species should be examined. Reproductive fluids and tissues collected from adult fish of a susceptible species at spawning should be included in at least one of the sampling periods each year.

Annex 17 (contd)

7. Corroborative diagnostic criteria

7.1. Definition of suspect case

A suspect case is defined as the presence of typical, gross clinical signs of the disease in a population of susceptible fish, OR a typical internal histopathological presentation among susceptible species, OR detection of antibodies against IHNV in a susceptible species, OR typical cytopathic effect in cell culture without identification of the agent, OR a single positive result from one of the diagnostic assays ranked as 'a' or 'b' in Table 5.1.

7.2. Definition of confirmed case

A confirmed case is defined as a suspect case that has EITHER: 1) produced typical cytopathic effect in cell culture with subsequent identification of the agent by one of the antibody-based or molecular tests listed in Table 5.1., OR: 2) a second positive result from a different diagnostic assay ranked as 'a' or 'b' in the last column of Table 5.1.

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* *

NB: There are OIE Reference Laboratories for infection with infectious haematopoietic necrosis virus (see Table at the end of this *Aquatic Manual* or consult the OIE web site for the most up-to-date list: http://www.oie.int/en/scientific-expertise/reference-laboratories/list-of-laboratories/). Please contact the OIE Reference Laboratories for any further information on infection with infectious haematopoietic necrosis virus.

NB: FIRST ADOPTED IN 1995 AS INFECTIOUS HAEMATOPOIETIC NECROSIS; MOST RECENT UPDATES ADOPTED IN 2012.

Annex 18

CHAPTER 10.2.

INFECTION WITH *APHANOMYCES INVADANS* (EPIZOOTIC ULCERATIVE SYNDROME)

Article 10.2.1.

For the purposes of the *Aquatic Code*, infection with *Aphanomyces invadans* means ~~all *infections* caused by~~ *infection* with the *pathogenic agent* ~~*Aphanomyces*~~ *A. invadans* (syn. *A. piscicida*). The *disease* was previously referred to as epizootic ulcerative syndrome.

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

Article 10.2.2.

Scope

The recommendations in this chapter apply to: yellowfin seabream *(Acantopagrus australis)*, climbing perch *(Anabas testudineus)*, eels (~~*Anguillidae*~~ Anguillidae), bagrid catfishes (~~*Bagridae*~~Bagridae), silver perch *(Bidyanus bidyanus)*, Atlantic menhaden *(Brevoortia tyrannus)*, jacks (*Caranx* spp.), catla *(Catla catla)*, striped snakehead *(Channa striatus)*, mrigal *(Cirrhinus mrigala)*, torpedo-shaped catfishes (*Clarius* spp.), halfbeaks flying fishes (~~*Exocoetidae*~~ Exocoetidae), tank goby *(Glossogobius giuris)*, marble goby *(Oxyeleotris marmoratus)*, gobies (~~*Gobiidae*~~ *Gobiidae*), rohu *(Labeo rohita)*, rhinofishes (*Labeo* spp.), barramundi and giant sea perch *(Lates calcarifer)*, striped mullet *(Mugil cephalus)*, mullets *(Mugilidae)* (*Mugil* spp. and *Liza* spp.), ayu *(Plecoglossus altivelis)*, pool barb *(Puntius sophore)*, barcoo grunter *(Scortum barcoo)*, sand whiting *(Sillago ciliata)*, ~~wells~~ catfishes (~~*Siluridae*~~ Siluridae spp.), snakeskin gourami *(Trichogaster pectoralis)*, common archer fish *(Toxotes chatareus)*, silver barb *(Puntius gonionotus)*, spotted scat *(Scatophagus argus)*, giant gourami *(Osphronemus goramy)*, dusky flathead *(Platycephalus fuscus)*, spiny turbot (*Psettodes* sp.), Tairiku-baratanago *(Rhodeus ocellatus)*, Keti-Bangladeshi (*Rohtee* sp.), rudd *(Scaridinius erythrophthalmus)*, therapon (*Terapon* sp.) and three-spot gouram~~y~~i *(Trichogaster trichopterus)*. These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

TILAPIA LAKE VIRUS (TiLV)–A NOVEL ORTHOMYXO-LIKE VIRUS

PATHOGEN INFORMATION

1. CAUSATIVE AGENT

1.1. Pathogen type

Virus.

1.2. Disease name and synonyms

Tilapia lake virus (TiLV) disease, syncytial hepatitis of tilapia (SHT).

1.3. Pathogen common names and synonyms

Tilapia lake virus (TiLV).

1.4. Taxonomic affiliation

The taxonomic affiliation has not been definitively concluded; however, TiLV has been described as a novel virus in the Family *Orthomyxoviridae* (Eyngor *et al.*, 2014).

1.5. Authority (first scientific description, reference)

The virus was first described by Eyngor *et al.* (2014).

1.6. Pathogen environment (fresh, brackish, marine waters)

Fresh and brackish water.

2. MODES OF TRANSMISSION

2.1. Routes of transmission (horizontal, vertical, indirect)

Co-habitation studies have demonstrated that direct horizontal transmission is an important route of transmission. There is no evidence of vertical transmission. The biophysical characteristics of the virus are not well characterised so it is difficult to determine the significance of indirect transmission by fomites.

2.2. Reservoir

Infected populations of fish, both farmed and wild, are the only established reservoirs of infection. The original source of TiLV is not known.

2.3 Risk factors (temperature, salinity, etc.)

Disease has been associated with transfer between ponds and thus may be associated with stress (Ferguson *et al.*, 2014; Dong *et al,* 2017). No other risk factors (temperature, salinity, etc.) have been identified as potential risk factors.

3. HOST RANGE

3.1. Susceptible species

Mortalities attributed to TiLV have been observed in wild tilapia *Sarotherodon (Tilapia) galilaeus*, farmed tilapia *Oreochromis niloticus* and commercial hybrid tilapia (*O. niloticus* X *O. aureus*) (Bacharach *et al.*, 2016; Ferguson *et al.*, 2014; Eyngor *et al.*, 2014). To date only tilapines have been shown to be susceptible. It is possible that other species will be found to be susceptible.

3.2. Affected life stage

In the outbreak reported by Ferguson *et al.* (2014) and Dong *et al.* (2017) fingerlings were mainly affected. Dong *et al.* (2017) reported approximately 90% mortality in red tilapia fingerlings within one months of stocking into cages. Mortality just over 9% in medium to large sized Nile tilapia was noted by Fathi *et al.* (2017). Other reports have not commented on different levels of mortality by life stage (Eyngor *et al.*, 2014).

3.3. Additional comments

There is some evidence that certain genetic strains of tilapia are resistant. Ferguson *et al.* (2014) noted that one strain of tilapia (genetically male tilapia) incurred a significantly lower level of mortality (10-20%) compared with other strains.

4. GEOGRAPHICAL DISTRIBUTION

TiLV has been reported in Colombia, Ecuador and Israel (Bacharach *et al.*, 2016; Ferguson *et al.*, 2014; Tsofack *et al.*, 2016); and most recently, in Egypt (Fathi *et al.*, 2017), Thailand (Dong *et al.*, 2017), India (Behera *et al.*, 2018), Malaysia (Amal *et al.*, 2018), and the Philippines (OIE, 2017), Uganda and Tanzania (Mugimba *et al.*, 2018). However, a lack of thorough investigation of all mortality incidents means that the geographic distribution of TiLV may be wider than currently. For example, reports of mortality in tilapia in Ghana and Zambia in 2016 have not been attributed to TiLV but the available information does not indicate that the presence of the virus has been investigated. A partial genome from Thailand showed relatively high variation to strains from Israel (around 97% nucleotide identity) (Dong *et al.,* 2017).

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5. CLINICAL SIGNS AND CASE DESCRIPTION

5.1. Host tissues and infected organs

The main organs where pathology is observed are the eyes, brain and liver (Eyngor *et al.*, 2014).

5.2. Gross observations and macroscopic lesions

Gross lesions included ocular alterations, including opacity of the lens and in advanced cases ruptured lens. Other lesions included skin erosions, haemorrhages in the leptomeninges and congestion of the spleen (Eyngor *et al.*, 2014).

5.3. Microscopic lesions and tissue abnormality

Histologic lesions have been observed in the brain, eye and liver (Eyngor *et al.*, 2014). Lesions in the brain included oedema, focal haemorrhages in the leptomeninges, and capillary congestion in both the white and grey matter *and* neural degeneration. Foci of gliosis and occasional perivascular cuffs of lymphocytes have been detected. Ocular lesions included ruptured lenticular capsule and cataractous changes. Foci of hepatocellular swelling were observed. The spleen was hyperplastic, with proliferating lymphocytes. Melanomacrophage centres (MMCs) were increased in size and number in both the liver and the spleen. Transmission electron microscopy confirmed the

presence of an orthomyxo-like virus within diseased hepatocytes and thus confirmed earlier reports of syncytial hepatitis (del-Pozo *et al.*, 2016).

5.4. OIE status

Under consideration for listing but currently does not meet all of the criteria for listing as described in Chapter 1.2. of the *Aquatic Animal Health Code* (OIE, 2016).

6. SOCIAL AND ECONOMIC SIGNIFICANCE

Tilapines, comprising more than 100 species, are the second most import group of farmed fish worldwide after carp. Global production is estimated at 4.5 million metric tons with a current value in excess of U.S.$7.5 billion (FAO, 2014). In some regions they are ecologically important (algae and mosquito control and habitat maintenance for shrimp farming) and an important wild capture species. Introduction of the virus has been shown to cause significant mortality (up to 90%) and thus result in serious economic losses to both farmers and fishers (Eyngor *et al.*, 2014; Dong *et al.*, 2017).

7. ZOONOTIC IMPORTANCE

None.

8. DIAGNOSTIC METHODS

8.1. Definition of suspicion

High levels of mortality in tilapine species, associated with ocular alterations (opacity of the lens or more severe pathology), should be considered suspicious of TiLV. Skin erosions, haemorrhages in the leptomeninges and moderate congestion of the spleen and kidney may be observed on post-mortem.

8.2. Presumptive test methods

TiLV can be cultured in primary tilapia brain cells or in an E-11 cell line, inducing a cytopathic effect at 3-10 days (Eyngor *et al.*, 2014) (Liamnimitr *et al.*, 2017). Tsofack *et al.* (2016) describe optimal conditions for culturing TiLV.

8.3. Confirmatory test methods

A PCR primer set has been designed and a reverse transcriptase (RT) PCR has been developed (Eyngor *et al.*, 2014). A more sensitive, nested RT-PCR has been published and is suitable for the detection of TiLV in clinical cases (Tsofack *et al.*, 2016). Most recently a semi-nested RT-PCR with an improved analytical sensitivity (7.5 viral copies per reaction)~~, has been published~~ (Dong *et al.,* 2017)~~, as has~~ a real-time SYBR assay with analytical sensitivity of 2 copies of plasmid (Tattiyapong *et al.*, 2017) and a TaqMan RT-qPCR assay (Waiymitra *et al.*, 2018) have been described. All molecular tests require further validation.

9. CONTROL METHODS

Restrictions on the movement of live tilapines from farms and fisheries where the virus is known to occur will limit the spread of the disease. Generic biosecurity measures to minimise fomite spread via equipment, vehicles or staff (i.e. cleaning and disinfection) should also be implemented.

Currently, no published methods have been shown to be effective in limiting the impact of an outbreak on an infected farm. It has been suggested that breeding for resistance or the development of a vaccine may offer the long term prospects for managing the disease (Ferguson *et al.*, 2014). A breeding programme would need to select and test a range of different strains of tilapia with a view to finding those least susceptible.

10. TRANSMISSION RISK

As TiLV has been horizontally transmitted through cohabitation, disease transmission is likely with movement of live aquatic animals. There is limited information about TiLV biophysical properties and the risks associated with aquatic animal products. However, it may be assumed that it will share properties of other aquatic orthomyxoviruses, such as infectious salmon anaemia virus. Current evidence suggests that the eye, brain and liver are likely to contain highest concentrations of TiLV and thus solid and liquid waste is likely to be contaminated. However, it is possible that the pathogenic agent may also be found in musculature of infected fish. TiLV has been detected by real-time RT-PCR and virus isolation in mucous but not faeces (Liamnimitr *et al.*, 2017).

11. ADDITIONAL USEFUL INFORMATION

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Annex 20

Original: English

September 2018

REPORT OF THE MEETING OF THE OIE *AD HOC* GROUP ON SUSCEPTIBILITY OF FISH SPECIES TO INFECTION WITH OIE LISTED DISEASES

Paris, 2–4 May 2018

The OIE *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases (the *ad hoc* Group) met for their fourth meeting at OIE Headquarters from 2 to 4 May 2018.

The list of participants and the Terms of Reference are presented in Annex I and Annex II, respectively.

Dr Stian Johnsen, Standards Department, welcomed members to this meeting and thanked the *ad hoc* Group for their ongoing work on this important topic.

The chair of the *ad hoc* Group, Dr Mark Crane, clarified that the primary purpose of this meeting was to finalise applying the criteria to host species to determine susceptibility to infection with infectious haematopoietic necrosis virus (IHNV) and to start work on applying the criteria to determine susceptibility to infection with viral haemorrhagic septicaemia virus.

The *ad hoc* Group applied the three-stage approach, outlined in Article 1.5.3. of the *Aquatic Code*, to assess susceptibility of a species to IHNV, as described below:

1) criteria to determine whether the route of transmission is consistent with natural pathways for the infection (as described in Article 1.5.4.);

2) criteria to determine whether the pathogenic agent has been adequately identified (as described in Article 1.5.5.);

3) criteria to determine whether the evidence indicates that presence of the pathogenic agent constitutes an infection (as described in Article 1.5.6.).

Stage 1: criteria to determine whether the route of transmission is consistent with natural pathways for the infection (as described in Article 1.5.4.)

Route of infection Key

N: Natural infection.

E: Experimental (non-invasive).

EI: Experimental (invasive).

Most references that reported invasive experimental procedures as the route of transmission were not assessed beyond Stage 1 (i.e. Article 1.5.4.).

Annex 20 (contd)

Stage 2: criteria to determine whether the pathogenic agent has been adequately identified as described in Article 1.5.5.

Accurate pathogen identification might not have been carried out in older publications because molecular typing techniques were not available at the time. In these circumstances a weight of evidence approach, using combined data from relevant studies, were considered and used to assess susceptibility.

Stage 3: criteria to determine whether the evidence indicates that presence of the pathogenic agent constitutes an infection as described in Article 1.5.6.

Criteria A to D in Article 1.5.6. were used to determine if there was sufficient evidence for infection with the pathogenic agent in the suspect host species. Evidence to support criterion A alone was sufficient to determine infection. In the absence of evidence to meet criterion A, satisfying at least two of criteria B, C or D were required to determine infection.

A. The pathogenic agent is multiplying in the host, or developing stages of the pathogenic agent are present in or on the host;

B. Viable pathogenic agent is isolated from the proposed susceptible species, or infectivity is demonstrated by way of transmission to naïve individuals;

C. Clinical or pathological changes are associated with the infection;

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

Hosts that were classified as susceptible species (as described in Article 1.5.7.) were proposed for inclusion in Article 10.6.2. of Chapter 10.6. 'Infection with infectious haematopoietic necrosis virus' of the *Aquatic Code.*

Hosts that were classified as species for which there is partial evidence for susceptibility (as described in Article 1.5.8.) were proposed for inclusion in a new Section 2.2.2. *Species with incomplete evidence for susceptibility* of Chapter 2.3.4. 'Infectious haematopoietic necrosis' of the *Aquatic Manual*.

The detailed assessments for IHNV assessed by the *ad hoc* Group are provided in Annex III.

The *ad hoc* Group wished to note the following:

The *ad hoc* Group requested that another physical meeting be held in 2018 to continue assessments for infection with viral haemorrhagic septicaemia virus and to start assessments for infection with red sea bream iridovirus.

_____

…/Annexes

Annex 20 (contd)

Annex I

REPORT OF THE MEETING OF THE OIE *AD HOC* GROUP ON SUSCEPTIBILITY OF FISH SPECIES TO INFECTION WITH OIE LISTED DISEASES

Paris, 2–4 May 2018

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Annex 20 (contd)

Annex II

REPORT OF THE MEETING OF THE OIE *AD HOC* GROUP ON SUSCEPTIBILITY OF FISH SPECIES TO INFECTION WITH OIE LISTED DISEASES

Paris, 2–4 May 2018

Terms of reference

Background

A new Chapter 1.5. 'Criteria for listing species as susceptible to infection with a specific pathogen' was introduced in the 2014 edition of the *Aquatic Code*. The purpose of this chapter is to provide criteria for determining which host species are listed as susceptible in Article X.X.2. of each disease-specific chapter in the *Aquatic Code.* The criteria are to be applied progressively to each disease-specific chapter in the *Aquatic Code*.

These assessments will be undertaken by *ad hoc* Groups and the assessments will be provided to Member Countries for comment prior to any change in the list of susceptible species in Article X.X.2. of the disease-specific chapters in the *Aquatic Code.*

For species where there is some evidence of susceptibility but insufficient evidence to demonstrate susceptibility through the approach described in Article 1.5.3., information will be included in the relevant disease-specific chapter in the *Aquatic Manual*.

Purpose

The *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases will undertake assessments for the ten OIE listed fish diseases.

Terms of Reference

1. Consider evidence required to satisfy the criteria in Chapter 1.5.

2. Review relevant literature documenting susceptibility of species for OIE listed fish diseases.

3. Propose susceptible species for OIE listed diseases for fish based on Article 1.5.7.

4. Propose susceptible species for OIE listed diseases for fish based on Article 1.5.8.

Expected outputs of the *ad hoc* Group

1. Develop a list of susceptible species for inclusion in the relevant Article X.X.2. of fish disease-specific chapters in the *Aquatic Code.*

2. Develop a list of species with incomplete evidence for susceptibility for inclusion in Section 2.2.2. of the *Aquatic Manual.*

3. Draft a report for consideration by the Aquatic Animals Commission at their September 2018 meeting.

Annex 20 (contd)

Annex III

ASSESSMENT OF HOST SUSCEPTIBILITY TO INFECTION WITH INFECTIOUS HAEMATOPOIETIC NECROSIS VIRUS

Criteria for susceptibility to infection with infectious haematopoietic necrosis virus (IHNV) are detailed in Table 1 (as per Article 1.5.6. of the *Aquatic Code*). This table includes Replication (A), Viability/Infectivity (B), Pathology/Clinical Signs (C) and Location (D). Hosts were considered to be infected with IHNV if they fulfilled either criterion A, or at least two of criteria B, C and D (as per point 3 of Article 1.5.7. of the *Aquatic Code*).

Table 1. *Criteria for susceptibility to infection with IHNV*

A: Replication	B: Viability / Infectivity *	C: Pathology / Clinical signs	D: Location*
Sequential virus titration showing increase in viral titres or high virus titres in internal organs (>10^5 $TCID_{50}$/g) **OR** TEM **OR** Immunohistochemistry **OR** Product of virus replication detected	Isolation of virus from internal organs by cell culture **OR** Passage to a susceptible host	Gross signs include lethargy interspersed with bouts of frenzied, abnormal activity, darkening of the skin, pale gills, ascites, distended abdomen, exophthalmia, and petechial haemorrhages externally. Internally, pathology includes petechial haemorrhages in visceral organs and/or muscle and heart; necrotic kidney, spleen, liver, swollen spleen, anaemia and ascites.	Recover virus from internal organs **OR** RT-PCR from internal organs

* Where gills and intestine are used, surface contamination should be ruled out.

Pathogen identification for IHNV:

Pathogen isolation on EPC, FHM, or CHSE cell lines with confirmation using immunological or molecular test. Immunological test could include virus neutralization, IFAT, or ELISA. Molecular tools include RT-PCR, DNA probes, sequencing. RT-PCR could also be done directly on infected tissues.

Annex 20 (contd)

Annex III (contd)

ASSESSMENT FOR HOST SUSCEPTIBILITY

The assessments for host susceptibility to infection with IHNV are provided in Table 2.

Table 2. *Outcome of assessments for host susceptibility to infection with IHNV*

Genus	Species	Common name	Stage 1: Transmission[1]	Stage 2: Pathogen identification	Stage 3: Evidence for infection[2]				Outcome	References
					A	B	C	D[3]		
Salmo	*salar*	Atlantic Salmon	N and E	Culture, neutralisation and RT-PCR	ND	Y	Y	Y	1	Armstrong *et al.*, 1993; St-Hilaire *et al.*, 2002
Salmo	*trutta*	Brown trout	E and N	Culture confirmed with serum neutralization published in other LaPatra *et al.*, 1990 paper	ND	Y	Y	N	1	LaPatra *et al.*, 1990; Rexhepi *et al.*, 2011
Salmo	*marmoratus*	Marble trout	E	Culture and PCR	ND	Y	Y	Y	1	Pascoli *et al.*, 2015
Salvelinus	*namaycush*	Lake trout	E	Culture and DNA probe	Y	Y	Y	N	1	Follett *et al.*, 1997
Salvelinus	*fontinalis*	Brook trout	N	Culture and RT-PCR and IFAT	Y	Y	Y	Y	1	Zhu *et al.*, 2013; Bootland *et al.*, 1994
Salvelinus	*alpinus*	Arctic char	E	Culture and ELISA	ND	Y	Y	N	1	McAllister *et al.*, 2000

Annex 20 (contd)

Annex III (contd)

Genus	Species	Common name	Stage 1: Transmission[1]	Stage 2: Pathogen identification	Stage 3: Evidence for infection[2]				Outcome	References
					A	B	C	D[3]		
Oncorhynchus	*tshawytscha*	Chinook salmon	N	Culture and serum neutralisation	Y	Y	Y	N	1	Follett *et al.*, 1987; Arkush *et al.*, 2004; St-Hilaire *et al.*, 2001
Oncorhynchus	*keta*	Chum salmon	N	Culture and serum neutralisation	ND	Y	Y	N	1	Follett *et al.*, 1987; Yoshimizu *et al.*, 1993
Oncorhynchus	*kisutch*	Coho	N	Culture and serum neutralisation	Y	Y	N	Y	1	Eaton *et al.*, 1991; LaPatra *et al.*, 1989; Helmick *et al.*, 1995; Hedrick *et al.*, 1995
Oncorhynchus	*masou*	Masu salmon	N	Culture and immunoassay	ND	Y	N	Y	1	Yoshimizu *et al.*, 1993
Oncorhynchus	*mykiss*	Rainbow trout	E and N	Culture and RT-PCR	ND	Y	Y	Y	1	Pascoli *et al.*, 2015; LaPatra *et al.*, 1993; Haenen *et al.*, 2016
Oncorhynchus	*nerka*	Sockeye salmon	E	Culture and DNA probe	Y	Y	Y	N	1	Follett *et al.*, 1997; Yoshimizu *et al.*, 1993
Oncorhynchus	*masou rhodurus*	Biwa trout subspecies of Masu	Subspecies of Masu (see Masu salmon)						1	Yamazaki & Motonishi, 1992
Oncorhynchus	*clarkii*	Cutthroat trout	E	Culture of known isolate 220-90	ND	Y	Y	N	1	LaPatra *et al.*, 1994
Clupea	*pallasii*	Pacific Herring	N	Virus culture and DNA probe or neutralization test	ND	Y	N	N	2	Kent *et al.*, 1998; Hart *et al.*, 2011
Cymatogaster	*aggregata*	Shiner perch	N	Virus culture and DNA probe or Neutralization test	ND	Y	N	N	2	Kent *et al.*, 1998

Annex 20 (contd)
Annex III (contd)

Genus	Species	Common name	Stage 1: Transmission[1]	Stage 2: Pathogen identification	Stage 3: Evidence for infection[2]				Outcome	References
					A	B	C	D[3]		
Aulorhynchus	*flavidus*	Tube-snout	N	Virus culture and DNA probe or neutralization test	ND	Y	N	N	2	Kent *et al.*, 1998
Acipenser	*transmontanus*	White sturgeon	E/EI	Cell culture but no confirmation	Y	Y	N	N	2	LaPatra *et al.*, 1995
Esox	*lucius*	Northern pike	N	Culture and ELISA	ND	Y	Y	N	2	Reschova *et al.*, 2008 ; Dorson *et al.*, 1987
Lota	*maxima*	Burbot	E	Culture and PCR	ND	Y	N	N	2	Polinski *et al.*, 2010
Oncorhynchus	*gorbuscha*	Pink salmon	E	Culture negative	ND	N	N	N	3	Follettt *et al.*, 1997
Thymallus	*thymallus*	Grayling	E	Culture negative	ND	N	N	N	3	Follett *et al.*, 1997
Plecoglossus	*altivelis*	Ayu sweetfish	N	Gene sequencing on stored isolate	ND	N	N	N	3	Nishizawa *et al.*, 2006
Anguilla	*anguilla*	European eel	N	Culture but not confirmed	ND	N	N	N	3	Bergmann *et al.*, 2003; Jorgensen *et al.*, 1994
Cyprinus	*carpio*	Common carp	E	Culture and qRT-PCR	ND	N	N	N	3	Palmer *et al.*, 2014
Perca	*flavescens*	American yellow perch	E	Culture and qRT-PCR	ND	N	N	N	3	Palmer *et al.*, 2014
Lepeophtheirus	*salmonis*	Salmon lice	E	Culture and PCR	ND	Y	N	N	#	Jakob *et al.*, 2011
Callibaetis	sp.	Mayfly	N	Culture and antibody neutralisation	ND	Y	N	N	#	Shors & Winston, 1988

Invertebrate species.

Annex 20 (contd)

Annex III (contd)

[1] **Route of Infection Key**
N: Natural infection.
E: Experimental (non-invasive).
EI: Experimental (invasive).

[2] **Evidence for Infection Key**
ND: Not determined.
N: Evidence do not indicate that presence of the pathogenic agent constitutes an infection.
Y: Evidence do indicate that presence of the pathogenic agent constitutes an infection.

[3] **"N"** in this column captures cases in which the tissue tested included external organs such as skin, gill, or gastrointestinal tissues or cases in which tested tissues were found negative.

Outcome system

The following outcome were assigned to each host evaluated based on the rules outlined above.

1: Meets the criteria for susceptibility; and be included in Article 10.6.2. of Chapter 10.6. 'IHNV' of the *Aquatic Code*.

2: Some but not all the criteria have been met; and be proposed for inclusion in a new Section 2.2.2. *Species with incomplete evidence for susceptibility* of Chapter 2.3.4. 'IHNV' of the *Aquatic Manual*.

3: Criteria for susceptibility have not been met.

4: Evidence of non-susceptibility.

Additional information relevant to assessments for IHNV

For species within the Salmonidae family, the *ad hoc* Group agreed that susceptibility could be determined based on only one study because of the broad host range of IHNV within this family. However, for animals belonging to other families the *ad hoc* Group required two studies to demonstrate susceptibility to infection with the pathogenic agent.

Turbot (*Psetta maxima*)

The *ad hoc* Group agreed the outcome for turbot be a "2" because there was no definitive identification of the virus from the fish that died in the study and the life stage of fish suggested they were not immunocompetent.

Recommendations

The *ad hoc* Group recommended that invertebrate species assessed and listed in Table 2 be included in Section 2.2.6. *Vectors* of Chapter 2.3.4. 'Infectious haematopoietic necrosis' of the *Aquatic Manual*. The *ad hoc* Group considered invertebrate species to be vectors for transmission of IHNV rather than true susceptible species because it was difficult to determine viral replication within the insect.

Annex 20 (contd)

Annex III (contd)

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Annex 21

Original: English

February to August 2018

REPORT OF THE ELECTRONIC *AD HOC* GROUP ON TILAPIA LAKE VIRUS

February to August 2018

The OIE electronic *ad hoc* Group on Tilapia lake virus (TiLV) was established in November 2017 to evaluate published and unpublished methods for detection of TiLV, describe the level of validation of each method and determine additional validation requirements, recommend any additional assays that may need to be developed, facilitate the sourcing and distribution of well-characterised positive control material for method evaluation, and implement inter-laboratory comparability studies.

This report covers activities and achievements of the *ad hoc* Group from February to August 2018. The list of participants and Terms of Reference are presented in Annexes I and II.

Activities, achievements and next steps

Following a request by the Aquatic Animal Health Standards Commission, in May 2018 countries who had reported the presence of Tilapia lake virus (TiLV) were contacted by the OIE Director General requesting them to consider providing positive TiLV control material to the OIE Collaborating Centre for New and Emerging Diseases and Diagnostic Test Validation, at the CSIRO Australian Animal Health Laboratory (AAHL) for molecular test evaluation and inter-laboratory comparability studies.

The *ad hoc* Group was pleased to report that to date, material containing infectious TiLV has been received (or is in the process of being organised) from Chinese Taipei, Israel, Peru and Thailand.

The *ad hoc* Group's work is currently focused on point 5. of the Terms of Reference which is being led by the OIE Collaborating Centre for New and Emerging Diseases, Australian Animal Health Laboratory, Australia, i.e.

TOR 5: Develop and implement a work plan for inter-laboratory comparability studies lAim: To develop and implement a plan for investigation of the inter-laboratory comparability of the following assays for the molecular detection of TiLV:

1. Conventional semi-nested assay (RT-nPCR) described by Dong *et al.* (2017).

2. Real-time SYBR assay (RT-qPCR) described by Tattiyapong *et al.* (2017).

3. Real-time probe-based assay (RT-qPCR) which is unpublished and has been provided by the *ad hoc* Group member Dr Prof. Hong Liu.

Annex 21 (contd)

Methodology:

Receipt of materials at AAHL

Material to be used in the inter-laboratory comparability studies will be sourced by *ad hoc* Group members and sent to the CSIRO Australian Animal Health Laboratory (AAHL) in Geelong, Australia. Material Transfer Agreements will be established to ensure the material supplied is only used to support the activities of the *ad hoc* Group. On arrival at AAHL, the presence of TILV in the materials will be confirmed by conventional PCR and sequence analysis, the virus will be amplified in E-ll cell cultures and stored in liquid nitrogen.

Amplification of TiLV and generation of positive control material

TiLV will be amplified in E-ll cell cultures and the end-point dilution determined using 10-fold dilutions with each of the TiLV molecular tests to ensure the material is suitable for preparation of the inter-laboratory comparability panel. This will also enable comparison of the analytical sensitivity (ASe) of the molecular assays. Clarified cell culture supernatant will be gamma-irradiated at 50kGy and tested to determine the degree of degradation of the TiLV RNA by the gamma-irradiation. Previous work done at AAHL with other finfish viruses suggests this will not render the material unable to be used.

If the TiLV molecular tests perform as expected preliminary assessment of analytical specificity (ASp) will be undertaken for each of the TiLV molecular tests using a nucleic acid extracted from a number of finfish viruses held at AAHL.

Inter-laboratory comparability panel

The inter-laboratory comparability panel would consist of 20 positive and 10 negative samples that would include:

1. 10-fold dilution series (7 samples) to enable estimates of efficiency of real-time molecular assays;
2. Strong positive (at least 2 samples);
3. Medium positive (at least 2 samples);
4. Weak positive (at least 2 samples);
5. 10-fold dilution of medium and low positive;
6. Positive samples with various viral concentrations to make up the 20 positive samples;
7. Negative samples consisting of supernatant of uninfected cell culture (10 samples).

Material will be provided as a gamma-irradiated cell culture supernatant with 50µL extracted and tested.

Not explicitly stating the planned composition of the panel is not to act as a test of the capability of the *ad hoc* Group member's laboratories, it is simply that it is good laboratory practice to provided "blinded" samples to participants undertaking this kind of test evaluation. Multiple aliquots of each of the different samples will be stored.

Homogeneity testing will be undertaken using 10 aliquots of each different concentration, with a coefficient of variation of <5% indicating homogeneity of the samples. Additional stability testing, using three aliquots of each sample will be undertaken at -20°C, 4°C and 22°C, at Day 0, Day 7 and Day 14, to make sure there are no stability issues with transport delays of the inter-laboratory comparability panel to *ad hoc* Group member's laboratories. Stability testing will also be undertaken when all laboratories have reported results to check the stability of stored aliquots at AAHL.

Participating laboratories will receive the samples as numbered tubes to test them blind with panels tested at least three times. Results will be reported back to the Chair of the *ad hoc* Group for collation and reporting back as uncoded results to the participating laboratories for discussion. Use of duplicate samples and 10-fold dilutions of samples will enable statistical analysis to determine repeatability and reproducibility.

Annex 21 (contd)

Initially, the inter-laboratory comparability panel will use different dilutions of a single TiLV isolate. When additional TiLV isolates are obtained from different geographical locations, ASp and ASe will be determined. Depending of the results new assays may need to be designed if not all isolates are detected by one, or more, of the molecular tests. Regardless, a second inter-laboratory comparability panel could be prepared as described above, but using several different geographical isolates of TiLV, to provide greater confidence on the robustness and repeatability of the tests.

The inter-laboratory comparability panel will also be provided to laboratories outside the *ad hoc* Group if these laboratories provide TiLV material can be used to support the activities of the *ad hoc* Group.

Result reporting

A result reporting sheet will be prepared which will request as much information as possible about the testing process in each laboratory. Information will include extraction methods/kits used and volume extracted, and volume eluted, molecular test reagents/kits used and reaction volume and template volume used and result recoding and interpretation, including threshold for real-time tests and cut-off determination. This will be provided prior to the shipment of the inter-laboratory comparability panel and a draft will be provided to *ad hoc* Group members for comment. It is highly desirable to have all testing and reporting submitted within one month of receipt of the panel.

Next steps

The *ad hoc* Group will continue their work and will report back progress to the next meeting of the Aquatic Animals Commission in February 2019.

References:

DONG, H., SIRIROOB, S., MEEMETTA, W., SANTIMANAWONG., W., GANGNONNGIW, W., PIRARAT, N., KHUNRAE, P., & RATTANAROJPONG, T., VANICHVIRIYAKIT, R. & SENAPIN, S. (2017). Emergence of tilapia lake virus in Thailand and an alternative semi-nested RT-PCR for detection. *Aquaculture*, **476**, 111–118.

TATTIYAPONG P., SIRIKANCHANA K. & SURACHETPONG W. (2017). Development and validation of a reverse transcription quantitative polymerase chain reaction for tilapia lake virus detection in clinical samples and experimentally challenged fish. *Journal of Fish Diseases*, **41(2)**, 255–261.

…/Annexes

Annex 21 (contd)

Annex I

REPORT OF THE OIE *AD HOC* GROUP ON TILAPIA LAKE VIRUS

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Annex 21 (contd)

Annex I (contd)

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Annex 21 (contd)

Annex II

Terms of Reference

The electronic *ad hoc* Group should:

1. Critically review the available literature regarding detection methods for TiLV and any unpublished methods that may also be available.

2. Provide recommendations on additional method development requirements.

3. Provide recommendations on method validation requirements.

4. Determine sources of well-characterised viable and non-viable positive control material for use in method evaluation and implementation in laboratories.

5. Develop a work plan for inter-laboratory comparability studies.

6. Draft a report by the end of January 2018 to be considered by the Commission when they meet in February 2019.

Ad hoc Group members should be familiar with Chapter 1.2. *Criteria for listing aquatic animal diseases* and the use of relevant glossary definitions in the *Aquatic Code*, and with the principles and methods of validation of diagnostic essays for infectious diseases in Chapter 1.1.2. of the *Aquatic Manual*.

Annex 22

AQUATIC CODE					
Chapter/Subject	**Task**	**Status at September 2018**			
		Member Country comments considered by AAC	*Revised by the AAC*	*Number of times circulated for comments*	*Circulated for information*
Definition of 'basic biosecurity conditions'	Amend the definition to make it more explicit in its application to compartments	✔	✔	2	
Criteria for listing species as susceptible (Chapter 1.5.)	Develop a new Article 1.5.9. to address diseases with a wide host range/review existing text	✔	✔	4	
Infection with salmonid alphavirus (Articles 10.5.1. and 10.5.2.)	Amend after consideration of the work of the *ad hoc* Group; horizontal changes	✔	✔	2	
Infection with infectious haematopoietic necrosis virus (Article 10.6.2.)	Amend after consideration of the work of the *ad hoc* Group; apply horizontal changes and change name of the pathogenic agent		✔	1	
Infection with koi herpesvirus (Article 10.7.2.)	Amend after consideration of the work of the *ad hoc* Group; horizontal changes	✔	✔	2	
Infection with spring viraemia of carp virus (Article 10.9.2.)	Amend after consideration of the work of the *ad hoc* Group; applied horizontal changes	✔	✔	2	
Infection with ranavirus (Chapter 8.3.)	Amend text to align with revised listed name, i.e. Infection with *Ranavirus* species		✔	1	
Acute hepatopancreatic necrosis disease (Chapter 9.1.)	Review use of AHPND and AHPND (VP) throughout the chapter		✔	1	
Infection with *Aphanomyces invadans* (Articles 10.2.1. and 10.2.2.)	Amend to ensure consistency with other amended fish disease-specific chapters; amend to remove the use of italics on Family names for fish				✔
Model Article X.X.8.	Review M 'lifelong holding' and address the safe disposal of dead aquatic animals or products derived thereof	✔	✔	1	
New draft chapter on Biosecurity for Aquaculture Establishments	Develop draft chapter on Biosecurity for aquaculture establishments		✔	1	
Approaches for determining periods required to demonstrate disease freedom	Develop approaches for determining periods required to demonstrate disease freedom		✔	1	

Annex 22 (cont.)

UNDER CONSIDERATION FOR FUTURE WORK		
New chapter on emergency disease preparedness		To be convened in line with the AAHSC work programme
New chapter on disease outbreak management	Support articles in each disease-specific chapter on returning to freedom following an outbreak	To be convened in line with the AAHSC work programme
New chapter on application of zoning	Chapter specific to the application of zoning to provide clearer guidance on establishing zones for trade and disease control purposes	To be convened in line with the AAHSC work programme
Review structure and application for different trade purposes of articles in disease-specific chapters	Re-structure disease-specific articles for aquatic animal products and live aquatic animals not for human consumption, including ornamentals	To be convened in line with the AAHSC work programme

***AD HOC* GROUPS**		
Topic	**Last meeting**	**Next meeting**
***Ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases**	May 2018	November 2019
Electronic *ad hoc* Group on Tilapia lake virus	Reported to the AAHSC September 2018 meeting	Report to the AAHSC February 2019 meeting
***Ad hoc* Group on Safe commodities**	To be convened in line with the AAHSC work programme	
***Ad hoc* Group on Susceptibility of amphibian species to infection with OIE listed diseases**	To be convened in line with the AAHSC work programme	
***Ad hoc* Group on Susceptibility of mollusc species to infection with OIE listed diseases**	To be convened in line with the AAHSC work programme	

Annex 22 (cont.)

AQUATIC MANUAL					
Chapter/Subject	**Task**	**Status at September 2018**			
		Member Country comments considered by AAC	*Revised by the AAC*	*Number of times circulated for comments*	*Circulated for information*
Chapter 2.2.9. Infection with yellow head virus genotype 1 (Sections 2.2.1. and 2.2.2.)	Sort lists of species in the Sections in alphabetic order	✔	✔	2	
Chapter 2.3.6. Infection with salmonid alphavirus (Sections 2.2.1., 2.2.2., 4.3.1.1.2. and table 5.1.)	Amend after consideration of the work of the *ad hoc* Group; update information about the real-time reverse-transcription polymerase chain reaction; amend the ranking of histopathology for both presumptive and confirmatory diagnosis	✔	✔	2	
Chapter 2.3.7. Infection with koi herpesvirus (Sections 1, 2.2.1. and 2.2.2.)	Amend after consideration of the work of the *ad hoc* Group	✔	✔	2	
Chapter 2.3.4. Infection with infectious haematopoietic necrosis virus (Sections 1, 2.2.1., 2.2.2. and 2.2.6.)	Amend after consideration of the work of the *ad hoc* Group		✔	1	
Review of chapters that had been updated and reformatted using the new disease chapter template	Revise and reformat using the new template: Chapters 2.3.8. *Infection with red sea bream iridovirus* and 2.3.9. *Infection with spring viraemia of carp virus*.		✔		

Annex 22 (cont.)

UNDER CONSIDERATION FOR FUTURE WORK		
New draft chapter on Infection with *Batrachochytrium salamandrivorans*	Consider new draft chapter developed by some OIE experts	Comments for author. Next version to be considered at the AAHSC February 2019 meeting
Chapters 2.3.3 Infection with *Gyrodactylus salaris* and 2.3.5. Infection with HPR-deleted or HPR0 infectious salmon anaemia virus	Further review and update these two chapters	Updates put on hold until members of the *ad hoc* Group are available to assist with reformatted the chapters using the new template
AD HOC GROUPS		
Topic		**Status**
***Ad hoc* Group on the new Aquatic Manual template**		Commence work on applying the new chapter template to disease-specific chapters
REFERENCE CENTRES		
Topic	**Task**	**Status**
Applications for OIE Reference Centre status or changes of experts	Review applications for OIE Reference Centre status or nominations for changes of expert at the OIE Reference Laboratories	On-going on a case-by-case basis
OTHER WORK		
Topic	**Task**	**Status**
OIE Global Conference on Aquatic Animal Health (2–4 April 2019) in Santiago (Chile)	Work on finalising the programme for the OIE Global Conference	
Technical disease cards	Review and update disease cards for tilapia lake virus and Infection with *Batrachochytrium salamandrivorans*	Technical disease card revised at the AAHSC September 2018 meeting and sent for Member Country information. No amendments needed for *B. sal.*