Organisation Mondiale de la Santé Animale | World Organisation for Animal Health | Organización Mundial de Sanidad Animal

Annex 2

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February 2018

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

EU comment
The EU would like to commend the OIE Aquatic Animal Health Standards Commission for its work and for having taken into consideration EU comments on the Aquatic Code and Manual submitted previously.
A number of general comments on this report of the February 2018 meeting of the Aquatic Animals Commission as well as the intended positions of the EU on the draft Aquatic Code and Manual chapters proposed for adoption at the 86th OIE General Session are inserted in the text below, while specific comments are inserted in the text of the respective annexes to the report.
The EU would like to stress again its continued commitment to participate in the work of the OIE and to offer all technical support needed by the Aquatic Animals Commission and its *ad hoc* groups for future work on the Aquatic Code and Manual.

The OIE Aquatic Animal Health Standards Commission (hereinafter referred to as the Aquatic Animals Commission) met at OIE Headquarters in Paris from 14 to 21 February 2018. The list of participants is attached as **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 2017 meeting: Argentina, Australia, Canada, Chile, Chinese Taipei, Costa Rica, Fiji, Guatemala, Japan, Mexico, New Caledonia, Norway, Singapore, Switzerland, Thailand, the United States of America (USA) and the Member States of the European Union (EU).

The Aquatic Animals Commission reviewed comments that Member Countries had submitted and amended texts in the OIE *Aquatic Code* and *Aquatic Manual* where appropriate. The amendments are shown in the usual manner by 'double underline' and '~~strikethrough~~' and may be found in the Annexes to this report. The amendments made at this meeting are highlighted with a coloured background in order to distinguish them from those made at the September 2017 meeting.

The Aquatic Animals Commission considered all Member Country comments that were supported by a rationale. However, the Commission was not able to draft a detailed explanation of the reasons for accepting or not accepting every comment received.

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 relevant reports of *ad hoc* Groups, which include important information and are provided as annexes to Commission's reports. The Commission encourages Member Countries to review these reports together with the report of the Commission. The Commission reminded Member Countries that all of the OIE *ad hoc* Group reports (since 2016) are also provided on the OIE website as stand-alone documents (available at

http://www.oie.int/en/standard-setting/specialists-commissions-working-groups/scientific-commission-reports/ad-hoc-groups-reports/).

The table below lists the texts as presented in the Annexes. Member Countries should note that texts in **Annexes 3 to 28** are proposed for adoption at the 86th General Session in May 2018; **Annexes 29 to 36** are presented for Member Country comment; and **Annexes 37 to 40** are presented for information.

The Aquatic Animals Commission strongly encourages Member Countries to participate in the development of the OIE's international standards by preparing to participate in the process of adoption of texts in Annexes **3 to 28** at the 86th General Session. The Commission also invites the submission of comments on **Annexes 29 to 36** of this report. Comments should be submitted as specific proposed text changes, supported by a scientific rationale. Proposed deletions should be indicated by '~~strikethrough~~' and proposed additions with 'double underline'. 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 Commission's working documents.

Comments on **Annexes 29 to 36** of this report must reach OIE Headquarters by the **30 July 2018** to be considered at the September 2018 meeting of the Aquatic Animals Commission. Comments received after the due date will not be submitted to the Commission for consideration at their September 2018 meeting.

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

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1.7.	Criteria to assess the safety of aquatic animal commodities (Chapter 5.4.)	Annex 7	47
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1.10.2.	Infection with *Aphanomyces invadans* (epizootic ulcerative syndrome) (Chapter 10.2.)	Annex 13	77
1.10.3.	Infection with *Gyrodactylus salaris* (Chapter 10.3.)	Annex 14	83
1.10.4.	Infection with infectious salmon anaemia virus (Chapter 10.4.)	Annex 15	91

1.10.5.	Infection with salmonid alphavirus (Chapter 10.5.)	Annex 16	101
1.10.6.	Infectious haematopoietic necrosis (Chapter 10.6.)	Annex 17	109
1.10.7.	Koi herpesvirus disease (Chapter 10.7.)	Annex 18	117
1.10.8.	Red sea bream iridoviral disease (Chapter 10.8.)	Annex 19	123
1.10.9.	Spring viraemia of carp (Chapter 10.9.)	Annex 20	129
1.10.10.	Viral haemorrhagic septicaemia (Chapter 10.10.)	Annex 21	137
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4.2.	White spot disease (Chapter 2.2.8.)	Annex 23	145
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	Texts for Member Country comment:	**Annex number**	**Page number**
AQUATIC CODE			
1.3.	Glossary	Annex 29	225
1.5.	Criteria for listing species as susceptible (Chapter 1.5.); clean (A) and track changes (B)	Annexes 30 A and 30 B	227
1.10.5.	Article 10.5.2. Infection with salmonid alphavirus (Chapter 10.5.)	Annex 31	235
1.10.7.	Article 10.7.2. Koi herpesvirus disease (Chapter 10.7.)	Annex 32	237
1.10.9.	Article 10.9.2. Spring viraemia of carp (Chapter 10.9.)	Annex 33	239
AQUATIC MANUAL			
4.6.1.	Infection with salmonid alphavirus (Chapter 2.3.6.)	Annex 34	241
4.6.2.	Koi herpesvirus disease (Chapter 2.3.7.)	Annex 35	255
4.7.	Infection with yellow head virus genotype 1 (Chapter 2.2.9.) (Section 2.2.2.)	Annex 36	275
Item	**Annexes for Member Country information:**		**Page number**
3.1.	Report of the *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases	Annex 37	277
3.4.	Report of the electronic *ad hoc* Group on Tilapia lake virus	Annex 38	311
5.1.	Proposed new *Aquatic Manual* disease chapter template	Annex 39	321
J	Aquatic Animals Commission Work Plan for 2017/2018	Annex 40	339

A. MEETING WITH THE DIRECTOR GENERAL

The Aquatic Animals Commission met with Dr Monique Eloit, Director General, on 19 February 2018. Dr Eloit welcomed the Commission members and thanked them for their ongoing support and commitment noting that this was the last meeting of the current Commission.

Dr Eloit provided an update on the new process for election to the OIE Specialist Commissions. She noted that the report of the Evaluation Committee on the nomination for election to the OIE Specialist Commissions will be considered by the Council at their February 2018 meeting. She informed the Commission that the final list will be provided to OIE Delegates prior to the 2018 General Session. Dr Eloit also mentioned that preparations are underway for the development of the 7th Strategic Plan.

B. ADOPTION OF THE AGENDA

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

C. 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: alignment of relevant revised text in the User's Guide and Chapter 5.3. of the *Aquatic Code* and *Terrestrial Code* and the development of a guidance document on the application of the criteria for listing an OIE disease.

D. MEETING WITH THE PRESIDENT OF THE OIE BIOLOGICAL STANDARDS COMMISSION

The President of the Aquatic Animals Commission held a tele-conference with the President of the Biological Standards Commission during the week of the Aquatic Animals Commission meeting. The Presidents discussed issues of mutual interest in the *Aquatic* and *Terrestrial Manuals*, notably: 1. the diagnostic test kit evaluation process for the OIE Register; 2. the new template for *Aquatic Manual* disease-specific chapters; 3. the draft SOPs for Collaborating Centres and; 4. how the Aquatic Animals Commission is implementing the newly adopted SOPs for Reference Laboratories.

E. OIE *AQUATIC ANIMAL HEALTH CODE*

1. Texts circulated for Member Country comments at the September 2017 meeting

1.1. General comments

A Member Country requested clarification regarding the listed disease red sea bream iridovirus (RSIV) noting that Chapter 2.3.8. in the *Aquatic Manual* also references infectious spleen and kidney necrosis virus (ISKNV) which is beyond the definition of the disease as described in the *Aquatic Code*. They also recommended that the Aquatic Animals Commission consider listing infection with ISKNV as a separate disease in the near future. The Commission acknowledged that there are errors in the *Aquatic Manual* Chapter for RSIV, in particular with respect to how other megalocytiviruses are referred to. The Commission noted that this issue will be addressed when the chapter is revised following the assessments of susceptible species for RSIV by the *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases. The Commission also acknowledged that other megalocytiviruses (non-RSIV) are significant pathogens and proposed that the next Commission consider assessing ISKNV against the criteria for listing an aquatic animal disease.

In response to a Member Country comment to include antiparasitic agents when addressing the issue of antimicrobial resistance, the Aquatic Animals Commission suggested that this issue would need to be considered in the context of risks to animal and human health.

1.2. User's Guide

Comments were received from Australia, EU, New Caledonia and USA.

The Aquatic Animals Commission reviewed amendments that had been adopted in the User's Guide of the *Terrestrial Code* in 2016 and made amendments to the User's Guide in the *Aquatic Code* to ensure alignment, where relevant. In addition, the Commission also considered Member Country comments.

Some Member Countries did not support the proposal to change the wording from 'more stringent' to 'that exceed' in point 4 of Section B and point 4 of Section C (now point 6) of the User's Guide in the *Aquatic Code*. Although the Aquatic Animals Commission considered that 'exceed' was a more appropriate term than 'stringent' they agreed that this proposal should not be made, at this time, in order to ensure alignment between the *Aquatic* and *Terrestrial Codes*. The President of the Aquatic Animals Commission discussed this point with the President of the Code Commission and they agreed to reconsider this at a future time.

In response to a Member Country comment, and to improve readability, the Aquatic Animals Commission amended text in the first paragraph of point 5 (now point 7) in Section C on the safety of aquatic animal products for trade.

The two Presidents also agreed to align several amendments that had been suggested by Member Countries to the Aquatic Animals Commission but that were also relevant to the *Terrestrial Code* User's Guide.

The revised User's Guide is attached at **Annex 3** and is proposed for adoption at the 86th General Session in May 2018.

EU position

The EU thanks the OIE and in general supports the adoption of this modified User's guide.

A comment is inserted in the text below.

1.3. Glossary

No comments were received from Member Countries on the proposed amendments to Glossary definitions for aquatic animal health status, biosecurity, biosecurity plan, self-declaration of freedom from disease and susceptible species.

The revised Glossary definitions for aquatic animal health status, biosecurity, biosecurity plan, self-declaration of freedom from disease and susceptible species are attached at **Annex 4** and are proposed for adoption at the 86th General Session in May 2018.

EU position

The EU supports the adoption of this modified Glossary.

Basic biosecurity conditions

The Aquatic Animals Commission agreed to amend the definition for 'basic biosecurity conditions' having recognised that the definition needed to be more explicit in its application to compartments.

The term 'import' is commonly understood to mean movements of goods across international borders and, therefore, is not appropriate to describe the movements of aquatic animals or products into compartments. Although the word 'import' has been removed, it should be noted that the 'requirements to prevent introduction of the pathogenic agent' apply to movements of aquatic animals or products into a country, a zone or a compartment as relevant (to maintain a disease-free status at the appropriate level). The definition has also been amended to refer to requirements to prevent the spread of pathogenic agents from areas where they have been detected, i.e. infected and protection zones.

The Aquatic Animals Commission agreed that the amended definition is more appropriate for current uses of 'basic biosecurity conditions' throughout the *Aquatic Code*.

The revised Glossary definition for 'basic biosecurity conditions' is attached at **Annex 29** for Member Country comments.

EU comment

The EU in general supports the proposed changes to the Glossary.

A comment is inserted in the text of Annex 29.

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

Comments were received from EU and Thailand.

No comments were received regarding the proposed name changes, therefore the amended names in Article 1.3.1. will be proposed for adoption.

Tilapia lake virus

The Aquatic Animals Commission reconsidered the assessment of Tilapia lake virus (TiLV) against the criteria for listing an aquatic animal disease in light of the report of the *ad hoc* Group on Tilapia lake virus (see Agenda Item 3.4.). The Commission agreed that there remains insufficient information concerning analytical and diagnostic specificity and sensitivity of the assay to meet criterion 3 of the listing criteria.

The Aquatic Animals Commission noted that new outbreaks of TiLV continue to be reported and once again reminded Member Countries that TiLV meets the definition of an "emerging disease" and, as such, should be reported in accordance with Article 1.1.4. of the *Aquatic Code*.

The Aquatic Animals Commission also encouraged Member Countries to investigate mortality and morbidity events in tilapines, emphasising that an understanding of the geographic distribution of TiLV is essential for efforts to control its possible spread.

The revised Chapter 1.3. *Diseases listed by the OIE* is attached at **Annex 5** and is proposed for adoption at the 86th General Session in May 2018.

EU position

The EU thanks the OIE and supports the adoption of this modified chapter.

1.5. Criteria for listing species as susceptible to infection with a specific pathogen (Chapter 1.5.)

Comments were received from Australia, Canada, EU, Thailand and USA.

In response to Member Country comments the Aquatic Animals Commission added some new text in Article 1.5.1. to clarify the purpose of the chapter. The Commission also amended text in the scope to improve readability.

The Aquatic Animals Commission noted that of the Member Country comments received, all but one was in support of the intent of the proposed new Article 1.5.9. The article includes criteria to determine susceptibility of host species at a taxonomic ranking higher than species, for diseases that have a broad host range.

Whilst most Member Countries supported the intent of the new article, the Aquatic Animals Commission recognised that there remained some uncertainty about how the article would apply in practice. The Commission considered Member Country comments, made relevant amendments to clarify the text, and provided further explanation below.

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 provided the following examples of how the threshold for application of Article 1.5.9. might apply.

Disease	**Number of host families susceptible (based on *ad hoc* Group assessments against Articles 1.5.1. to 1.5.8.)**	**Outcome**
Infection with infectious salmon anaemia	1 (Salmonidae)*	Article 1.5.9. does not apply. All susceptible species to be listed individually.
Infection with salmonid alphavirus	2 (Salmonidae and Pleuoronectidae)**	Article 1.5.9. does not apply. All susceptible species to be listed individually.
Infection with *Aphanomyces astaci*	More than 3 (e.g. Astacidae, Cambaridae, Parastacidae, Potamidae)#	Article 1.5.9. would be applied. If the criteria of 1.5.9. were met listing of susceptibility may be at a taxonomic ranking higher than species.

Infection with white spot syndrome virus	More than 3 (e.g. Penaeidae, Paniluridae, Portunidae, Palaemonidae)[##]	Article 1.5.9. would be applied. If the criteria of 1.5.9. were met listing of susceptibility may be at a taxonomic ranking higher than species.

* Refer to the April 2017 report of the *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases report available at: http://www.oie.int/en/standard-setting/specialists-commissions-working-groups/scientific-commission-reports/ad-hoc-groups-reports/

** Refer to the November 2017 report of the *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases report attached at Annex 37.

[#] Refer to the October 2015 report of the *ad hoc* Group on Susceptibility of crustacean species to infection with OIE listed diseases report available at: http://www.oie.int/en/standard-setting/specialists-commissions-working-groups/scientific-commission-reports/ad-hoc-groups-reports/

[##] Refer to the June 2016 report of the *ad hoc* Group on Susceptibility of crustacean species to infection with OIE listed diseases report available at: http://www.oie.int/en/standard-setting/specialists-commissions-working-groups/scientific-commission-reports/ad-hoc-groups-reports/

The revised Chapter 1.5. *Criteria for listing species as susceptible to infection with a specific pathogen* is attached at **Annexes 30A and 30B** for Member Country comments.

EU comment

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

A comment is inserted in the text of Annex 30B.

1.6. OIE procedures relevant to the Agreement on the Application of Sanitary and Phytosanitary Measures of the World Trade Organization (Chapter 5.3.)

Comments were received from Australia, EU and Switzerland.

The Aquatic Animals Commission considered Member Country comments and amended the text, as appropriate, to improve readability whilst ensuring alignment, where relevant, to the corresponding chapter in the *Terrestrial Code*.

Some Member Countries did not support the proposal to change the wording from 'more stringent' to 'that exceed' in Articles 5.3.1. and 5.3.5. Although the Aquatic Animals Commission considered that 'exceed' was a more appropriate term than 'stringent' they agreed that this proposal should not be made, at this time, in order to ensure alignment between the *Aquatic* and *Terrestrial Codes*. The President of the Aquatic Animals Commission discussed this point with the President of the Code Commission and they agreed to reconsider this at a future time.

The revised Chapter 5.3. *OIE procedures relevant to the Agreement on the Application of Sanitary and Phytosanitary Measures of the World Trade Organization* is attached at **Annex 6** and is proposed for adoption at the 86th General Session in May 2018.

EU position

The EU thanks the OIE and supports the adoption of this modified chapter.

1.7. Criteria to assess the safety of aquatic animal commodities (Chapter 5.4.)

Comments were received from Australia, EU and Thailand.

The Aquatic Animals Commission considered Member Country comments and amended the text, as appropriate, to improve readability.

The Aquatic Animals Commission did not agree with a Member Country that products for retail trade are excluded from the scope of Article 5.4.1. The Commission clarified that Article 5.4.1. applies to aquatic animal products 'for any purpose' and therefore aquatic animal products meeting the criteria in Article 5.4.1. would be safe for retail trade. It reminded Member Countries that Article 5.4.2. applies only to aquatic animal products that are for retail trade for human consumption.

The Aquatic Animals Commission did not agree with a Member Country comment that point 1 a) in Article 5.4.1. should include text referring to amounts of pathogenic agent able to cause infection so as to align with text in Chapter 2.2. of the *Terrestrial Code.* The Commission wished to note that the criteria in Chapter 5.4. of the *Aquatic Code* were developed prior to those in the *Terrestrial Code* and take into account risk factors associated with determining the safety of assessed aquatic animal products. For Article 5.4.1. products need to have been either i) treated to inactivate the pathogenic agent or ii) have no pathogenic agent present. If the pathogenic agent were present even at low levels then disease risk would need to be evaluated in consideration of a variety of factors, such as potential pathways for introduction, presence of susceptible host species, or environmental conditions.

In response to several Member Country comments, the Aquatic Animals Commission agreed to amend criterion 2 in Article 5.4.2., to improve the interpretation of this criterion, by removing the word 'small' and instead referring to the intended outcome, i.e. 'that is unlikely to result in the introduction and establishment of the pathogenic agent'.

The Aquatic Animals Commission noted that the proposed amendment to criterion 2 would not change the result of previous assessments because the intended outcome of this criterion (i.e. whether the product can transmit the pathogenic agent) is unchanged.

The revised Chapter 5.4. *Criteria to assess the safety of aquatic animal commodities* is attached at **Annex 7** and is proposed for adoption at the 86th General Session in May 2018.

EU position

The EU thanks the OIE and supports the adoption of this modified chapter.

1.8. Amendments to amphibian disease-specific chapters

Comments were received from Australia and EU.

Horizontal amendments

The Aquatic Animals Commission agreed with a Member Country comment to italicise the family name i.e. '*Iridoviridae'* in 8.X.1. and would ensure that this is applied in all disease-specific chapters for viral diseases.

The Aquatic Animals Commission applied amendments proposed in the model Articles X.X.8., X.X.9., X.X.10. and X.X.11. in the following chapters (see Agenda Item 1.11.).

1.8.1. New draft chapter for Infection with *Batrachochytrium salamandrivorans* (Chapter 8.X.)

The Aquatic Animals Commission agreed to put 'under study' wherever there is a reference to the corresponding chapter in the *Aquatic Manual*, given that this chapter has not yet been developed.

In response to a Member Country comment on the proposed list of susceptible species, the Aquatic Animals Commission noted that the proposed list of susceptible species in Article 8.X.2. is based on a recent European Food Safety Authority report. As these species have not been assessed against the criteria in Chapter 1.5., the Commission placed these species 'under study'. The Commission requested that an *ad hoc* Group be convened to undertake these assessments. The Commission also requested that the *ad hoc* Group undertake assessments for the list of susceptible species for Chapter 8.1. *Infection with Batrachochytrium dendrobatidis* considering that the two species are

closely related. OIE Headquarters informed the Commission the request to convene a new *ad hoc* Group would have to be prioritised against other work being undertaken by *ad hoc* Groups.

The Aquatic Animals Commission did not agree with a Member Country comment to delete 'basic biosecurity conditions' in point 4 *c)* in Articles 8.X.4. and 8.X.5., as they considered that basic biosecurity conditions are as applicable to wildlife as they are to aquaculture animals e.g. movement requirements.

The Aquatic Animals Commission did not agree to delete point 4 *b)* in Articles 8.X.4. and 8.X.5. as they considered that eradication, although less likely in wild populations, would be feasible in some situations e.g. public aquaria or zoos, and therefore was important to maintain.

In response to a Member Country comment, the Aquatic Animals Commission clarified that Article 8.X.10. does not apply to ornamental or hobby uses because risk management measures would need to be specific for these purposes. The Commission noted that a risk management option for ornamental trade is to source animals from countries that are free from infection with *B. salamandrivorans*. The Commission acknowledged that there is a need to develop specific guidance as to how disease risks associated with ornamental trade can be managed.

EU comment

The EU thanks the OIE for the above clarification. However, since according to the AAHSC Article 8.X.10. does not apply to movements of animals for "ornamental and hobby uses", the delimitations should clearly be stated in the chapter. Furthermore, it should also be defined in the Code what "ornamental and hobby uses" means, especially if this term also refers to animals that are moved with a commercial purpose, but at its final owner will be kept as a pet or an ornament.

The Aquatic Animals Commission agreed to consider how best to address this in their future work and will add this item to their work programme. The Commission reminded Member Countries that the definition for aquatic animals does include those used for ornamental purposes.

The revised Chapter 8.X. *Infection with Batrachochytrium salamandrivorans* is attached at **Annex 8** and is proposed for adoption at the 86th General Session in May 2018.

EU position

The EU thanks the OIE and in general supports the adoption of this modified chapter. The EU comment above should however be addressed by the AAHSC at its next meeting.

1.8.2. Infection with *Batrachochytrium dendrobatidis* (Chapter 8.1.)

No additional comments were received on the proposed amendments.

The revised Chapter 8.1. *Infection with Batrachochytrium dendrobatidis* is attached at **Annex 9** and is proposed for adoption at the 86th General Session in May 2018.

EU position

The EU supports the adoption of this modified chapter.

1.8.3. Infection with ranavirus (Chapter 8.2.)

No additional comments were received on the proposed amendments.

The revised Chapter 8.2. *Infection with ranavirus* is attached at **Annex 10** and is proposed for adoption at the 86th General Session in May 2018.

EU position

The EU supports the adoption of this modified chapter.

1.9. Infection with infectious hypodermal and haematopoietic necrosis virus (Articles 9.4.1. and 9.4.2.)

Comments were received from Mexico and New Caledonia.

At the request of a Member Country the Aquatic Animals Commission amended the name of the virus and the genus in Article 9.4.1. in line with the International Committee on Taxonomy of Viruses.

In response to a Member Country comment to change nomenclature for *Penaeus* to *Litopenaeus*, the Aquatic Animals Commission reminded Member Countries that they had reviewed literature on penaeid taxonomy and reported on their decision to use the *Penaeus* taxonomy in their September 2016 report (see page 11–12 http://www.oie.int/en/international-standard-setting/specialists-commissions-groups/aquatic-animal-commission-reports/meeting-reports/

The revised Articles 9.4.1. and 9.4.2. of the *Infection with infectious hypodermal and haematopoietic necrosis virus* chapter are attached at **Annex 11** and are proposed for adoption at the 86th General Session in May 2018.

EU position

The EU thanks the OIE and supports the adoption of this modified chapter.

1.10. Amendments to fish disease-specific chapters

Comments were received from Australia, Canada, Chinese Taipei, EU and Japan.

Horizontal amendments

The Aquatic Animals Commission agreed with a Member Country to italicise the family name e.g. '*Iridoviridae'* in Article 8.X.1. and requested that this be applied in all disease-specific chapters for viral diseases, including the fish chapters.

The Aquatic Animals Commission agreed with a Member Country that there was an inconsistency in the use of italics for parasite taxa at the level of family and above and made relevant amendments.

The Aquatic Animals Commission applied amendments proposed in the model Articles X.X.8, X.X.9., X.X.10. and X.X.11. in the following chapters (see Agenda Item 1.11.) with the exception of Article X.X.8. for *G. salaris* (see Agenda Item 1.10.3.).

The Aquatic Animals Commission did not agree with a Member Country comment to change, for example, 'the infection with *G. salaris* status' to 'the *G. salaris* infection status', as this expression is in line with rules previously agreed when applying the naming convention 'infection with [pathogenic agent]' to all disease-specific chapters.

In response to several Member Country comments, the Aquatic Animals Commission amended relevant text to ensure consistency between disease-specific chapters, e.g. ensuring the list of susceptible species in Article X.X.2. are in alphabetical order by common name.

1.10.1. Epizootic haematopoietic necrosis (Chapter 10.1.)

No additional comments specific to this chapter were received.

The revised Chapter 10.1. *Epizootic haematopoietic necrosis* is attached at **Annex 12** and is proposed for adoption at the 86th General Session in May 2018.

EU position

The EU supports the adoption of this modified chapter.

1.10.2. Infection with *Aphanomyces invadans* (epizootic ulcerative syndrome) (Chapter 10.2.)

No additional comments specific to this chapter were received.

The revised Chapter 10.2. *Infection with Aphanomyces invadans* is attached at **Annex 13** and is proposed for adoption at the 86th General Session in May 2018.

EU position

The EU supports the adoption of this modified chapter.

1.10.3. Infection with *Gyrodactylus salaris* (Chapter 10.3.)

In response to a Member Country comment to delete point 2) in Articles 10.3.4. and 10.3.5. (historical freedom) the Aquatic Animals Commission noted that a self-declaration of freedom could only be made if there are conditions conductive to clinical expression of the disease. If conditions are not conductive to clinical disease, point 3 of these articles (requiring active surveillance) may need to be applied.

EU comment

The EU does not agree with this rationale. Since conditions are not conductive to clinical expression of this disease, the point needs to be deleted as it is not relevant for this disease. Keeping it would create confusion and could deter countries from applying active surveillance when it is in fact required.

The Aquatic Animals Commission agreed that the model Article X.X.8. did not describe relevant risk mitigation measures for infection with *G. salaris.* The Commission revised the article to include previously adopted risk mitigation measures for *G. salaris* for the importation of aquatic animals for aquaculture purposes. In addition, the Commission also noted that there had been an error in the application of the new model Article X.X.8. to this chapter and made appropriate corrections.

The revised Chapter 10.3. *Infection with Gyrodactylus salaris* is attached at **Annex 14** and is proposed for adoption at the 86th General Session in May 2018.

EU position

The EU notes that none of its previous comments (available here https://ec.europa.eu/food/sites/food/files/safety/docs/ia_standards_oie_eu_position_aahsc-report_201709.pdf) were taken into account.

Comments are inserted in the text of Annex 14.

1.10.4. Infection with infectious salmon anaemia virus (Chapter 10.4.)

The Aquatic Animals Commission agreed with a Member Country comment to amend text in Article 10.4.1. to ensure consistency regarding the difference between non-pathogenic and pathogenic genotypes of infectious salmon anaemia virus.

The Aquatic Animals Commission agreed to delete the tissues 'ovarian fluid and milt' listed in point 1 *b)* of Article 10.4.17. noting that the most appropriate tissues for detection of infection are provided in the corresponding chapter in the *Aquatic Manual*.

The Aquatic Animals Commission disagreed with a Member Country comment to remove Articles 10.4.5., 10.4.7. and 10.4.11. noting that the provisions of Chapter 10.4. are provided in recognition of three possible levels of disease status with respect to ISAV.

The revised Chapter 10.4. *Infection with infectious salmon anaemia virus* is attached at **Annex 15** and is proposed for adoption at the 86th General Session in May 2018.

EU position

The EU thanks the OIE and supports the adoption of this modified chapter.

1.10.5. Infection with salmonid alphavirus (Chapter 10.5.)

Regarding the list of susceptible species listed in Article 10.5.2., the Aquatic Animals Commission noted that two of the three species currently listed were assessed and met the criteria for listing as susceptible species as described in Chapter 1.5. (see Agenda Item 3.1.). The Aquatic Animals Commission noted that brown trout *(Salmo trutta)* currently listed in Article 10.5.2. did not meet the criteria for listing as a susceptible species and was therefore proposed to be deleted from Article 10.5.2. (see Agenda Item 3.1.).

The Aquatic Animals Commission also noted that a new species, common dab *(Limanda limanda)*, met the criteria for listing as a susceptible species and was therefore proposed to be added to Article 10.5.2.

The Aquatic Animals Commission wished to advise Member Countries that the revised Chapter 10.5. (with horizontal amendments) would be proposed for adoption in May 2018; however, the revised Article 10.5.2. (with revised susceptible species) would not be proposed for adoption but would rather be provided to Member Countries for comment (see Annex 31).

The revised Article 10.5.2. is attached at **Annex 31** for Member Country comment.

EU comment

The EU supports the proposed changes to this chapter.

The revised Chapter 10.5. *Infection with salmonid alphavirus* is attached at **Annex 16** and is proposed for adoption at the 86th General Session in May 2018.

EU position

The EU supports the adoption of this modified chapter.

1.10.6. Infectious haematopoietic necrosis (Chapter 10.6.)

No additional comments specific to this chapter were received.

The revised Chapter 10.6. *Infectious haematopoietic necrosis* is attached at **Annex 17** and is proposed for adoption at the 86th General Session in May 2018.

EU position

The EU in general supports the proposed changes to this chapter.

Comments are inserted in the text of Annex 17.

1.10.7. Koi herpesvirus disease (Chapter 10.7.)

Regarding the susceptible species listed in Article 10.7.2., the Aquatic Animals Commission noted that all species currently listed were assessed and met the criteria for listing as susceptible species as described in Chapter 1.5. (see Agenda Item 3.1.).

The Aquatic Animals Commission agreed to delete ghost carp (*Cyprinus carpio goi*) as they considered this name to be a junior synonym for common carp (*Cyprinus carpio carpio*).

The Aquatic Animals Commission wished to advise Member Countries that the revised Chapter 10.7. (with horizontal amendments) would be proposed for adoption in May 2018; however, the revised Article 10.7.2. (with revised susceptible species) would not be proposed for adoption but would rather be provided to Member Countries for comment (see Annex 32).

The revised Article 10.7.2. is attached at **Annex 32** for Member Country comments.

EU comment

The EU in general supports the proposed changes to this chapter.

Comments are inserted in the text of Annex 32.

The revised Chapter 10.7. *Koi herpesvirus disease* is attached at **Annex 18** and is proposed for adoption at the 86th General Session in May 2018.

EU position

The EU in general supports the adoption of this modified chapter.

A comment is inserted in the text of Annex 18.

1.10.8. Red sea bream iridoviral disease (Chapter 10.8.)

No comments specific to this chapter were received.

The revised Chapter 10.8. *Red sea bream iridoviral disease* is attached at **Annex 19** and is proposed for adoption at the 86th General Session in May 2018.

EU position

The EU supports the adoption of this modified chapter.

1.10.9. Spring viraemia of carp (Chapter 10.9.)

Regarding the list of susceptible species listed in Article 10.9.2., the Aquatic Animals Commission noted that six of the ten species currently listed were assessed and met the criteria for listing as susceptible species as described in Chapter 1.5. (see Agenda Item 3.1.).

The Aquatic Animals Commission noted that four of the ten species currently listed in Article 10.9.2., did not meet the criteria for listing as a susceptible species and were, therefore, proposed to be deleted from Article 10.9.2. (see Agenda Item 3.1.).

The Aquatic Animals Commission noted that additional species –bream *(Abramis brama),* fathead minnow *(Pimephales promelas),* golden shiner *(Notemigonus crysoleucas),* roach *(Rutilus rutilus)* and zebrafish *(Sander vitreus)*– met the criteria for listing as a susceptible species and were, therefore, proposed to be added to Article 10.9.2. (see Agenda Item 3.1.).

The Aquatic Animals Commission wished to advise Member Countries that the revised Chapter 10.9. (with horizontal amendments) would be proposed for adoption in May 2018; however, the revised Article 10.9.2. (with revised susceptible species) would not be proposed for adoption but would rather be provided to Member Countries for comment (see Annex 33).

The revised Article 10.9.2. is attached at **Annex 33** for Member Country comments.

EU comment

The EU in general supports the proposed changes to this chapter.

Comments are inserted in the text of Annex 33.

The revised Chapter 10.9. *Spring viraemia of carp* is attached at **Annex 20** and is proposed for adoption at the 86th General Session in May 2018.

EU position

The EU in general supports the adoption of this modified chapter.

A comment is inserted in the text of Annex 20.

1.10.10. Viral haemorrhagic septicaemia (Chapter 10.10.)

No additional comments specific to this chapter were received.

The revised Chapter 10.10. *Viral haemorrhagic septicaemia* is attached at **Annex 21** and is proposed for adoption at the 86th General Session in May 2018.

EU position

The EU in general supports the adoption of this modified chapter.

A comment is inserted in the text of Annex 21.

1.11. Model Articles X.X.8, X.X.9., X.X.10. and X.X.11.

Comments were received from Australia.

The Aquatic Animals Commission agreed to apply model Articles X.X.8., X.X.9., X.X.10. and X.X.11. to all disease-specific chapters in Sections 8, 9 and 10 of the *Aquatic Code*, once adopted, with the exception of Article X.X.8. for the infection with *G. salaris* chapter (see Agenda Item 1.10.3.). The Commission agreed to make these amendments in the mollusc disease-specific chapters when work on susceptible species commences for these diseases.

In response to a Member Country comment to re-structure articles for aquatic animal products and live aquatic animals not for human consumption, including ornamentals, the Aquatic Animals Commission agreed that the structure of articles in the disease-specific chapters would benefit from re-organisation. The Commission agreed to put this issue on their future work programme.

The revised model Articles X.X.8., X.X.9., X.X.10. and X.X.11. are attached at **Annex 22** and are proposed for adoption at the 86th General Session in May 2018.

EU position

The EU in geneal supports the adoption of these modified model articles.

A comment is inserted in the text below.

2. Other issues

2.1. Acute hepatopancreatic necrosis disease (Chapter 9.1.)

In light of recent publications of new non-*Vibrio* species that cause acute hepatopancreatic necrosis disease (AHPND), the Aquatic Animals Commission continued to review this information (see papers reviewed below) and determined that no amendments to the scope in Article 9.1.1. were required.

While recent publications demonstrated the presence of the plasmid carrying the PirA and PirB toxin genes in other species of *Vibrio*, none of the studies described the re-isolation and identification of the bacteria in order to demonstrate definitively that these bacterial species could reproduce the

disease AHPND. The Aquatic Animals Commission noted that given the potentially widespread presence of the PirA and PirB genes in nature it is important to ensure that, before expanding the scope of the disease, there is definitive evidence to support any bacterial species as a pathogenic agent. Re-isolation and identification of the bacterial agent after determining the presence of the toxin genes as well as evidence to demonstrate that the bacterial species is the cause of AHPND (e.g. bioassay) is required to fulfil Koch's postulates.

References:

Dong *et al.* (2017) pirABvp-Bearing Vibrio parahaemolyticus and Vibrio campbellii pathogens isolated from the same AHPND-affected pond possess highly similar pathogenic plasmids. *Frontiers in Microbiology*, **8**, 1859

Liu *et al.* (in press) A *Vibrio owensii* strain as the causative agent of AHPND in cultured shrimp, *Litopenaeus vannamei*. *Journal of Invertebrate Pathology*, https://doi.org/10.1016/j.jip.2018.02.005

2.2. Technical disease cards

2.2.1. Tilapia lake virus

The Aquatic Animals Commission reviewed the Technical Disease Card for TiLV, taking into consideration new scientific information and updated the text, where relevant, and the list of references.

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/

2.2.2. *Batrachochytrium salamandrivorans*

The Aquatic Animals Commission reviewed the Technical Disease Card for *B. salamandrivorans*, taking into consideration new scientific information and updated the text, were relevant, and the list of references.

The updated technical disease card will be made available, once revised, on the OIE website at: http://www.oie.int/en/international-standard-setting/specialists-commissions-groups/aquatic-animal-commission-reports/disease-information-cards/

3. *Ad hoc* Groups

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 held from 28–30 November 2017. The Commission commended the *ad hoc* Group for their substantial work.

The OIE *ad hoc* Group had undertaken assessments of susceptible species 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 fish disease-specific chapters in the *Aquatic Code* and *Aquatic Manual* for infection with salmonid alphavirus (Chapter 10.5. and Chapter 2.3.6., respectively), koi herpesvirus disease (Chapter 10.9. and Chapter 2.3.7., respectively), and spring viraemia of carp (Chapter 10.9. and Chapter 2.3.9., respectively).

The Aquatic Animals Commission agreed with the *ad hoc* Group recommendation to include information on species found to be refractory to infection in each disease-specific chapter of the *Aquatic Manual.* This information would be included in a new Section 2.2.3. but not until the proposed new Article 1.5.9. has been adopted.

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 attached at **Annex 37** for Member Country information.

3.2. Report of the *ad hoc* Group on Demonstration of disease freedom

The Aquatic Animals Commission considered the work undertaken by the *ad hoc* Group on Demonstration of disease freedom who worked electronically from November 2017 to January 2018 to develop the principles for demonstrating disease freedom that could be used to amend relevant text in all disease-specific chapters. The Commission acknowledged the work undertaken by this *ad hoc* Group on this complex topic.

The Aquatic Animals Commission agreed that the work done to date was not sufficiently advanced to present an approach to Member Countries at this time. The Commission agreed that it would undertake further work on this issue with a view to providing Member Countries with an approach for determining periods required to demonstrate disease freedom.

3.3. Report of the *ad hoc* Group on Aquatic animal biosecurity for aquaculture establishments

The Aquatic Animals Commission reviewed the report of the meeting of the *ad hoc* Group on Aquatic animal biosecurity for aquaculture establishments, held from 16–18 January 2018. The Commission commended the group on their work.

The Aquatic Animals Commission reviewed the report and draft chapter on aquatic animal biosecurity for aquaculture establishments and wished to make some further amendments before providing to Member Countries. The Commission agreed to work to finalise the draft chapter and provide it for the next Commission meeting to be held in September 2018.

3.4. 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 November 2017 to January 2018 to assess TiLV diagnostics and validation. The Commission commended the *ad hoc* Group for their substantial work that demonstrated excellent collaboration between a number of laboratories worldwide.

The *ad hoc* Group reviewed the available published and unpublished information regarding detection methods for TiLV, provided advice on additional method development and validation requirements for a number of molecular detection tests, and identified sources of well-characterised viable and non-viable positive control material. The *ad hoc* Group also established a work plan for inter-laboratory method evaluation and comparability studies as a first stage in the method validation process.

In response to a recommendation by the *ad hoc* Group, the Aquatic Animals Commission requested that OIE Headquarters contact the OIE Delegates of Member Countries where TiLV has been reported and request that positive control material be provided to Dr Colling at the Collaborating Centre for New and Emerging Diseases, Australian Animal Health Laboratory (CSIRO Livestock Industries, Private Bag 24, Geelong 3220, Victoria, AUSTRALIA, Tel: +61-3 52.27.51.60, Fax: +61-3 52.27.55.55). This material would be necessary for molecular test evaluation and inter-laboratory comparability studies.

The Aquatic Animals Commission revised the *ad hoc* Group's Terms of Reference and requested that the *ad hoc* Group continue this important work and report back to the next meeting of the Commission in September 2018.

The report of the OIE *ad hoc* Group on Tilapia lake virus is attached at **Annex 38** for Member Country information.

The Aquatic Animals Commission reminded Member Countries that *ad hoc* Group reports will be uploaded onto 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/

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

4. Texts circulated for Member Country comments at the September 2017 meeting

Comments were received from Australia, Canada, China (People's Rep. of), Costa Rica, Guatemala, Japan, Mexico, Singapore, Thailand, USA and EU.

4.1. General comments

The Aquatic Animals Commission wished to remind Member Countries that the key amendments being made to the *Aquatic Manual* chapters (as annexed to the Commission's September meeting report) include changes to the susceptible species for each disease and application of the naming convention "infection with [pathogenic agent]" throughout the chapters.

Two *ad hoc* Groups, one on crustacean diseases and the second on fish diseases, had undertaken in-depth assessments by applying the *Criteria for listing species as susceptible to infection with a specific pathogen* (Chapter 1.5. of the *Aquatic Code*). As a result of this work, the lists of susceptible species in the *Aquatic Code* chapters were being proposed for amendment and the Aquatic Animals Commission considered it important to amend the corresponding section of the *Aquatic Manual* chapters—Section 2.2.1. *Susceptible host species*. A new Section 2.2.2. *Species with incomplete evidence for susceptibility* was also being included in *Aquatic Manual* chapters.

In addition the Commission reviewed chapters to determine the need for any urgent updates particularly for Table 5.1. *Methods for targeted surveillance and diagnosis*, Section 6. *Test*(*s*) *recommended for targeted surveillance to declare freedom from* [*disease name*] and the definitions of a suspect and a confirmed case in Section 7. *Corroborative diagnostic criteria*.

The Aquatic Animals Commission noted that it is not possible to undertake extensive review of all chapters at this time; however, it is intended that all chapters will be thoroughly updated when the new template for disease-specific chapters, developed by the *ad hoc* Group on the *Aquatic Manual*, is applied (see Agenda Item 5.1.). When a chapter is identified for update, members of the *ad hoc* Group would work with the Reference Laboratory experts to apply the template.

The Aquatic Animals Commission wished to assure Member Countries that a number of their comments that seem to not have been addressed would in fact be addressed in future revisions of the disease-specific *Aquatic Manual* chapters using the new template.

In response to a general comment, the Aquatic Animals Commission noted that reports of meetings of the OIE *ad hoc* Groups are available on line at: http://www.oie.int/en/standard-setting/specialists-commissions-working-groups/scientific-commission-reports/ad-hoc-groups-reports/

4.2. White spot disease (Chapter 2.2.8.)

The Aquatic Animals Commission reviewed all Member Country comments on Chapter 2.2.8. *Infection with white spot syndrome virus* (WSSV) and made relevant amendments.

In response to a Member Country query, the Aquatic Animals Commission noted that the proposed name, infection with white spot syndrome virus, incorporates the recognised name of the pathogenic agent of white spot disease.

In Section 2.2.1. *Susceptible host species*, a Member Country requested that the word "resistant" be maintained rather than replaced by the word "refractory" in the sentence "…no decapod (order Decapoda) crustacean from marine and brackish or freshwater sources has been reported to be refractory ~~resistant~~ to infection with WSSV…". The Aquatic Animals Commission did not believe that the words are synonyms and felt that "refractory" was the more appropriate word.

In Section 3.3. *Pooling of samples*, a Member Country asked that the proposed text on the lack of evaluation of the effect of pooling on diagnostic sensitivity, and the recommendation that larger life stages should be processed, and tested individually be applied to all *Aquatic Manual* chapters. The Aquatic Animals Commission noted that this issue would be addressed when the chapters are revised using the new template (see Agenda Items 4.1. and 5.1.).

The Aquatic Animals Commission did not accept suggested amendments to Section 7.2. *Definition of confirmed case,* noting that all proposals for changes to case definitions would be addressed once the new chapter template is implemented. Regarding the requirement to undertake sequence analysis following real-time polymerase chain reaction (PCR) to confirm a case, a Member Country commented that it may not be possible to obtain sufficient target nucleic acid to conduct sequence analysis for weak positive cases of WSSV. The Commission agreed that this may be a problem and noted that if a weak positive result is obtained, and there is insufficient target nucleic acid for confirmation by other methods, the suspect population should be re-sampled and tested.

The revised Chapter 2.2.8. *Infection with white spot syndrome virus* is attached as **Annex 23** and is proposed for adoption at the 86th General Session in May 2018.

EU position

The EU thanks the OIE and supports the adoption of this modified chapter.

4.3. Amended fish disease-specific chapters

4.3.1. Epizootic haematopoietic necrosis virus (Chapter 2.3.1.)

The Aquatic Animals Commission reviewed all Member Country comments on Chapter 2.3.1. *Infection with epizootic haematopoietic necrosis virus* (EHNV) and made relevant amendments.

For Section 2.2.1. *Susceptible host species*, a Member Country did not agree with some of the proposed additions to the list of species susceptibility to EHNV. The Aquatic Animals Commission noted that the *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases had assessed these susceptible species thoroughly. The Commission is satisfied with the findings of the *ad hoc* Group which can be found in Annex 37 of the Commission's September 2017 meeting.

For Section 2.2.2. *Species with incomplete evidence for susceptibility*, a Member Country proposed additional species. The Aquatic Animals Commission did not believe that the information presented warranted reassessment by the *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases.

The Aquatic Animals Commission added a new paragraph to Section 2.2.2. regarding species in which pathogen-specific positive PCR results have been reported but an active infection has not been demonstrated. The list of species proposed was assessed by the *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases.

A Member Country proposed adding two new subsections on new species to Section 2.2.6. *Persistent infection*. The Aquatic Animals Commission did not accept the proposal as the section would be revised when the new template for disease-specific chapters of the *Aquatic Manual* is applied to this chapter.

In Section 4.3.1.2.3.1. *PCR and restriction endonuclease analysis* (*REA*)*: technical procedure,* a Member Country requested that the REA procedure be reinstated. The Aquatic Animals Commission did not agree to this request given that sequence analysis is a more commonly used and preferable technique.

A proposal to include the real-time PCR in Section 6. *Test*(*s*) *recommended for targeted surveillance to declare freedom from EHN* was accepted in principle. The real-time PCR protocol will be included in the chapter when it is revised using the new template.

The revised Chapter 2.3.1. *Infection with epizootic haematopoietic necrosis virus* is attached as **Annex 24** and is proposed for adoption at the 86th General Session in May 2018.

EU position

The EU supports the adoption of this modified chapter.

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

The Aquatic Animals Commission reviewed all Member Country comments on Chapter 2.3.3. *Infection with Gyrodactylus salaris* and made relevant amendments.

The Aquatic Animals Commission added a new paragraph to Section 2.2.2. *Species with incomplete evidence for susceptibility,* regarding species in which the parasite can survive for short periods but not indefinitely. The Commission decided to place the paragraph regarding pathogen-specific positive PCR results that have been reported, but where an active infection has not been demonstrated, 'under study' so that it could undertake further assessments of the list of species.

In response to a Member Country comment, the Aquatic Animals Commission reworded a sentence in Section 2.2.5. *Target organs and infected tissue*, to clarify that the distribution of the parasite on the host may vary depending on the intensity of infection.

A Member Country disagreed with the statement in Section 2.3.5. *Environmental factors* regarding the temperature dependence of survival of the parasite at higher salinities. The Aquatic Animals Commission believed that the Member Country had misinterpreted the reference and left the text unchanged.

The Aquatic Animals Commission agreed to send technical comments submitted by a Member Country on Section 4.3.1.2.3.2. *Analysis of the mitochondrial cytochrome oxidase I gene* to the OIE Reference Laboratory expert for response.

The Aquatic Animals Commission also agreed to send comments on Section 7.2. *Definition of confirmed case* to the OIE Reference Laboratory expert with the request that he clarify the criteria.

The revised Chapter 2.3.3. *Infection with Gyrodactylus salaris* is attached as **Annex 25** and is proposed for adoption at the 86th General Session in May 2018.

EU position

The EU thanks the OIE and in general supports the adoption of this modified chapter.

Comments are inserted in the text of Annex 25.

4.3.3. Infection with infectious salmon anaemia virus (Chapter 2.3.5.)

The Aquatic Animals Commission reviewed all Member Country comments on Chapter 2.3.5. *Infection with infectious salmon anaemia virus* (ISAV) and made relevant amendments.

In the chapter, the term "infection with ISAV" refers to infection with both HPR0 and HPR-deleted ISAV while clinical disease only occurs with HPR-deleted variants. To eliminate any potential confusion, the Aquatic Animals Commission amended the text throughout the chapter to improve clarity, for example by inserting "HPR-deleted" into "infection with ISAV" where relevant.

The Aquatic Animals Commission rejected a proposal to add the words "but evidence is not considered definitive" following the words "A link between non-pathogenic HPR0 ISAV

and pathogenic HPR-deleted ISAV, with some outbreaks potentially occurring as a result of the emergence of HPR-deleted ISAV from HPR0 ISAV has been suggested" because there is a large body of evidence that forms the basis for strain differentiation in the chapter.

The Aquatic Animals Commission added a new paragraph to Section 2.2.2. *Species with incomplete evidence for susceptibility* regarding species in which pathogen-specific positive PCR results have been reported but an active infection has not been demonstrated. The list of species proposed was assessed by the *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases.

In Section 3.3. *Pooling of samples*, a Member Country proposed that it is acceptable to pool from 1 to 3 fish for surveillance protocols, but that individual samples are required for confirmatory purposes when the disease is suspected and for molecular studies. The Aquatic Animals Commission believed that the original text is sound but would welcome information on the validation of assays to demonstrate the diagnostic performance of assays for pooled samples.

There were a number of comments in Section 4.3.1.2.3.2 *Real-time RT-PCR*. The Aquatic Animals Commission agreed that these would be addressed when the chapter is revised using the new template. In the meantime, the Commission agreed that this section should remain unchanged from the last adopted version (2014).

The revised Chapter 2.3.5. *Infection with infectious salmon anaemia virus* is attached as **Annex 26** and is proposed for adoption at the 86th General Session in May 2018.

EU position

The EU thanks the OIE and in general supports the adoption of this modified chapter.

Comments are inserted in the text of Annex 26.

4.4. Infection with infectious hypodermal and haematopoietic necrosis virus (Chapter 2.2.3.)

No Member Country comments had been received on the proposal to move *Macrobrachium rosenbergii* from Section 2.2.1. *Susceptible host species* to Section 2.2.2. S*pecies with incomplete evidence for susceptibility* following an assessment of the *ad hoc* Group on Susceptibility of crustacean species to infection with OIE listed diseases.

The revised Sections 2.2.1. and 2.2.2. are presented as **Annex 27** and are proposed for adoption at the 86th General Session in May 2018.

EU position

The EU supports the adoption of this modified chapter.

4.5. Acute hepatopancreatic necrosis disease (Chapter 2.2.1.)

No Member Country comments had been received regarding the proposed addition of *P. japonicus* to Section 2.2.2.

The Aquatic Animals Commission did not agree with a proposal to add the words "or other bacterial strains carrying the plasmid that are capable of producing the infection" after the words "Acute hepatopancreatic necrosis disease (AHPND) means infection with strains of *Vibrio parahaemolyticus* (*Vp*$_{AHPND}$)" in the scope of the chapter. The Commission reviewed the published literature on AHPND and found that *Vibrio parahaemolyticus* remains the only bacterial species for which there is sound evidence as a causative agent of the disease (see also Agenda Item 2.1.).

The revised Sections 2.2.1. and 2.2.2. are presented as **Annex 28** and are proposed for adoption at the 86th General Session in May 2018.

EU position

The EU supports the adoption of this modified chapter.

4.6. Chapters for which the *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases had reviewed the list of susceptible species

4.6.1. Infection with salmonid alphavirus (Chapter 2.3.6.)

As above, the Aquatic Animals Commission amended 2.2.1. *Susceptible host species* and Section 2.2.2. *Species with incomplete evidence for susceptibility* after consideration of the work of the *ad hoc* Group (see also Agenda Item 1.10.5.).

The Aquatic Animals Commission also reviewed and amended Section 1. *Scope*, Section 2.4.7. *Disinfection of eggs and larvae*, and Section 3.3. *Pooling of samples*. Finally, the Commission replaced "subtype" with "genotype" throughout the chapter.

The revised Chapter 2.3.6. *Infection with salmonid alphavirus* is presented in **Annex 34** for Member Country comment.

EU comment

The EU supports the proposed changes to this chapter.

4.6.2. Koi herpesvirus disease (Chapter 2.3.7.)

The Aquatic Animals Commission amended 2.2.1. *Susceptible host species,* Section 2.2.2. *Species with incomplete evidence for susceptibility* and Section 2.2.8. *Known or suspected wild aquatic animal carriers* after consideration of the work of the *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases, which had applied the 'Criteria for listing species as susceptible to infection with a specific pathogen' (Chapter 1.5.) (see Agenda Item 1.10.7.). Amendments to Section 2.2.2. included species for which pathogen-specific PCR results have been reported but an active infection has not been demonstrated.

The Aquatic Animals Commission applied the naming convention "infection with [pathogenic agent]" to the title of the chapter and throughout. The Commission also reviewed and amended Section 1. *Scope*, Section 2.2.6. *Vectors*, Section 3.3. *Pooling of samples*, and harmonised the title of the chapter with the name of the disease and ensured the correct use of the disease name throughout the chapter.

The revised Chapter 2.3.7. *Infection with koi herpesvirus* is presented in **Annex 35** for Member Country comment.

EU comment

The EU in general supports the proposed changes to this chapter.

Comments are inserted in the text of Annex 35.

4.6.3. Spring viraemia of carp (Chapter 2.3.9.)

Instead of circulating the chapter for Member Country comment, the Aquatic Animals Commission agreed that it should be thoroughly updated using the new template. Members of the *ad hoc* Group that developed the template will assist the OIE Reference Laboratory experts with this task (see Agenda Item 5.1.).

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

Since adopting the revised Section 2.2.2. *Species with incomplete evidence for susceptibility*, the Aquatic Animals Commission had added a new paragraph to this Section in the other crustacean disease-specific chapters. The new paragraph concerned species for which pathogen-specific positive PCR results have been reported but an active infection has not been demonstrated. The Commission agreed to amend Section 2.2.2. of Chapter 2.2.9. *Infection with yellow head virus genotype 1* to include this list of species so as to align it with the other disease-specific chapters. The list of species proposed was assessed by the *ad hoc* Group on Susceptibility of crustacean species to infection with OIE listed diseases.

The revised Section 2.2.2. of Chapter 2.2.9. *Infection with yellow head virus genotype 1* is attached at **Annex 36** for Member Country comment.

EU comment

The EU supports the proposed changes to this chapter.

5. Other issues

5.1. Review of the final version of the *Aquatic Manual* disease chapter template proposed by the *ad hoc* Group

The Aquatic Animals Commission approved the *Aquatic Manual* disease chapter template following consideration of revisions that had been made by the *ad hoc* Group (the template is attached for information to this report in Annex 39). The template contains detailed guidance to the experts to ensure a high level of consistency between chapters. The structure of the chapter has been simplified and overlap between sections has been removed.

A key driver in developing the template has been to ensure that only tests that appear in the table of OIE recommended diagnostic methods are described and these tests are used to determine a suspect or confirmed case. There is a requirement that each test must be validated (based on Chapter 1.1.2. *Principles and methods of validation of diagnostic assays for infectious diseases* of the *Aquatic Manual*) for use in i) apparently healthy animals and ii) clinically diseased animals.

For section 6 (corroborative diagnostic criteria), the template includes appendices to provide guidance on the combinations of tests used in the definitions of suspect and confirmed cases. This guidance will ensure consistent approaches are used for case definitions in each disease-specific chapter.

The Aquatic Animals Commission identified four diseases to which the template would be initially applied: Infectious haematopoietic necrosis, Red sea bream iridoviral disease, Spring viraemia of carp, and Viral haemorrhagic septicaemia. Members of the *ad hoc* Group will work with OIE Reference Laboratory experts to apply the template, and the work would be coordinated with the *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases so that the sections on susceptible host species and species with incomplete evidence for susceptibility could be revised at the same time.

The Aquatic Animals Commission reviewed the Terms of Reference (ToR) for the *ad hoc* Group on the *Aquatic Manual* to include the requirement to work with Reference Laboratory experts to apply the template to each of the disease-specific chapters.

The template is presented in **Annex 39** for Member Country information.

EU comment

The EU thanks the OIE for providing the Aquatic Manual disease chapter template for member country information. We nevertheless have a few comments that are inserted in the text of Annex 39.

5.2. Proposal for amendments in Chapter 2.3.10. on Viral haemorrhagic septicaemia (VHS) in the OIE Aquatic Manual

The OIE Reference Laboratory expert, in conjunction with a candidate reference laboratory, through an OIE twinning project, had developed and validated a novel conventional one-step reverse-transcription PCR method for detecting viral haemorrhagic septicaemia virus (VHSV). This new method was developed to address specificity issues that had been identified for the existing method in the *Aquatic Manual*. The new method has been submitted for publication and the experts requested that it be included in the *Aquatic Manual* chapter replacing the current RT-PCR method.

The Aquatic Animals Commission wished to thank the experts for their contribution to improving the methods in Chapter 2.3.10. The Commission agreed that it was appropriate to await publication of the method prior to inclusion in Chapter 2.3.10. The Commission would consider incorporation of the new method into Chapter 2.3.10. at its next meeting in September, pending publication of the method.

5.3. New draft chapter for Infection with *Batrachochytrium salamandrivorans* (Chapter 2.1.X.) – identification of potential contributors

In the absence of a Reference Laboratory for *Batrachochytrium salamandrivorans,* the Aquatic Animals Commission identified experts who could be invited to prepare a draft *Aquatic Manual* chapter using the new template that could be reviewed at its September 2018 meeting.

The Aquatic Animals Commission reminded the OIE Delegates that it would welcome applications from suitable laboratories to become a Reference Laboratory for *B. salamandrivorans.*

G. OIE REFERENCE CENTRES

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

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

OIE *Reference Laboratory for Koi herpesvirus disease*
Friedrich-Loeffler-Institut (FLI), Federal Research Institute for Animal Health, Institute of Infectology, Insel Riems, Germany.
Designated Reference Expert: Dr Sven M. Bergmann.

OIE *Reference Laboratory for* Viral haemorrhagic septicaemia
Aquatic Animal Quarantine Laboratory, General Service Division, National Fishery Products Quality Management Service, Ministry of Oceans and Fisheries, Busan, Korea (Rep. of).
Designated Reference Expert: Dr Hyoung Jun Kim.

OIE *Collaborating Centre for Emerging Aquatic Animal Diseases*
Centre for Environment, Fisheries and Aquaculture Sciences (CEFAS), The Nothe, Dorset, United Kingdom.
NB: if adopted, this Collaborating Centre would replace the existing one on Information on Aquatic Animal Diseases.

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

Taura syndrome
Dr Arun K. Dhar to replace Dr Kathy Tang-Nelson at the Aquaculture Pathology Laboratory, School of Animal and Comparative Biomedical Sciences, University of Arizona, United States of America.

Other applications were received for Reference Laboratory status or for replacement experts that were not supported by the Commission. The Commission agreed that these applications did not provide sufficient evidence of experience with diagnosing the diseases in question.

7. Feedback from the Biological Standards Commission: proposed SOPs[1]: *Procedures for designation of OIE Collaborating Centres*

The Biological Standards Commission had drafted a document entitled *Procedures for designation of OIE Collaborating Centres*. The purpose of the document is to have clear criteria and procedures for designation and de-listing OIE Collaborating Centres. The document outlines the steps that need to be followed by applicants for OIE Collaborating Centre status, the roles of the Specialist and Regional Commissions, the Council and the Assembly.

The Aquatic Animals Commission provided feedback on the draft document. In particular, given that designation would now be for a five-year period, the Commission proposed including in the Guidelines for applicants, a new point requesting a work plan for that period. The Commission also requested that for applications that cover both terrestrial and aquatic animals, a lead Specialist Commission be identified.

The Aquatic Animals Commission approved the document (see Annex 4 of the report of the February 2018 meeting of the Biological Standards Commission), which would be presented by the Biological Standards Commission for adoption at the 86th General Session in May 2018.

8. Review of annual reports of Reference Centre activities in 2017

In May 2017, the *Procedures for designation of OIE Reference Laboratories* (the SOPs) were adopted by Resolution of the Assembly and made available online at: http://www.oie.int/fileadmin/Home/eng/Our_scientific_expertise/docs/pdf/ANG_SOP_RL_applications.pdf . The Aquatic Animals Commission began the procedure of implementing the SOPS by evaluating the reports against the performance criteria.

8.1. Requirement to be accredited to ISO 17025 or equivalent quality management system

In accordance with the SOPs, laboratories that had not achieved accreditation to ISO 17025 or equivalent quality management system (QMS) would have their OIE Reference Laboratory status suspended, with the possibility to reinstate it within two years should they achieve accreditation in that time. Laboratories that have still not achieved accreditation two years after suspension, would have to re-apply for OIE Reference Laboratory status, once accreditation is achieved.

The Aquatic Animals Commission reviewed the annual reports of all 39 OIE Reference Laboratories for aquatic animal diseases, paying particular attention of the QMS and accompanying certificate, and identified 12 laboratories for suspension because they were not yet accredited. These laboratories would receive a letter from the Director General of the OIE explaining the decision and procedure and emphasising the importance and benefit of having an accredited quality management system, particularly for confidence in test results.

A further 15 laboratories had reported that they were accredited but either did not attach a certificate or the certificate did not include information on the scope of accreditation. These laboratories would be given until mid-April to submit a certificate or details of the scope of their accreditation. The Aquatic Animals Commission would review all replies received and decide a course of action. The Assembly would be informed of the final outcome of these evaluations.

9. Twinning projects

The Aquatic Animals Commission was updated on the status of aquatic animal disease twinning projects.

Since the September 2017 meeting of the Aquatic Animals Commission, the twinning project between Norway and Brazil for infectious salmon anaemia has been completed. Five projects are still in progress (Japan with Indonesia for Koi herpesvirus; USA with Indonesia for shrimp diseases; USA with Saudi Arabia for shrimp diseases; Denmark with Republic of Korea for Viral haemorrhagic septicaemia and Italy with Tunisia for Viral encephalopathy and retinopathy).

[1] SOPs: Standard Operating Procedures.

The final report of the twinning project between Norway and Brazil for infectious salmon anaemia which concluded its activities in January 2018 was shared with the Aquatic Animals Commission for information. The Commission wished to congratulate the laboratories on a successful project and an excellent final report.

H. OTHER ISSUES

10. Draft guidelines on application of criteria for listing aquatic animal diseases

The Aquatic Animals Commission continued to work on the development of the draft guidelines on the application of the criteria for listing aquatic animal diseases. The Commission will review the document at their September 2018 meeting and propose to provide the document to Member Countries for information as part of their September 2018 meeting report. The Commission will also share it with the Code Commission who are interested in developing a similar guidance document.

The Aquatic Animals Commission reminded Member Countries that this guidance is intended to be used by the Commission or relevant *ad hoc* Groups when applying the criteria to the possible listing or delisting of an aquatic animal disease.

11. New procedure for Self-declaration of disease freedom

The Aquatic Animals Commission was informed that the new 'Procedure for submission of a self-declaration of disease freedom for publication by the OIE' has been finalised. The procedure relates to self-declaration of disease freedom for a country, zone or compartment for OIE listed aquatic and terrestrial animal diseases. It describes the process for the preparation, screening and publication of self-declarations of freedom from any disease, other than those diseases for which the OIE has put in place a specific procedure for official recognition of disease status.

The procedure is available on the OIE website at: http://www.oie.int/en/animal-health-in-the-world/self-declared-disease-status/

12. *Aquatic Manual* chapters for delisted or unlisted diseases

The Aquatic Animals Commission agreed that they would remove any chapters for delisted or unlisted diseases from the *Aquatic Manual* that do not have a Reference Laboratory expert. The rationale for removing these chapters is that without a Reference Laboratory expert there would be no clear mechanism for maintaining the quality of these chapters.

The Aquatic Animals Commission agreed to implement this decision after the review of Reference Laboratories against their ISO17025 laboratory requirements.

13. Antimicrobial resistance

In response to the Aquatic Animals Commission suggestion, in their September 2017 report, that the designation of a Collaborating Centre for antimicrobial resistance (AMR) in aquatic animals may be useful to assist in managing risks associated with AMR in aquatic animals, Norway proposed the possibility of extending their Collaborating Centre for Aquatic animal epidemiology and risk to also include the topic of AMR. The Commission welcomed this possibility and invited them to submit an application for their consideration.

I. OIE GLOBAL CONFERENCE ON AQUATIC ANIMAL HEALTH

The Aquatic Animals Commission was informed that the next OIE Global conference on aquatic animal health will be held in Santiago, Chile, from 9 to 11 April 2019. The Commission is excited about this event which will provide an opportunity for OIE Member Countries to engage on important aquatic animal health issues.

J. WORK PLAN OF THE OIE AQUATIC ANIMAL HEALTH STANDARDS COMMISSION FOR 2017/2018

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

The revised work programme is attached as **Annex 40** for Member Country information.

EU comment

The EU thanks the OIE for providing the AAHSC work programme for member country information. We nevertheless have a comment that is inserted in the text of Annex 40.

K. ACTIVITIES OF THE MEMBERS OF THE OIE AQUATIC ANIMAL HEALTH STANDARDS COMMISSION

The Aquatic Animals Commission wished to inform Member Countries of activities that Commission Members have undertaken in their role as Commission Members since the last meeting in September 2017.

Dr Alicia Gallardo Lagno represented the Aquatic Animals Commission in the FAO workshop on the use of antimicrobials in Aquaculture in Latin America**,** which was held in Lima (Peru) from 22 to 24 November 2017. She also attended the International Conference on Aquatic Animal Health 2017: Recent Advances, which was held in Lima (Peru) from 31 October to 2 November 2017. Dr Edmund Peeler attended and contributed to the OIE National Focal Points for Aquatic Animals held in Qingdao (People's Republic of China) from 12 to 14 December 2017.

L. NEXT MEETING

The Aquatic Animals Commission was informed that the dates for the next meetings would be decided by the OIE Headquarters pending the election of new members. Members elected to the Specialist Commissions in May 2018 will be advised in writing of the dates once they are confirmed. However, tentative dates being considered are 10 to 17 September 2018 to facilitate orientation for new members of the four Specialist Commissions and a specific training for the Presidents.

/Annexes

Annex 1

MEETING OF THE OIE
AQUATIC ANIMAL HEALTH STANDARDS COMMISSION

Paris, 14–21 February 2018

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Annex 2

MEETING OF THE OIE
AQUATIC ANIMAL HEALTH STANDARDS COMMISSION

Paris, 14–21 February 2018

Adopted agenda

A. MEETING WITH THE DIRECTOR GENERAL

B. ADOPTION OF THE AGENDA

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

D. MEETING WITH THE PRESIDENT OF THE OIE BIOLOGICAL STANDARDS COMMISSION

E. OIE *AQUATIC ANIMAL HEALTH CODE*

1. Texts circulated for Member Country comments at the September 2017 meeting

1.1. General comments

1.2. User's Guide

1.3. Glossary

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

1.5. Criteria for listing species as susceptible to infection with a specific pathogen (Chapter 1.5.)

1.6. OIE procedures relevant to the Agreement on the Application of Sanitary and Phytosanitary Measures of the World Trade Organization (Chapter 5.3.)

1.7. Criteria to assess the safety of aquatic animal commodities (Chapter 5.4.)

1.8. Amendments to amphibian disease-specific chapters

1.8.1. New draft chapter for Infection with *Batrachochytrium salamandrivorans* (Chapter 8.X.)

1.8.2. Infection with *Batrachochytrium dendrobatidis* (Chapter 8.1.)

1.8.3. Infection with ranavirus (Chapter 8.2.)

1.9. Infection with infectious hypodermal and haematopoietic necrosis virus (Articles 9.4.1. and 9.4.2.)

1.10. Amendments to fish disease-specific chapters

1.10.1. Epizootic haematopoietic necrosis (Chapter 10.1.)

1.10.2. Infection with *Aphanomyces invadans* (epizootic ulcerative syndrome) (Chapter 10.2.)

1.10.3. Infection with *Gyrodactylus salaris* (Chapter 10.3.)

1.10.4. Infection with infectious salmon anaemia virus (Chapter 10.4.)

1.10.5. Infection with salmonid alphavirus (Chapter 10.5.)

1.10.6. Infectious haematopoietic necrosis (Chapter 10.6.)

1.10.7. Koi herpesvirus disease (Chapter 10.7.)

1.10.8. Red sea bream iridoviral disease (Chapter 10.8.)

1.10.9. Spring viraemia of carp (Chapter 10.9.)

1.10.10. Viral haemorrhagic septicaemia (Chapter 10.10.)

1.11. Model Articles X.X.8., X.X.9., X.X.10. and X.X.11.

2. Other issues

2.1. Acute hepatopancreatic necrosis disease (Chapter 9.1.)

2.2. Technical disease cards

2.2.1. Tilapia lake virus

2.2.2. *Batrachochytrium salamandrivorans*

3. *Ad hoc* Groups

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

3.2. Report of the *ad hoc* Group on Demonstration of disease freedom

3.3. Report of the *ad hoc* Group on Aquatic animal biosecurity for aquaculture establishments

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

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

4. Texts circulated for Member Country comments at the September 2017 meeting

4.1. General comments

4.2. White spot disease (Chapter 2.2.8.)

4.3. Amended fish disease-specific chapters

4.3.1. Epizootic haematopoietic necrosis virus (Chapter 2.3.1.)

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

4.3.3. Infection with infectious salmon anaemia virus (Chapter 2.3.5.)

4.4. Infection with infectious hypodermal and haematopoietic necrosis virus (Chapter 2.2.3.)

4.5. Acute hepatopancreatic necrosis disease (Chapter 2.2.1.) – addition of *P. japonicus* Section 2.2.2.

4.6. Chapters for which the *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases had reviewed the list of susceptible species

4.6.1. Infection with salmonid alphavirus (Chapter 2.3.6.)

4.6.2. Koi herpesvirus disease (Chapter 2.3.7.)

4.6.3. Spring viraemia of carp (Chapter 2.3.9.)

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

5. Other issues

5.1. Review of the final version of the *Aquatic Manual* disease chapter template proposed by the *ad hoc* Group

5.2. Proposal for amendments in Chapter 2.3.10. on Viral haemorrhagic septicaemia (VHS)

5.3. New draft chapter for Infection with *Batrachochytrium salamandrivorans* (Chapter 2.1.X.)

G. OIE REFERENCE CENTRES

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

7. Feedback from the Biological Standards Commission: proposed SOPs[2]: *Procedures for designation of OIE Collaborating Centres*

8. Review of annual reports of Reference Centre activities in 2017

8.1. Requirement to be accredited to ISO 17025 or equivalent quality management system

9. Twinning projects

H. OTHER ISSUES

10. Draft guidelines on application of criteria for listing aquatic animal diseases

11. New procedure for Self-declaration of disease freedom

12. *Aquatic Manual* chapters for delisted or unlisted diseases

13. Antimicrobial resistance

I. OIE GLOBAL CONFERENCE ON AQUATIC ANIMAL HEALTH

J. WORK PLAN OF THE OIE AQUATIC ANIMAL HEALTH STANDARDS COMMISSION FOR 2017/2018

K. ACTIVITIES OF THE MEMBERS OF THE OIE AQUATIC ANIMAL HEALTH STANDARDS COMMISSION

L. NEXT MEETING

[2] SOPs: Standard Operating Procedures

Annex 3

USER'S GUIDE

EU position

The EU thanks the OIE and in general supports the adoption of this modified User's guide.

A comment is inserted in the text below.

A. Introduction

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

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

3) OIE standards are based on the most recent scientific and technical information. Correctly applied, they protect aquatic animal health during the production and trade in aquatic animals and aquatic animal products as well as the welfare of farmed fish.

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

~~4bis~~ 5) The year that a chapter was first adopted and the year of last revision are noted at the end of each chapter.

~~5~~6) The complete text of the *Aquatic Code* is available on the OIE website and individual chapters may be downloaded from: http://www.oie.int.

B. *Aquatic Code* content

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

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

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

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

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

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

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

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

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

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

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

C. Specific issues

1) Notification

Chapter 1.1. describes Member Countries' obligations under OIE Organic Statutes. Listed diseases, as prescribed in Chapter 1.1., are compulsorily notifiable. Member Countries are encouraged to also provide information to the OIE on other aquatic animal health events of epidemiological significance, including occurrence of emerging diseases.

Chapter 1.2. describes the criteria for the inclusion of a disease listed by the OIE.

Chapter 1.3. specifies the diseases that are listed by the OIE. Diseases are divided into four sections corresponding to amphibian, crustacean, fish and molluscan hosts, respectively.

2) Diagnostic tests

Methods for diagnosis of listed diseases are provided in the OIE *Manual of Diagnostic Tests for Aquatic Animals* (hereafter referred to as the *Aquatic Manual*). Experts responsible for laboratory testing should be fully conversant with the methods in the *Aquatic Manual*.

3) Freedom from a disease

Article 1.4.6. provides general principles for declaring a country, ~~or~~ zone or compartment free from infection with a pathogenic agent. This article applies when there is no disease-specific chapter.

~~2~~4) Pathogen differentiation

Some pathogens have one or more variants. Existence of highly pathogenic variants and the need to differentiate them from more benign variants are recognised in the *Aquatic Code*. When pathogenic agents have strains that are stable, possess characteristics that can be used for diagnostic purposes, and display different levels of pathogenicity, different standards providing protection proportionate to the risk posed by the different strains should be applied. Infection with infectious salmon anaemia virus is the first listed disease for which risk management options based on strain differentiation are provided.

35) Determining the susceptibility of species to listed diseases

~~The *Aquatic Code* proposes the use of criteria to assess the susceptibility of host species to the pathogenic agents of diseases listed in the *Aquatic Code*.~~

Chapter 1.5. provides criteria for determining which species are listed as susceptible in Article X.X.2. of each disease-specific chapter in the *Aquatic Code*. This is important in the aquaculture context, given the large number of existing and new aquaculture species.

This is work in progress and the list of susceptible species in some chapters is yet to be assessed against the criteria in Chapter 1.5.

46) Trade requirements

Aquatic animal health measures related to international trade should be based on OIE standards. A Member Country may authorise the importation of aquatic animals or aquatic animal products into its territory under conditions different from those recommended by the *Aquatic Code*. To scientifically justify ~~more stringent~~ more stringent measures ~~that exceed OIE standards~~, the importing country should conduct a risk analysis in accordance with OIE standards, as described in Chapter 2.1. Members of the WTO should refer to the Agreement on the Application of Sanitary and Phytosanitary Measures.

Chapters 5.1. to 5.3. describe the obligations and ethical responsibilities of importing and exporting countries in international trade. Competent Authorities and all veterinarians and certifying officials directly involved in international trade should be familiar with these chapters. Chapter 5.3. also describes the OIE informal procedure for dispute mediation.

Disease-specific chapters in the *Aquatic Code* include articles listing the aquatic animal products that are considered safe for trade without the imposition of disease-specific sanitary measures, regardless of the status of the exporting country or zone for the pathogenic agent in question. Where such a list is present, importing countries should not require any conditions related to the agent in question with respect to the listed aquatic animal products.

57) Safety of ~~Trade in~~ aquatic animal products for trade ~~commodities~~

Chapter 5.4. describes the criteria ~~(Articles 5.4.1 and 5.4.2.)~~ used to assess the safety of aquatic animal products. ~~commodities that are considered safe for trade regardless of the disease status of the country, zone or compartment without the need for additional risk mitigation measures for the disease.~~ The aquatic animal products that have been assessed and found to meet these criteria are listed in each disease-specific chapter. Article 5.4.1. describes criteria to assess the safety of aquatic animal products for any purpose ~~without the need for additional risk mitigation measures~~. Article 5.4.2. describes criteria to assess the safety of aquatic animal products for the purpose of retail trade for human consumption.

Article X.X.3. lists aquatic animal products that may be imported for any purpose regardless of the disease status of the exporting country, zone or compartment for the disease in question. The inclusion of an aquatic animal product in Article X.X.3. is based on evidence that demonstrates the absence of the pathogenic agent in that product or the inactivation of the pathogenic agent by physical, chemical or biological means.

~~Based on assessments using criteria in Article 5.4.1., in all disease specific chapters, point 1 of Article X.X.3. lists aquatic animal commodities that may be imported for any purpose from a country, zone or compartment not declared free from the disease in question. The criteria for inclusion of aquatic animal commodities in point 1 of Article X.X.3. are based on the absence of the pathogenic agent or inactivation of the pathogenic agent by treatment or processing.~~

Article X.X.11. (crustacean, fish and mollusc chapters), Article X.X.12. (amphibian chapters) and Article 10.4.15. (infection with ISAV chapter) list aquatic animal products that may be imported for retail trade for human consumption regardless of the disease status of the exporting country, zone or compartment for the disease in question. The assessment for inclusion of aquatic animal products in these articles is based on the form and presentation of the product, the expected volume of waste tissues generated by the consumer and the likely presence of viable pathogenic agent in the waste.

Annex 3 (contd)

EU comment
In line with proposed section number changes in the Fish Code chapters, the following adjustments will be required to the numbering and sections references of the paragraph above, sa follows:
"Article X.X.11. (crustacean~~, fish~~ and mollusc chapters), Article X.X.12. (amphibian and fish chapters) and Article 10.4.16~~15~~".
This comment is made in anticipation that the proposed changes to the fish Code chapters are adopted in May 2018, and if so, we expect this will be picked up as a matter of course when the final edits of the 2018 edition of the Aquatic Code are made.

~~Based on assessments using criteria in Article 5.4.2, in all disease specific chapters, point 1 of Article X.X.12. (for Chapter 10.4. the relevant Article is 10.4.15.) lists aquatic animal commodities for retail trade for human consumption from a country, zone or compartment not declared free from the disease in question. The criteria for inclusion of aquatic animal commodities in point 1 of Article Article X.X.12. include consideration of the form and presentation of the product, the expected volume of waste tissues generated by the consumer and the likely presence of viable pathogenic agent in the waste.~~

~~6~~8) International aquatic animal health certificates

An international aquatic animal health certificate is an official document that the Competent Authority of the exporting country issues in accordance with Chapter 5.1. and Chapter 5.2. It lists aquatic animal health requirements for the exported commodity. The quality of the exporting country's Aquatic Animal Health Services is essential in providing assurances to trading partners regarding the safety of exported aquatic animal products. This includes the Aquatic Animal Health Services' ethical approach to the provision of international health certificates and their history in meeting their notification obligations.

International health certificates underpin international trade and provide assurances to the importing country regarding the health status of the aquatic animals and aquatic animal products imported. ~~The measures prescribed should take into account the health status of both exporting and importing countries and be based upon the standards in the *Aquatic Code*.~~ The measures prescribed should take into account the health status of both exporting and importing countries and be based upon the standards in the *Aquatic Code*.

The following steps should be taken when drafting international aquatic animal health certificates:

a) identify the diseases, from which the importing country is justified in seeking protection because of its own aquatic animal health status. Importing countries should not impose measures in regards to diseases that occur in their own territory but are not subject to official control programmes;

b) for aquatic animal products capable of transmitting these diseases through international trade, the importing country should apply the relevant articles in the disease-specific chapters. The application of the articles should be adapted to the disease status of the ~~exporting~~ country, zone or compartment of origin. Such a status should be established in accordance with Article 1.4.6. except when articles of the relevant disease chapter specify otherwise;

c) when preparing international aquatic animal health certificates, the importing country should endeavour to use terms and expressions in accordance with the definitions given in the Glossary. ~~As stated in Article 5.2.3., international~~ International aquatic animal health certificates should be kept as simple as possible and should be cl

early worded, to avoid misunderstanding of the importing country's requirements;

d) Chapter 5.10. provides, as further guidance to Member Countries, model health certificates that should be used as a baseline.

~~7~~9) Guidance notes for importers and exporters

It is recommended that Competent Authorities prepare 'guidance notes' to assist importers and exporters to understand trade requirements. These notes should identify and explain the trade conditions, including the measures to be applied before and after export and during transport and unloading, and the relevant legal obligations and operational procedures. The guidance notes should advise on all details to be included in the health certification accompanying the consignment to its destination. Exporters should also be reminded of the International Air Transport Association rules governing air transport of aquatic animals and aquatic animal products.

Annex 4

GLOSSARY

EU position

The EU supports the adoption of this modified Glossary.

AQUATIC ANIMAL HEALTH STATUS

means the status of a country, *zone* or *compartment* with respect to a *disease* in accordance with the criteria listed in the relevant disease-specific chapter or Chapter 1.4. of the *Aquatic Code* ~~dealing with the *disease*~~.

BIOSECURITY

means a set of management and physical measures designed to ~~reduce~~ mitigate the *risk* of introduction~~, establishment and spread~~ of *pathogenic agents* ~~to~~ into, ~~from and within~~ or spread within, or release from, ~~an~~ *aquatic animal* populations ~~population~~.

BIOSECURITY PLAN

means a ~~plan~~ document that identifies ~~significant~~ potential pathways for the introduction of p*athogenic agents* into, ~~and~~ or spread within, or release from, ~~of *disease* in~~ a *zone*, ~~or~~ *compartment*~~,~~ or *aquaculture establishment*; and describes the measures ~~which are being, or will be,~~ applied to mitigate the identified *risks*~~,~~ ~~to introduce and spread *disease*~~, in accordance with ~~taking into consideration~~ the recommendations in the *Aquatic Code*. ~~The plan should also describe how these measures are audited, with respect to both their implementation and their targeting, to ensure that the *risks* are regularly re-assessed and the measures adjusted accordingly.~~

SELF-DECLARATION OF FREEDOM ~~FROM DISEASE~~

means declaration by the *Competent Authority* of the Member Country concerned that the country, *zone* or *compartment* is free from a *listed disease* based on implementation of the provisions of the *Aquatic Code* and the *Aquatic Manual*. [NOTE: The Member Country is encouraged to inform the OIE of its claimed status and the OIE may publish the claim but publication does not imply OIE endorsement of the claim.]

SUSCEPTIBLE SPECIES

means ~~a~~ species of *aquatic animals* ~~*animal* in which *infection*~~ that have ~~has~~ been demonstrated as susceptible to *infection* with a specific *pathogenic agent*, in accordance with Chapter 1.5. ~~by the occurrence of natural *cases* or by experimental exposure to the *pathogenic agent* that mimics natural transmission pathways~~.

Annex 5

CHAPTER 1.3.

DISEASES LISTED BY THE OIE

EU position

The EU thanks the OIE and supports the adoption of this modified chapter.

~~Preamble:~~ The ~~following~~ *diseases* in this chapter ~~are~~ have been assessed in accordance with Chapter 1.2. and ~~constitutes~~ constitute ~~are listed by~~ the OIE list of *aquatic animal diseases* ~~according to the criteria for listing an aquatic animal disease~~ (see ~~Article 1.2.2.~~).

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

Article 1.3.1.

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

– Infection with ~~Epizootic~~ epizootic haematopoietic necrosis virus ~~disease~~

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

– Infection with *Gyrodactylus salaris*

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

– Infection with salmonid alphavirus

- ~~Infectious~~ Infection with infectious haematopoietic necrosis virus

– ~~Koi~~ Infection with koi herpesvirus ~~disease~~

– Infection with red ~~Red~~ sea bream iridovirus ~~iridoviral disease~~

– ~~Spring~~ Infection with spring viraemia of carp virus

– ~~Viral~~ Infection with viral haemorrhagic septicaemia virus

Article 1.3.2.

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

– Infection with abalone herpesvirus

– Infection with *Bonamia ostreae*

– Infection with *Bonamia exitiosa*

– Infection with *Marteilia refringens*

– Infection with *Perkinsus marinus*

– Infection with *Perkinsus olseni*

Annex 5 (contd)

Article 1.3.3.

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

– Acute hepatopancreatic necrosis disease

– Infection with *Aphanomyces astaci* (crayfish plague)

– Infection with *Hepatobacter penaei* (necrotising hepatopancreatitis)

– Infection with infectious hypodermal and haematopoietic necrosis virus

– Infection with infectious myonecrosis virus

– Infection with *Macrobrachium rosenbergii* nodavirus (white tail disease)

– Infection with Taura syndrome virus

– Infection with white spot syndrome virus

– Infection with yellow head virus genotype 1.

Article 1.3.4.

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

– Infection with *Batrachochytrium dendrobatidis*

– Infection with *Batrachochytrium salamandrivorans*

– Infection with *Ranavirus* species.

Annex 6

CHAPTER 5.3.

OIE PROCEDURES RELEVANT TO THE AGREEMENT ON THE APPLICATION OF SANITARY AND PHYTOSANITARY MEASURES OF THE WORLD TRADE ORGANIZATION

EU position

The EU thanks the OIE and supports the adoption of this modified chapter.

Article 5.3.1.

The Agreement on the Application of Sanitary and Phytosanitary Measures and role and responsibility of the OIE

The *Agreement on the Application of Sanitary and Phytosanitary Measures* (SPS Agreement) specifically encourages the Members of the World Trade Organization to base their *sanitary measures* on international standards, guidelines and recommendations, where they exist. Members may choose to implement *sanitary measures* ~~that exceed more stringent~~ more stringent ~~adopt a higher level of protection than that provided by~~ than those in international standards, ~~texts~~ if these are deemed necessary to protect *aquatic animal* or human health and are scientifically justified by a *risk analysis* ~~there is a scientific justification or if the level of protection provided by the relevant international texts is considered to be inappropriate~~. In such circumstances, Members ~~are subject to obligations relating to *risk assessment* and to~~ should adopt a consistent approach ~~of~~ to *risk management*.

~~The SPS Agreement encourages Governments to make a wider use of risk analysis: WTO Members shall undertake an assessment as appropriate to the circumstances of the actual risk involved.~~

To promote transparency, ~~The~~ the SPS Agreement, in Article 7, obliges WTO Members to notify changes in, and provide relevant information on, *sanitary measures* ~~which~~ that may, directly or indirectly, affect international trade.

The SPS Agreement recognises the OIE as the relevant international organisation responsible for the development and promotion of international animal health standards, guidelines, and recommendations affecting trade in ~~live~~ *aquatic animals* and *aquatic animal products*.

Article 5.3.2.

Introduction ~~on~~ to the ~~judgement~~ determination of the equivalence of sanitary measures

The importation of *aquatic animals* and *aquatic animal products* involves a degree of *risk* to ~~the~~ *aquatic animal ~~health status~~* and human health ~~status of~~ in an *importing country*. The estimation of that *risk* and the choice of the appropriate *risk management* option~~(s)~~ are made ~~more~~ difficult by differences among the *aquatic animal* health management systems and *aquatic animal* production and processing systems in Member Countries. However, ~~it is now recognised that~~ significantly different ~~animal health and production~~ systems and measures ~~can provide~~ may achieve equivalent *aquatic animal* and human health protection for the purposes of *international trade~~, with benefits to both the importing country and the exporting country~~*.

~~These~~ The recommendations in this chapter are intended to assist Member Countries to determine whether *sanitary measures* arising from different ~~animal health and production~~ systems ~~may provide~~ achieve the same level of *aquatic animal* and human health protection. ~~They discuss pinciples~~ Principles are provided ~~which might~~ that may be utilised in a ~~judgement~~ determination of equivalence, and outline a step-wise process for trading partners to follow ~~in facilitating a judgement of~~ ~~equivalence~~. These provisions are applicable whether equivalence applies ~~at the level of~~ to specific measures, specific *commodities* or on a systems-wide basis~~, and whether equivalence applies to specific areas of trade or aquatic animal products, or~~ in ~~generally general~~.

Annex 6 (contd)

Article 5.3.3.

General considerations on the ~~judgement~~ determination of the equivalence of sanitary measures

Before trade in *aquatic animals* or their products ~~may~~ occurs, an *importing country* ~~must~~ should be ~~satisfied~~ assured that ~~its~~ *aquatic animal* ~~*health status*~~ and human health in its *territory* will be appropriately protected. In most cases, the *risk management* measures adopted ~~drawn up~~ will rely in part on judgements made about the *aquatic animal* health management and *aquatic animal* production system~~(s)~~ in the *exporting country* and the effectiveness of *sanitary measures* ~~procedures~~ applied ~~undertaken~~ there. Systems operating in the *exporting country* may differ from those in the *importing country* and from those in other countries with which the *importing country* has traded. Differences may be ~~with respect to~~ in infrastructure, policies ~~and/~~or operating procedures, laboratory systems, approaches to control of ~~the pests and~~ *diseases* ~~present~~, border security and internal movement controls.

~~International recognition of the legitimacy of different approaches to achieving the importing country's appropriate level of protection (ALOP) has led to the principle of equivalence being included in trade agreements, including the SPS Agreement of the WTO.~~

If trading partners agree that the measures applied achieve the same level of health protection, these measures are considered equivalent. Benefits of applying equivalence may include:

1) minimising costs associated with *international trade* by ~~tailoring~~ allowing *sanitary measures* to be tailored ~~animal health measures~~ to local circumstances;

2) maximising *aquatic animal* health outcomes for a given level of resource input;

3) facilitating trade by achieving the required health protection through less trade restrictive *sanitary measures*; and

4) decreased reliance on relatively costly ~~*commodity*~~ testing ~~and isolation procedures~~ ~~in bilateral or multilateral agreements~~.

The *Aquatic Code* recognises equivalence by recommending alternative *sanitary measures* for many *diseases*. Equivalence may be ~~gained~~ achieved, for example, by enhanced *surveillance* and monitoring, by the use of alternative test, treatment or isolation procedures, or by combinations of the above. To facilitate the ~~judgement~~ determination of equivalence, Member Countries should base their *sanitary measures* on the OIE standards~~,~~ and guidelines ~~and recommendations of the OIE~~.

~~It is essential to apply a scientific~~ Member Countries should use *risk analysis* to ~~the extent practicable in~~ establish~~ing~~ the basis for a ~~judgement~~ determination of equivalence.

Article 5.3.4.

Prerequisite considerations ~~in a judgement~~ for the determination of equivalence

1) Application of risk assessment

~~Application of the discipline of *risk*~~ *Risk assessment* provides a structured basis for judging equivalence among different *sanitary measures* as it allows a comparison ~~close examination to be made~~ of the effect of a measure~~(s)~~ on a particular step~~(s)~~ in the importation pathway~~, and the relative~~ with the effect~~s~~ of a proposed alternative measure~~(s) on the same or related steps~~.

A ~~judgement~~ determination of equivalence should ~~needs to~~ ~~assess~~ compare the effectiveness of the *sanitary measures* ~~in terms of its effectiveness~~ against ~~regarding~~ the particular *risk* or group of *risks* against which ~~it~~ ~~the measure~~ ~~is~~ they are designed to protect. ~~Such an assessment may include the following elements: the purpose of the measure, the level of protection achieved by the measure and the contribution the measure makes to achieving the ALOP of the importing country.~~

2) Categorisation of sanitary measures

Proposals for equivalence may ~~be in terms of a measure comprising~~ consider a single component ~~of a measure~~ (e.g. an isolation or sampling procedure, a test or treatment requirement, a certification procedure) or multiple components (e.g. a production system for a *commodity*) of a *sanitary measure*, or a combination of *sanitary measures*. ~~Multiple components or combinations of measures~~ *Sanitary measures* ~~Measures~~ may be applied consecutively or concurrently.

Sanitary measures are ~~those~~ described in each disease-specific chapter ~~chapter~~ of the *Aquatic Code* ~~which are used for~~ to manage the *risks* ~~reduction and are appropriate for particular~~ posed by that *disease* ~~*diseases*~~. ~~*Sanitary measures* may be applied either alone or in combination and include test requirements, processing requirements, inspection or certification procedures, quarantine confinements, and sampling procedures.~~

For the purposes of ~~judging~~ determining equivalence, *sanitary measures* can be broadly categorised as:

a) infrastructure~~:~~ including the legislative base (e.g. *aquatic animal* health law) and administrative systems (e.g. organisation of *Veterinary Services* or *Aquatic Animal Health Services* ~~national and regional animal health authorities, emergency response organisations~~);

b) programme design and~~/~~implementation~~:~~ including documentation of systems, performance and decision criteria, laboratory capability, and provisions for certification, audit and enforcement;

c) specific technical requirement~~:~~ including requirements applicable to the use of secure facilities, treatment (e.g. retorting of cans), specific test (e.g. ELISA) and procedures (e.g. pre-export inspection).

~~A *sanitary*~~ *Sanitary measure*~~(*s*)~~ proposed for a ~~judgement~~ determination of equivalence may fall into one or more of these categories, which are not mutually exclusive.

In some cases, such as a method for inactivation of *pathogenic agents*, a comparison of specific technical requirements may suffice. In many instances, however, ~~a judgement as to~~ assessment of whether the same level of protection ~~is likely to~~ will be achieved may only be ~~able to be~~ determined through an evaluation of all relevant components of an *exporting country*'s *aquatic animal* health management systems and *aquatic animal* production systems. ~~For example, a judgement of equivalence for a specific sanitary measure at the programme design/implementation level may require a prior examination of infrastructure while a judgement of equivalence for a specific measure at the specific technical requirement level may require that the specific measure be judged in its context through examination of infrastructure and programmes.~~

Article 5.3.5.

Principles for ~~judgement~~ determination of equivalence

~~In conjunction with the above considerations,~~ ~~judgement~~ Determination of the equivalence of *sanitary measures* should be based on application of the following principles:

1) an *importing country* has the right to set the level of protection it deems appropriate ~~(its ALOP)~~ in relation to human and animal life and health in its *territory*; this ~~ALOP~~ may be expressed in qualitative or quantitative terms;

2) the *importing country* should be able to describe the reason for each *sanitary measure* i.e. the level of protection intended to be achieved by application of the identified measure against a ~~*hazard*~~ *risk*;

3) an *importing country* should recognise that *sanitary measures* different from the ones it has proposed may be capable of ~~providing~~ achieving the same level of protection, in particular, it should consider the existence of *free zones* or *compartments*, and of ~~*safe*~~ safe *aquatic animal products*;

4) the *importing country* should, upon request, ~~enter into consultations~~ consult with the *exporting country* with the aim of facilitating a ~~judgement~~ determination of equivalence;

5) any *sanitary measure* or combination of *sanitary measures* can be proposed for ~~judgement~~ determination of equivalence;

Annex 6 (contd)

6) an interactive process should be followed that applies a defined sequence of steps, and utilises an agreed process for exchange of information, so as to limit data collection to that which is necessary, to minimise administrative burden, and to facilitate resolution of claims;

7) the *exporting country* should be able to demonstrate objectively how the alternative *sanitary measure*~~(s)~~ proposed as equivalent will provide the same level of protection;

8) the *exporting country* should present a submission for equivalence in a form that facilitates ~~judgement~~ determination by the *importing country*;

9) the *importing country* should evaluate submissions for equivalence in a timely, consistent, transparent and objective manner, and in accordance with appropriate *risk assessment* principles;

10) the *importing country* should take into account any knowledge of and prior experience with the *Veterinary Authority* or other *Competent Authority* of the *exporting country*;

11) the *importing country* should take into account any arrangements it has with other *exporting countries* on similar issues;

12) the *importing country* may also take into account any knowledge of the *exporting country's* arrangements with other *importing countries*;

13) the *exporting country* should, upon request, provide the *importing country* access to information ~~access to enable~~ on the procedures or systems ~~which~~ that are the subject of the equivalence ~~judgement~~ determination ~~to be examined and evaluated upon request of the *importing country*~~;

14) the *importing country* should be the sole ~~determinant~~ judge of equivalence, but should provide to the *exporting country* a full explanation for its judgement;

15) to facilitate a ~~judgement~~ determination of equivalence, Member Countries should base their *sanitary measures* on relevant OIE standards and guidelines, where these exist. However, they may choose to implement more stringent *sanitary measures* ~~that exceed OIE standards~~ if these are scientifically justified by a *risk analysis*;

16) to allow the ~~judgement~~ determination of equivalence to be reassessed if necessary, the *importing country* and the *exporting country* should keep each other informed of significant changes to infrastructure, health status or programmes ~~which~~ that may bear on the ~~judgement~~ determination of equivalence; and

17) appropriate technical assistance from an *importing country*, following a ~~should give positive consideration to a~~ request by an *exporting* ~~developing~~ *country*, ~~for appropriate technical assistance that would~~ may facilitate the successful completion of a ~~judgement~~ determination of equivalence.

Article 5.3.6.

Sequence of steps to be taken in ~~judgement~~ determination of equivalence

There is no single sequence of steps ~~which~~ that ~~must~~ should be followed in all ~~judgements~~ determinations of equivalence. The steps that trading partners choose will generally depend on the circumstances and their trading experience. Nevertheless, ~~The~~ the interactive sequence of steps described below may be useful for assessing any ~~all~~ *sanitary measures* irrespective of their categorisation as infrastructure, programme design~~/~~ and implementation or specific technical requirement components of an *aquatic animal* health management system or ~~and~~ *aquatic animal* production system.

This sequence assumes that the *importing country* is meeting its obligations under the WTO SPS Agreement and has in place a transparent measure based either on an international standard or a *risk analysis*.

Annex 6 (contd)

Recommended steps are:

1) the *exporting country* identifies the measure~~(s)~~ for which it wishes to propose an alternative ~~measure(s),~~ and requests from the *importing country* a reason for its *sanitary measure* in terms of the level of protection intended to be achieved against a ~~*hazard*(s)~~ *risk*;

2) the *importing country* explains the reason for the measure~~(s),~~ in terms that ~~which~~ would facilitate comparison with an alternative *sanitary measure*~~(s)~~ and consistent with the principles set out in these provisions;

3) the *exporting country* demonstrates the case for equivalence of an alternative *sanitary measure*~~(s)~~ in a form ~~which~~ that facilitates evaluation ~~analysis~~ by an *importing country*;

4) the *exporting country* responds to any technical concerns raised by the *importing country* by providing relevant further information;

5) ~~judgement~~ determination of equivalence by the *importing country* should take~~s~~ into account as appropriate:

   a) the impact of biological variability and uncertainty;

   b) the expected effect of the alternative *sanitary measure*~~(s) on all relevant hazards~~;

   c) OIE standards and guidelines;

   d) ~~application of solely *qualitative* frameworks where it is not possible or reasonable to conduct *quantitative*~~ the results of a *risk assessment*;

6) the *importing country* notifies the *exporting country* of its judgement and its ~~the underlying~~ reasons within a reasonable period of time. The judgement:

   a) ~~recognition~~ recognises ~~of~~ the equivalence of the *exporting country's* alternative *sanitary measure*~~(s)~~; or

   b) request~~s~~ ~~for~~ further information; or

   c) ~~rejection~~ rejects ~~of~~ the case for equivalence of the alternative *sanitary measure*~~(s)~~;

7) an attempt should be made to resolve any differences of opinion over judgement of a case~~, either interim or final,~~ by using an agreed mechanism such as ~~to reach consensus (e.g.~~ the OIE informal procedure for dispute mediation~~), or by referral to an agreed expert~~ (Article 5.3.8.);

8) depending on the category of measures involved, the *importing country* and the *exporting country* may informally acknowledge the equivalence or enter into a formal agreement of equivalence ~~agreement~~ giving effect to the judgement ~~or a less formal acknowledgement of the equivalence of a specific measure(s) may suffice~~.

An *importing country* recognising the equivalence of an *exporting country's* alternative *sanitary measure*~~(s) needs to~~ should ensure that it acts consistently with regard to applications from third countries for recognition of equivalence applying to the same or a very similar measure~~(s)~~. Consistent action does not mean however that a specific measure~~(s)~~ proposed by several *exporting countries* should always be judged as equivalent because ~~as~~ a measure~~(s)~~ should not be considered in isolation but as part of a system of infrastructure, policies and procedures, in the context of the *aquatic animal* health situation in the *exporting country*.

Annex 6 (contd)

Article 5.3.7.

Sequence of steps to be taken in establishing a zone~~/~~ or~~—~~compartment and having it recognised for international trade purposes

The terms '*zone'* and 'zoning' in the *Aquatic Code* have the same meaning as 'region', 'area' and 'regionalisation' in the SPS Agreement of the WTO.

The requirements for establishing ~~There is no single sequence of steps which should be followed in establishing of~~ a ~~disease free~~ *zone* or a *compartment* declared free of a *disease* ~~is~~ are described in Chapter 4.1. and in each *disease*-specific chapter ~~Chapter 4.1.~~ and should be considered by trading partners when establishing *sanitary measures* for trade. ~~The steps that the Veterinary Services or *Aquatic Animal Health Services* of the importing country and the exporting country choose and implement will generally depend on the circumstances existing within the countries and at their borders, and their trading history. The recommended steps are~~ The requirements include:

1. For zoning

a) The *exporting country* identifies a geographical area within its *territory*, which, based on *surveillance*, it considers to contain an *aquatic animal subpopulation* with a distinct health status with respect to a specific *disease*./~~specific diseases based on surveillance~~.

b) The *exporting country* describes in the *biosecurity plan* for the *zone* the measures ~~which are being, or will be,~~ applied to distinguish such an area epidemiologically from other parts of its territory, in accordance with the recommendations in the *Aquatic Code*.

c) Upon request, the *exporting country* provides to the *importing country*:

i) ~~the above information to the *importing country*, with~~ an explanation of why the area, as described in points *a)* and *b)* above, can be treated as an epidemiologically separate *zone* for *international trade* purposes;

ii) ~~access to enable the procedures or systems that establish the *zone* to be examined and evaluated upon request by the *importing country*.~~ access to information on the procedures or systems that establish the *zone*.

d) The *importing country* determines whether it accepts such an area as a *zone* for the importation of *aquatic animals* ~~and~~ or *aquatic animal products*, taking into account:

i) an evaluation of the *exporting country's Veterinary Services* or *Aquatic Animal Health Services*;

ii) the result of a *risk assessment* based on the information provided by the *exporting country* and its own research;

iii) its own *aquatic animal* health situation with respect to the *disease*~~(s)~~ concerned; and

iv) other relevant OIE standards or guidelines.

e) The *importing country* notifies the *exporting country* of its ~~determination~~ judgement and ~~the underlying~~ its reasons, within a reasonable period of time, being:

i) recognition of the *zone*; or

ii) request for further information; or

iii) rejection of the area as a *zone* for *international trade* purposes.

f) An attempt should be made to resolve any differences over recognition of the *zone*, either in the interim or finally, by using an agreed mechanism to reach consensus such as the OIE informal procedure for dispute mediation (Article 5.3.8.).

g) The *Veterinary Authorities* or other *Competent Authorities* of the *importing* and *exporting countries* should enter into an formal agreement recognising the *zone*.

2. For compartmentalisation

a) Based on discussions with the relevant industry, the *exporting country* identifies within its territory a *compartment* comprising an *aquatic animal subpopulation* contained in one or more *establishments*, or and other premises operating under common management practices and related to *biosecurity plan*. The *compartment* contains an identifiable *aquatic animal subpopulation* with a distinct health status with respect to a specific *disease*(s). The *exporting country* describes how this status is maintained through a partnership between the relevant industry and the *Veterinary Authority* or other *Competent Authority* of the *exporting country*.

b) The *exporting country* examines the *compartment's biosecurity plan* and confirms through an audit that:

i) the *compartment* is epidemiologically closed throughout its routine operating procedures as a result of effective implementation of its *biosecurity plan*; and

ii) the *surveillance* and monitoring programme in place is appropriate to verify the status of such a *subpopulation* with respect to such the disease(s) in question.

c) The *exporting country* describes the *compartment*, in accordance with the recommendations in the *Aquatic Code* Chapters 4.1. and 4.2.

d) Upon request, the *exporting country* provides to the *importing country*:

i) ~~the above information to the *importing country*, with~~ an explanation of why such a *subpopulation*, as described in points *a)* and *b)* above, can be treated as an epidemiologically separate *compartment* for *international trade* purposes; and

ii) ~~access to enable the procedures or systems that establish the *compartment* to be examined and evaluated upon request by the *importing country*~~ access to information on the procedures or systems that establish the *compartment*.

e) The *importing country* determines whether it accepts such a *subpopulation* as a *compartment* for the importation of *aquatic animals* or and *aquatic animal products*, taking into account:

i) an evaluation of the *exporting country's Veterinary Service* or *Aquatic Animal Health Services*;

ii) the result of a *risk assessment* based on the information provided by the *exporting country* and its own research;

iii) its own *aquatic animal* health situation with respect to the disease(s) concerned; and

iv) other relevant OIE standards or guidelines.

f) The *importing country* notifies the *exporting country* of its determination judgement and the underlying its reasons, within a reasonable period of time, being:

i) recognition of the *compartment*; or

ii) request for further information; or

iii) rejection of such a *subpopulation* as a *compartment* for *international trade* purposes.

g) An attempt should be made to resolve any differences over recognition of the *compartment*, either in the interim or finally, by using an agreed mechanism to reach consensus such as the OIE informal procedure for dispute mediation (Article 5.3.8.).

h) The *Veterinary Authorities* or other *Competent Authorities* of the *importing* and *exporting countries* should enter into an formal agreement recognising the *compartment.*

Annex 6 (contd)

i) The *Veterinary Authority* or other *Competent Authorities* of the *exporting country* should promptly inform *importing countries* of any occurrence of a *disease* in respect of which the *compartment* was defined.

Article 5.3.8.

The OIE informal procedure for dispute mediation

The OIE ~~shall~~ maintains ~~its existing~~ a voluntary in-house mechanisms for assisting Member Countries to resolve differences. In-house procedures that ~~which~~ will apply are that:

1) Both parties agree to give the OIE a mandate to assist them in resolving their differences.

2) If considered appropriate, the Director General of the OIE recommends an expert, or experts, and a chairman, as requested, agreed by both parties.

3) Both parties agree on the terms of reference and working programme, and to meet all expenses incurred by the OIE.

4) The expert or experts are entitled to seek clarification of any of the information and data provided by either country in the assessment or consultation processes, or to request additional information or data from either country.

5) The expert or experts ~~shall~~ submit a confidential report to the Director General of the OIE, who ~~will~~ then transmits it to both parties.

Annex 7

CHAPTER 5.4.

CRITERIA TO ASSESS THE SAFETY OF AQUATIC ANIMAL PRODUCTS ~~COMMODITIES~~

EU position

The EU thanks the OIE and supports the adoption of this modified chapter.

Preamble: In the context of this chapter the word 'safety' is applied only to animal health considerations for *listed diseases*.

Article 5.4.1.

Criteria to assess the safety of ~~aquatic animals and~~ aquatic animal products imported (or transited) for any purpose regardless of the disease X status of the exporting ~~for any purpose from a~~ country, zone or compartment ~~not declared free from disease X~~

~~In~~ all ~~*disease* chapters,~~ ~~point~~ Point 1 of Article X.X.3. of all disease-specific chapters (Sections 8-11), lists ~~*aquatic animals* and~~ *aquatic animal products* that can be imported (or transited) for any purpose regardless of the *disease* X status of the *exporting* ~~traded for any purpose from a~~ *country*, *zone* or *compartment* ~~not declared free from *disease* X~~. The criteria for inclusion of ~~*aquatic animals* and~~ *aquatic animal products* in point 1 of Article X.X.3. are based on the absence of the *pathogenic agent* in the ~~traded *aquatic animals* and~~ *aquatic animal product~~s~~* or inactivation of the *pathogenic agent* by treatment or processing.

The assessment of the safety of the ~~*aquatic animals* and~~ *aquatic animal product~~s~~* using the criteria relating to treatment or processing can only be undertaken where treatments or processing are well defined. It may not be necessary to provide details of the entire treatment or process undertaken. However, the steps considered critical in the inactivation of the *pathogenic agent* of concern should be detailed.

It is assumed that treatment or processing prior to importation (i) is done by using standardised protocols, which include the steps considered critical in the inactivation of the *pathogenic agent* of concern; (ii) is conducted in accordance with good manufacturing practices ~~Good Manufacturing Practices~~; and (iii) that any other steps in the treatment, processing, and subsequent handling and transport of the *aquatic animal product* do not jeopardise the safety of the traded *aquatic animal product*.

Criteria

For an ~~*aquatic animal* or~~ *aquatic animal product* to be considered safe for *international trade* under the provisions of Article X.X.3., it should comply with the following criteria:

1) Absence of pathogenic agent in the traded ~~aquatic animal or~~ aquatic animal product:

   a) There is strong evidence that the *pathogenic agent* is not present in the tissues from which the ~~*aquatic animal* or~~ *aquatic animal product* is derived.

   AND

   b) The water (including ice) used to process or transport the ~~*aquatic animal* or~~ *aquatic animal product* is not contaminated with the *pathogenic agent* and the processing prevents cross contamination of the ~~*aquatic animal* or~~ *aquatic animal product* ~~to be traded~~.

OR

2) Even if the *pathogenic agent* is present in, or contaminates the tissues from which the ~~*aquatic animal* or~~ *aquatic animal product* is derived, *pathogenic agent* is inactivated by the treatment or processing methods to produce the ~~*aquatic animal* or~~ *aquatic animal product* ~~to be traded inactivate the *pathogenic agent*~~ such as:

   a) physical (e.g. temperature, drying, smoking);

   AND/OR

   b) chemical (e.g. iodine, pH, salt, smoke);

   AND/OR

c) biological (e.g. fermentation).

Article 5.4.2.

Criteria to assess the safety of ~~aquatic animals or~~ aquatic animal products imported (or transited) for retail trade for human consumption regardless of the disease X status of the exporting ~~from a~~ country, zone or compartment ~~not declared free from disease X~~

~~In all *disease* chapters, point~~ Point 1 of Article X.X.12. (amphibian ~~and fish~~ disease-specific chapters) and Article X.X.11. (crustacean, fish and mollusc disease-specific chapters) lists ~~*aquatic animals* or~~ *aquatic animal products* for retail trade for human consumption. The criteria for inclusion of ~~*aquatic animals* or~~ *aquatic animal products* in point 1 of Article X.X.12. (amphibian ~~and fish~~ disease-specific chapters) and Article X.X.11. (crustacean, fish and mollusc disease-specific chapters) include consideration of the form and presentation of the product, the expected volume of waste tissues generated by the consumer and the likely presence of viable *pathogenic agent* in the waste.

For the purposes of this criterion retail means the selling or provision of the ~~*aquatic animals* or~~ *aquatic animal products* directly to the consumer with the intended purpose of human consumption. The retail pathway may also include wholesale distribution of the products provided they are not further processed by the wholesale distributor or the retailer, i.e. are not subjected to actions such as gutting, cleaning, filleting, freezing, thawing, cooking, unpacking, packing or repackaging.

It is assumed that: (i) the ~~*aquatic animals* or~~ *aquatic animal products* are used for human consumption only; (ii) waste may not always be handled in an appropriate manner that mitigates the introduction of the *pathogenic agent*; the level of risk is related to the waste disposal practices in each Member's country or territory; (iii) treatment or processing prior to importation is conducted in accordance with good manufacturing practices ~~Good Manufacturing Practices~~, and (iv) any other steps in the treatment, processing and subsequent handling of the ~~*aquatic animals* or~~ *aquatic animal products* prior to importation do not jeopardise the safety of the ~~traded~~ ~~*aquatic animals* or~~ *aquatic animal products*.

Criteria

For ~~*aquatic animals* or~~ *aquatic animal products* to be considered safe for *international trade* under the provisions of point 1 of Article X.X.12. (amphibian ~~and fish~~ disease-specific chapters) and Article X.X.11. (crustacean, fish and mollusc disease-specific chapters), it should comply with the following criteria:

1) the ~~*aquatic animal* or~~ *aquatic animal product* is prepared and packaged for retail trade for human consumption; AND

EITHER

2) it includes an ~~only a small~~ amount of raw waste tissues generated by the consumer that is unlikely to result in the introduction and establishment of the *pathogenic agent*;

OR

3) the *pathogenic agent* is not normally found in the waste tissues generated by the consumer.

Annex 8

CHAPTER 8.X.

INFECTION WITH *BATRACHOCHYTRIUM SALAMANDRIVORANS*

EU position

The EU thanks the OIE and in general supports the adoption of this modified chapter. The EU comment included in the introduction to the report should however be addressed by the AAHSC at its next meeting.

Article 8.X.1.

For the purposes of the *Aquatic Code*, infection with *Batrachochytrium salamandrivorans* means *infection* with the *pathogenic agent Batrachochytrium salamandrivorans*, of the Division Chytridiomycota and Order Rhizophydiales.

[Information on methods for *diagnosis* is provided in the *Aquatic Manual*] (under development).

Article 8.X.2.

Scope

The recommendations in this chapter apply to the following species that meet the criteria for listing as susceptible in accordance with Chapter 1.5.: [alpine newt *(Ichthyosaura alpestris)*, blue-tailed fire-bellied newt *(Cynops cyanurus)*, fire salamander *(Salamandra salamandra)*, eastern newt *(Nothophthalmus viridescens)*, French cave salamander *(Hydromantes strinatii)*, Italian newt *(Lissotriton italicus)*, yellow spotted newt (*Neurergus crocatus*), Japanese fire-bellied newt *(Cynops pyrrhogaster)*, northern spectacle salamander *(Salamandrina perspicillata)*, Tam Dao salamander (*Paramesotriton deloustali*), rough-skinned newt *(Taricha granulosa)*, sardinian brook salamander *(Euproctus platycephalus)* and Spanish ribbed newt *(Pleurodeles waltl)*] (under study).

Article 8.X.3.

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

1) *Competent Authorities* should not require any conditions related to *B. salamandrivorans*, regardless of the infection with *B. salamandrivorans* 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.X.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 *B. salamandrivorans*);

   b) cooked amphibian 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 *B. salamandrivorans*);

   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 *B. salamandrivorans*);

   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 *B. salamandrivorans*);

   e) amphibian skin leather.

2) When authorising the importation or transit of *aquatic animal products* derived from a species referred to in Article 8.X.2., other than those referred to in point 1 of Article 8.X.3., *Competent Authorities* should require the conditions prescribed in Articles 8.X.7. to 8.X.12. relevant to the infection with *B. salamandrivorans* 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.X.2. but which could reasonably be expected to pose a *risk* of transmission of *B. salamandrivorans*, 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.X.4.

Country free from infection with *B. salamandrivorans*

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from infection with *B. salamandrivorans* if all the areas covered by the shared water bodies are declared countries or *zones* free from infection with *B. salamandrivorans* (see Article 8.X.5.).

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

1) none of the *susceptible species* referred to in Article 8.X.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.X.2. are present and the following conditions have been met:

   a) there has been no occurrence of infection with *B. salamandrivorans* 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*)] (under development); and

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

OR

3) the infection with *B. salamandrivorans* 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 *B. salamandrivorans*;

OR

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

   a) on detection of *B. salamandrivorans*, 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 *B. salamandrivorans,* 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 *B. salamandrivorans*; and

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

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.X.5.

Article 8.X.5.

Zone or compartment free from infection with *B. salamandrivorans*

If a *zone* or *compartment* extends over more than one country, it can only be declared a *zone* or *compartment* free from infection with *B. salamandrivorans* 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 *B. salamandrivorans* may be declared free by the *Competent Authority* of the country concerned if:

1) none of the *susceptible species* referred to in Article 8.X.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.X.2. are present in the *zone* or *compartment* and the following conditions have been met:

   a) there has been no occurrence of infection with *B. salamandrivorans* 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*)] (under development); and

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

OR

3) the infection with *B. salamandrivorans* 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 *B. salamandrivorans*;

OR

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

   a) on detection of *B. salamandrivorans*, 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 *B. salamandrivorans*, 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 *B. salamandrivorans*; and

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

Annex 8 (contd)

Article 8.X.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from infection with *B. salamandrivorans* following the provisions of points 1 or 2 of Articles 8.X.4. or 8.X.5. (as relevant) may maintain its status as free from infection with *B. salamandrivorans* provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from infection with *B. salamandrivorans* following the provisions of point 3 of Articles 8.X.4. or 8.X.5. (as relevant) may discontinue *targeted surveillance* and maintain its free status provided that conditions that are conducive to clinical expression of infection with *B. salamandrivorans*, [as described in the corresponding chapter of the *Aquatic Manual*)] (under development), 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 *B. salamandrivorans*, *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.X.7.

Importation of aquatic animals or aquatic animal products from a country, zone or compartment declared free from infection with *B. salamandrivorans*

When importing *aquatic animals* of a species referred to in Article 8.X.2., or *aquatic animal products* derived thereof, from a country, *zone* or *compartment* declared free from infection with *B. salamandrivorans*, 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.X.4. or 8.X.5. (as applicable) and 8.X.6., the place of production of the *aquatic animals* or *aquatic animal products* is a country, *zone* or *compartment* declared free from infection with *B. salamandrivorans*.

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.X.3.

Article 8.X.8.

Importation of aquatic animals for aquaculture from a country, zone or compartment not declared free from infection with *B. salamandrivorans*

When importing, for *aquaculture*, *aquatic animals* of a species referred to in Article 8.X.2. from a country, *zone* or *compartment* not declared free from infection with *B. salamandrivorans*, 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) the treatment of all transport water, equipment, effluent and waste material to inactivate *B. salamandrivorans* 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 *B. salamandrivorans*;

b) in the *importing country*:

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

ii) test the F-0 population for *B. salamandrivorans* 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 *B. salamandrivorans*, ~~(as described in Chapter 2.1.X. of the *Aquatic Manual*)~~ and sample and test for *B. salamandrivorans* in accordance with Chapter 1.4. of the *Aquatic Code* and ~~[~~ ~~(as described in~~ Chapter 2.1.X. of the *Aquatic Manual*~~)~~] (under development);

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

vi) if *B. salamandrivorans* 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 8.X.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 *B. salamandrivorans*

When importing, for processing for human consumption, *aquatic animals* of a species referred to in Article 8.X.2., or *aquatic animal products* derived thereof, from a country, *zone* or *compartment* not declared free from infection with *B. salamandrivorans*, 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 8.X.3. or in point 1 of Article 8.X.12, or other products authorised by the *Competent Authority*; and

2) all water (including ice), equipment, *containers* and packaging material used in transport are treated to ensure inactivation of *B. salamandrivorans* 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 *B. salamandrivorans* 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.X.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 *B. salamandrivorans*

When importing *aquatic animals* of a species referred to in Article 8.X.2., or *aquatic animal products* derived thereof, intended for uses other than human consumption, including animal *feed* and agricultural, industrial, research or pharmaceutical use, from a country, *zone* or *compartment* not declared free from infection with *B. salamandrivorans*, 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.X.3. or other products authorised by the *Competent Authority*; and

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

Article 8.X.11.

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

When importing, for use in laboratories or zoos**,** *aquatic animals* of a species referred to in Article 8.X.2. from a country, *zone* or *compartment* not declared free from infection with *B. salamandrivorans*, 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 *B. salamandrivorans* 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 *B. salamandrivorans* 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.X.12.

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

1) *Competent Authorities* should not require any conditions related to *B. salamandrivorans*, regardless of the infection with *B. salamandrivorans* status of the *exporting country*, *zone* or *compartment*, when authorising the importation (or transit) of amphibian meat (skin off and fresh or frozen) that has been prepared and packaged for retail trade and complies 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 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 8.X.2. from a country, *zone* or *compartment* not declared free from infection with *B. salamandrivorans*, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

Annex 9

CHAPTER 8.1.

INFECTION WITH *BATRACHOCHYTRIUM DENDROBATIDIS*

EU position

The EU supports the adoption of this modified chapter.

Article 8.1.1.

For the purposes of the *Aquatic Code*, infection with *Batrachochytrium dendrobatidis* means *infection* with the *pathogenic agent Batrachochytrium dendrobatidis* of the Division Chytridiomycota and Order Rhizophydiales.

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

Article 8.1.2.

Scope

The recommendations in this chapter apply to: all species of *Anura* (frogs and toads), *Caudata* (salamanders, newts and sirens) and *Gymnophiona* (caecilians). The recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

Article 8.1.3.

Importation or transit of ~~aquatic animals and~~ aquatic animal products for any purpose regardless of the infection with *B. dendrobatidis* status of the exporting country, zone or compartment

1) *Competent Authorities* should not require any conditions related to ~~infection with~~ *B. dendrobatidis*, regardless of the infection with *B. dendrobatidis* 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 8.1.2. that ~~which~~ are intended for any purpose and ~~which~~ 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 *B. dendrobatidis*);

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

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

   d) mechanically dried amphibian products (i.e. a heat treatment of 100°C for at least 30 minutes or any time/temperature equivalent ~~which~~ that has been demonstrated to inactivate *B. dendrobatidis*);

   e) amphibian skin leather.

2) When authorising the importation or transit of ~~*aquatic animals* and~~ *aquatic animal products* derived from a species referred to in Article 8.1.2., other than those referred to in point 1 of Article 8.1.3., *Competent Authorities* should require the conditions prescribed in Articles 8.1.7. to 8.1.12. relevant to the infection with *B. dendrobatidis* status of the *exporting country*, *zone* or *compartment*.

3) When considering the importation or transit of ~~*aquatic animals* and~~ *aquatic animal products* derived from a species not ~~covered~~ referred to in Article 8.1.2. but which could reasonably be expected to pose a *risk* of ~~spread~~ transmission of ~~infection with~~ *B. dendrobatidis*, 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 ~~assessment~~ analysis.

Article 8.1.4.

Country free from infection with *B. dendrobatidis*

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from infection with *B. dendrobatidis* if all the areas covered by the shared water bodies are declared countries or *zones* free from ~~the *zone* are declared~~ infection with *B. dendrobatidis* (see Article 8.1.5.).

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

1) none of the *susceptible species* referred to in Article 8.1.2. 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 8.1.2. are present and the following conditions have been met:

   a) there has been no ~~observed~~ occurrence of ~~the *disease*~~ infection with *B. dendrobatidis* 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 ~~disease~~ infection with *B. dendrobatidis* 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 ~~infection with~~ *B. dendrobatidis*;

OR

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

   a) on detection of *B. dendrobatidis* ~~the *disease*~~, 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 ~~have been destroyed or removed from the *infected zone*~~ by means that minimise the likelihood ~~*risk*~~ of further transmission ~~spread~~ of *B. dendrobatidis* ~~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 infection with *B. dendrobatidis* ~~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 ~~infection with~~ *B. dendrobatidis*.

In the meantime, part or all of the unaffected ~~non-affected~~ area may be declared a free *zone* provided that such a part meets the conditions in point 3 of Article 8.1.5.

Annex 9 (contd)

Article 8.1.5.

Zone or compartment free from infection with *B. dendrobatidis*

If a *zone* or *compartment* extends over more than one country, it can only be declared a ~~an infection with *B. dendrobatidis* free~~ *zone* or *compartment* free from infection with *B. dendrobatidis* 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 *B. dendrobatidis* 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 8.1.2. 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 8.1.2. are present in the *zone* or *compartment* and the following conditions have been met;

   a) there has ~~not~~ been no ~~any observed~~ occurrence of infection with *B. dendrobatidis* ~~the *disease*~~ 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 *B. dendrobatidis* ~~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 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 ~~infection with~~ *B. dendrobatidis*;

OR

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

   a) on detection of *B. dendrobatidis* ~~the *disease*~~, 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 ~~have been destroyed or removed from the *infected zone*~~ by means that minimise the likelihood ~~*risk*~~ of further transmission ~~spread~~ of *B. dendrobatidis* ~~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 infection with *B. dendrobatidis* ~~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 ~~infection with~~ *B. dendrobatidis*.

Annex 9 (contd)

Article 8.1.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from infection with *B. dendrobatidis* following the provisions of points 1 or 2 of Articles 8.1.4. or 8.1.5. (as relevant) may maintain its status as free from infection with *B. dendrobatidis* provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from infection with *B. dendrobatidis* following the provisions of point 3 of Articles 8.1.4. or 8.1.5. (as relevant) may discontinue *targeted surveillance* and maintain its free status ~~as free from infection with *B. dendrobatidis*~~ provided that conditions that are conducive to clinical expression of infection with *B. dendrobatidis*, as described in the corresponding chapter of the *Aquatic Manual*, ~~exist~~ and *basic biosecurity conditions* are continuously maintained.

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

Article 8.1.7.

Importation of aquatic animals ~~and~~ or aquatic animal products from a country, zone or compartment declared free from infection with *B. dendrobatidis*

When importing *aquatic animals* of a species referred to in Article 8.1.2., or ~~and~~ *aquatic animal products* derived thereof, from a country, *zone* or *compartment* declared free from infection with *B. dendrobatidis*, 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, ~~certifying that,~~ on the basis of the procedures described in Articles 8.1.4. or 8.1.5. (as applicable) and 8.1.6., the place of production of the *aquatic animals* or ~~and~~ *aquatic animal products* is a country, *zone* or *compartment* declared free from infection with *B. dendrobatidis*.

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* ~~*commodities*~~ listed ~~referred to~~ in point 1 of Article 8.1.3.

Article 8.1.8.

Importation of aquatic animals for aquaculture from a country, zone or compartment not declared free from infection with *B. dendrobatidis*

When importing, for *aquaculture*, *aquatic animals* of a species referred to in Article 8.1.2. from a country, *zone* or *compartment* not declared free from infection with *B. dendrobatidis*, 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) the treatment of all transport water, equipment, effluent and waste materials to inactive *B. dendrobatidis* 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 *B. dendrobatidis*;

b) in the *importing country*:

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

ii) test the F-0 population for *B. dendrobatidis* 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 ~~infection with~~ *B. dendrobatidis*, ~~(as described in Chapter 2.1.1. of the *Aquatic Manual*)~~ and sample and test for *B. dendrobatidis* in accordance with Chapter 1.4. of the *Aquatic Code* and Chapter 2.1.1. of the *Aquatic Manual*;

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

vi) if *B. dendrobatidis* 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 8.1.9.

Importation of aquatic animals or ~~and~~ aquatic animal products for processing for human consumption from a country, zone or compartment not declared free from infection with *B. dendrobatidis*

When importing, for processing for human consumption, *aquatic animals* of a species referred to in Article 8.1.2., or *aquatic animal products* derived thereof, from a country, *zone* or *compartment* not declared free from infection with *B. dendrobatidis*, 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 8.1.3.~~,~~ or ~~products described~~ in point 1 of Article 8.1.12., or other products authorised by the *Competent Authority*; and

2) all water (including ice), equipment, *containers* and packaging material used in transport ~~and all effluent and waste materials from the processing~~ are treated ~~in a manner that~~ to ensure~~s~~ inactivation of *B. dendrobatidis* or ~~is~~ disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and ~~that prevents contact of waste with *susceptible species*~~

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

For these *aquatic animals ~~commodities~~* 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 ~~commodity~~* being used for any purpose other than for human consumption.

Article 8.1.10.

Importation of aquatic animals or aquatic animal products intended for uses other than human consumption, including ~~use in~~ animal feed~~, or for~~ and agricultural, industrial, research or pharmaceutical use, from a country, zone or compartment not declared free from infection with *B. dendrobatidis*

When importing~~, for use in animal *feed* or for agricultural, industrial or pharmaceutical use,~~ *aquatic animals* of a species referred to in Article 8.1.2., 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 *B. dendrobatidis*, the *Competent Authority* of the *importing country* should require that:

1) the consignment ~~be~~ 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 8.1.3. or other ~~for slaughter and processing into~~ products authorised by the *Competent Authority*; and

2) all water (including ice), equipment, *containers* and packaging material used in transport are treated to ensure~~s~~ inactivation of *B. dendrobatidis* or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and ~~water and equipment used in transport and all effluent and waste materials from the processing facility be treated in a manner that inactivates *B. dendrobatidis*.~~

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

~~This article does not apply to *commodities* referred to in point 1 of Article 8.1.3.~~

Article 8.1.11.

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

When importing, for use in laboratories or zoos, *aquatic animals* of a species referred to in Article 8.1.2. from a country, *zone* or *compartment* not declared free from infection with *B. dendrobatidis*, the *Competent Authority* of the *importing country* should ensure:

1) the consignment is delivered directly to ~~direct delivery to~~, and ~~lifelong~~ held in, ~~holding~~ in ~~of the consignment~~, *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~~s~~ inactivation of *B. dendrobatidis* or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and ~~water and equipment used in transport and all effluent and waste materials from the processing facility be treated in a manner that inactivates *B. dendrobatidis.*~~

3) all effluent and waste materials from the *quarantine* facilities in the laboratories or zoos are treated to ensure inactivation of *B. dendrobatidis* or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.; and

~~2)~~ ~~the treatment of water and equipment used in transport and of all effluent and waste materials in a manner that inactivates *B. dendrobatidis*; and~~

~~3~~4) the carcasses are disposed of in accordance with Chapter 4.7.

Article 8.1.12.

Importation (or transit) of ~~aquatic animals and~~ aquatic animal products for retail trade for human consumption regardless of the infection with *B. dendrobatidis* status of the exporting ~~from a~~ country, zone or compartment ~~not declared free from infection with *B. dendrobatidis*~~

1) *Competent Authorities* should not require any conditions related to ~~infection with~~ *B. dendrobatidis*, regardless of the infection with *B. dendrobatidis* status of the *exporting country*, *zone* or *compartment*, when authorising the importation (or transit) of amphibian meat (skin off and fresh or frozen) that ~~which~~ has been prepared and packaged for retail trade and ~~which~~ complies 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* ~~*commodities*~~ Member Countries may wish to consider introducing internal measures to address the *risks* associated with the *aquatic animal product* ~~*commodity*~~ being used for any purpose other than for human consumption.

2) When importing ~~*aquatic animals* or~~ *aquatic animal products*, other than those referred to in point 1 above, derived from a ~~of~~ species referred to in Article 8.1.2. from a country, *zone* or *compartment* not declared free from infection with *B. dendrobatidis*, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

Annex 10

CHAPTER 8.2.

INFECTION WITH RANAVIRUS

EU position

The EU supports the adoption of this modified chapter.

Article 8.2.1.

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

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

Article 8.2.2.

Scope

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

Article 8.2.3.

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

1) *Competent Authorities* should not require any conditions related to ~~infection with~~ ranavirus, regardless of the infection with ranavirus status of the *exporting country*, *zone* or *compartment*, when authorising the importation or transit of the following *aquatic animal products* derived from a ~~from the~~ species referred to in Article 8.2.2. that ~~which~~ are intended for any purpose and ~~which~~ 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* ~~and Family Iridoviridae~~ [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 ~~which~~ has been demonstrated to inactivate all virus species of the genus *Ranavirus* ~~in the~~ and Family *Iridoviridae* [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 ~~which~~ has been demonstrated to inactivate all virus species of the genus *Ranavirus* ~~in the and Family Iridoviridae~~ [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 ~~which~~ has been demonstrated to inactivate all virus species of the genus *Ranavirus* ~~in the and Family Iridoviridae~~ [with the exception of epizootic haematopoietic necrosis virus and European catfish virus]).

2) When authorising the importation or transit of ~~*aquatic animals* and~~ *aquatic animal products* derived from a species referred to in Article 8.2.2., other than those referred to in point 1 of Article 8.2.3., *Competent Authorities* should require the conditions prescribed in Articles 8.2.7. to 8.2.12. relevant to the infection with ranavirus status of the *exporting country*, *zone* or *compartment*.

3) When considering the importation or transit of ~~*aquatic animals* and~~ *aquatic animal products* derived from a species not ~~covered~~ referred to in Article 8.2.2. but which could reasonably be expected to pose a *risk* of transmission ~~spread~~ of ~~infection with~~ ranavirus, 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 ~~assessment~~.

Article 8.2.4.

Country free from infection with ranavirus

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 if all the areas covered by the shared water bodies are declared countries or *zones* free from ~~the *zone* are declared~~ infection with ranavirus (see Article 8.2.5.).

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

1) none of the *susceptible species* referred to in Article 8.2.2. 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 8.2.2. are present and the following conditions have been met:

   a) there has been no ~~observed~~ occurrence of infection with ranavirus ~~the *disease*~~ 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 ~~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 two years; and

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

OR

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

   a) on detection of ranavirus ~~the *disease*~~, 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 ~~have been destroyed or removed from the *infected zone*~~ by means that minimise the likelihood ~~*risk*~~ of further transmission ~~spread~~ of ranavirus~~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 infection with ranavirus~~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 ~~infection with~~ ranavirus.

In the meantime, part or all of the unaffected ~~non-affected~~ area may be declared a free *zone* provided that such a part meets the conditions in point 3 of Article 8.2.5.

Article 8.2.5.

Zone or compartment free from infection with ranavirus

If a *zone* or *compartment* extends over more than one country, it can only be declared a ~~an infection with ranavirus free~~ *zone* or *compartment* free from infection with ranavirus 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 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 8.2.2. 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 8.2.2. are present in the *zone* or *compartment* and the following conditions have been met:

   a) there has ~~not~~ been no ~~any observed~~ occurrence of infection with ranavirus ~~the *disease*~~ 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 ~~disease~~ infection with ranavirus 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 ~~infection with~~ ranavirus;

OR

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

   a) on detection of infection with ranavirus~~the *disease*~~, 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 ~~have been destroyed or removed from the *infected zone*~~ by means that minimise the likelihood ~~*risk*~~ of further transmission ~~spread~~ of ranavirus~~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 infection with ranavirus~~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 ~~infection with~~ ranavirus.

Article 8.2.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from infection with ranavirus following the provisions of points 1 or 2 of Articles 8.2.4. or 8.2.5. (as relevant) may maintain its status as free from infection with ranavirus provided that *basic biosecurity conditions* are continuously maintained.

Annex 10 (contd)

A country, *zone* or *compartment* that is declared free from infection with ranavirus following the provisions of point 3 of Articles 8.2.4. or 8.2.5. (as relevant) may discontinue *targeted surveillance* and maintain its free status ~~as free from infection with ranavirus~~ provided that conditions that are conducive to clinical expression of infection with ranavirus, as described in the corresponding chapter of the *Aquatic Manual*, ~~exist~~ and *basic biosecurity conditions* are continuously maintained.

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

Article 8.2.7.

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

When importing *aquatic animals* of a species referred to in Article 8.2.2., or ~~and~~ *aquatic animal products* derived thereof, from a country, *zone* or *compartment* declared free from infection with ranavirus, 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* certifying that,~~ The *international aquatic animal health certificate* should state that, on the basis of the procedures described in Articles 8.2.4. or 8.2.5. (as applicable) and 8.2.6., the place of production of the *aquatic animals* or ~~and~~ *aquatic animal products* is a country, *zone* or *compartment* declared free from infection with ranavirus.

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 ~~*commodities* referred to~~ in point 1 of Article 8.2.3.

Article 8.2.8.

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

When importing, for *aquaculture*, *aquatic animals* of a species referred to in Article 8.2.2. from a country, *zone* or *compartment* not declared free from infection with ranavirus, 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) the treatment of all transport water, equipment, effluent and waste materials to inactivate ranavirus 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;

   b) in the *importing country*:

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

ii) test the F-0 population for ranavirus 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, ~~(as described in Chapter 2.1.2. of the *Aquatic Manual*)~~ and sample and test for ranavirus in accordance with Chapter 1.4. of the *Aquatic Code* and Chapter 2.1.2. of the *Aquatic Manual;*

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

vi) if ranavirus 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 8.2.9.

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

When importing, for processing for human consumption, *aquatic animals* of a species referred to in Article 8.2.2., or *aquatic animal products* derived thereof, from a country, *zone* or *compartment* not declared free from infection with ranavirus, 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 8.2.3., or ~~products described~~ in point 1 of Article 8.2.12., or other products authorised by the *Competent Authority*; and

2) all water (including ice), equipment, *containers* and packaging material used in transport ~~and all effluent and waste materials from the processing~~ are treated ~~in a manner that~~ to ensure~~s~~ inactivation of ranavirus or ~~is~~ disposed of in a biosecure manner ~~that prevents contact of waste with *susceptible species*~~ 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 or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

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

Article 8.2.10.

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

When importing *aquatic animals* of a species referred to in Article 8.2.2., or *aquatic animal products* derived thereof, intended for use~~s~~ other than human consumption, including ~~in~~ animal *feed* ~~or~~ and ~~for~~ agricultural, industrial, research or pharmaceutical use, ~~*aquatic animals* of the species referred to in Article 8.2.2.~~ from a country, *zone* or *compartment* not declared free from infection with ranavirus, the *Competent Authority* of the *importing country* should require that:

1) the consignment is ~~be~~ delivered directly to, and held in, *quarantine* or containment facilities until processed into one of the products referred to in point 1 of Article 8.2.3. or other ~~for slaughter and processing into~~ products authorised by the *Competent Authority*; and

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

4.7. and 5.5.; and ~~water and equipment used in transport and all effluent and waste materials from the processing facility be treated in a manner that inactivates ranavirus.~~

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

~~This article does not apply to *commodities* referred to in point 1 of Article 8.2.3.~~

Article 8.2.11.

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

When importing, for use in laboratories or zoos, *aquatic animals* of a species referred to in Article 8.2.2. from a country, *zone* or *compartment* not declared free from infection with ranavirus, the *Competent Authority* of the *importing country* should ensure:

1) the consignment is delivered directly to~~direct delivery to~~, and ~~lifelong~~ held ~~holding~~ in, ~~of the consignment~~ ~~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~~s~~ inactivation of ranavirus or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and ~~the treatment of water and equipment used in transport and of all effluent and waste materials in a manner that inactivates ranavirus; and~~

3) all effluent and waste materials from the *quarantine* facilities in the laboratories or zoos are treated to ensure inactivation of ranavirus or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7; and

~~3~~4) the ~~disposal of~~ carcasses are disposed of in accordance with Chapter 4.7.

Article 8.2.12.

Importation (or transit) of ~~aquatic animals and~~ aquatic animal products for retail trade for human consumption regardless of the infection with ranavirus status of the exporting ~~from a~~ country, zone or compartment ~~not declared free from infection with ranavirus~~

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

   – no *aquatic animal products* listed.

2) When importing ~~*aquatic animals* or~~ *aquatic animal products*, other than those referred to in point 1 above, derived from a ~~of~~ species referred to in Article 8.2.2. from a country, *zone* or *compartment* not declared free from infection with ranavirus, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

Annex 11

CHAPTER 9.4.

INFECTION WITH INFECTIOUS HYPODERMAL AND HAEMATOPOIETIC NECROSIS VIRUS

EU position

The EU supports the adoption of this modified chapter.

Article 9.4.1.

For the purposes of the *Aquatic Code*, infection with infectious hypodermal and haematopoietic necrosis virus means *infection* with the *pathogenic agent* *Decapod penstyldensovirus 1*, commonly known as infectious hypodermal and haematopoietic necrosis virus (IHHNV), of the Genus *Penstyldensovirus* ~~*Brevidensovirus*~~ and Family ~~Parvoviridae~~ *Parvoviridae*.

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

Article 9.4.2.

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 river prawn *(Macrobrachium rosenbergii)* (under study)~~, yellowleg shrimp *(Penaeus californiensis)*, giant tiger prawn *(Penaeus monodon)*, northern white shrimp *(Penaeus setiferus)*, blue shrimp *(Penaeus stylirostris)* and whiteleg shrimp *(Penaeus vannamei)*.

[…]

Annex 12

CHAPTER 10.1.

INFECTION WITH ~~THE~~ EPIZOOTIC HAEMATOPOIETIC NECROSIS VIRUS

EU position

The EU supports the adoption of this modified chapter.

Article 10.1.1.

For the purposes of the *Aquatic Code*, infection with epizootic haematopoietic necrosis virus ~~(EHN)~~ means *infection* with the *pathogenic agent* epizootic haematopoietic necrosis virus ~~EHN virus~~ (EHNV), of the Genus ~~genus~~ *Ranavirus*, ~~of the family~~ and Family ~~Iridoviridae~~ *Iridoviridae*.

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

Article 10.1.2.

Scope

The recommendations in this chapter apply to the following species that meet the criteria for listing as susceptible in accordance with Chapter 1.5.: black bullhead *(Ameiurus melas)*, crimson spotted rainbow fish *(Melanotaenia fluviatilis)*, eastern mosquito fish *(Gambusia holbrooki)*, European perch *(Perca fluviatilis)*, macquarie perch *(Macquaria australasica)*, mosquito fish *(Gambusia affinis)*, mountain galaxias *(Galaxias olidus)*, northern pike *(Esox lucius)*, pike-perch *(Sander lucioperca)*, ~~redfin perch *(Perca fluviatilis)* and~~ rainbow trout *(Oncorhynchus mykiss)* and silver perch *(Bidyanus bidyanus)*. ~~These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.~~

Article 10.1.3.

Importation or transit of ~~aquatic animals and~~ aquatic animal products for any purpose regardless of the infection with EHNV ~~epizootic haematopoietic necrosis virus~~ status of the exporting country, zone or compartment

1) *Competent Authorities* should not require any conditions related to EHNV, regardless of the infection with EHNV status of the *exporting country*, *zone* or *compartment*, when authorising the importation or transit of the following *aquatic animal products* derived from a ~~the~~ species referred to in Article 10.1.2. that ~~which~~ are intended for any purpose and ~~which~~ 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 EHNV);

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

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

   d) fish oil;

   e) fish *meal*;

   f) fish skin leather.

2) When authorising the importation or transit of ~~*aquatic animals* and~~ *aquatic animal products* ~~of~~ derived from a species referred to in Article 10.1.2., other than those referred to in point 1 of Article 10.1.3., *Competent Authorities* should require the conditions prescribed in Articles 10.1.7. to 10.1.~~11.~~12. relevant to the infection with EHNV status of the *exporting country*, *zone* or *compartment*.

3) When considering the importation or transit of ~~*aquatic animals* and~~ *aquatic animal products* ~~of~~ derived from a

species not referred to ~~covered~~ in Article 10.1.2. but which could reasonably be expected to pose a *risk* of transmission ~~spread~~ of EHNV, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this ~~assessment~~ analysis.

Article 10.1.4.

Country free from infection with EHNV ~~the epizootic haematopoietic necrosis virus~~

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from infection with EHNV if all the areas covered by the shared water bodies are declared countries or *zones* free from infection with EHNV (see Article 10.1.5.).

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

1) none of the *susceptible species* referred to in Article 10.1.2. 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 10.1.2. are present and the following conditions have been met:

   a) there has been no ~~observed~~ occurrence of infection with EHNV ~~the *disease*~~ 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 ~~past~~ last ten years;

OR

3) the infection with EHNV ~~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 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 EHNV;

OR

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

   a) on detection of EHNV ~~the *disease*~~, 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 ~~havebeen destroyed or removed from the *infected zone*~~ by means that minimise the likelihood ~~*risk*~~ of further ~~spread~~ transmission of EHNV ~~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 infection with EHNV ~~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 EHNV.

   In the meantime, part or all of the unaffected ~~non-affected~~ area may be declared a free *zone* provided that such a part meets the conditions in point 3 of Article 10.1.5.

Article 10.1.5.

Zone or compartment free from infection with EHNV ~~the epizootic haematopoietic necrosis virus~~

If a *zone* or *compartment* extends over more than one country, it can only be declared ~~an infection with EHNV~~

~~free~~ a *zone* or *compartment* free from infection with EHNV 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 EHNV 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 10.1.2. 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 10.1.2. are present in the *zone* or *compartment* and the following conditions have been met:

   a) there has been no ~~observed~~ occurrence of infection with EHNV 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 ~~disease~~ infection with EHNV 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 EHNV;

OR

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

   a) on detection of EHNV ~~the *disease*~~, 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 ~~been destroyed or removed from the *infected zone*~~ by means that minimise the likelihood ~~*risk*~~ of further ~~spread~~ transmission of EHNV ~~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 infection with EHNV ~~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 EHNV.

Article 10.1.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from infection with EHNV following the provisions of points 1 or 2 of Articles 10.1.4. or 10.1.5. (as relevant) may maintain its status as free from infection with EHNV provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from infection with EHNV following the provisions of point 3 of Articles 10.1.4. or 10.1.5. (as relevant) may discontinue *targeted surveillance* and maintain its free status ~~as free from EHNV~~ provided that conditions that are conducive to clinical expression of infection with EHNV, as described in the corresponding chapter of the *Aquatic Manual*, ~~exist~~ and *basic biosecurity conditions* are continuously maintained.

Annex 12 (contd)

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 EHNV, *targeted surveillance* ~~needs~~ should ~~to~~ be continued at a level determined by the *Aquatic Animal Health Service* on the basis of the likelihood of *infection*.

Article 10.1.7.

Importation of aquatic animals ~~and~~ or aquatic animal products from a country, zone or compartment declared free from infection with EHNV ~~the epizootic haematopoietic necrosis virus~~

When importing *aquatic animals* of a species referred to in Article 10.1.2., or ~~and~~ *aquatic animal products* ~~of species referred to in Article 10.1.2.~~ derived thereof, from a country, *zone* or *compartment* declared free from infection with EHNV, 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, ~~certifying that,~~ on the basis of the procedures described in Articles 10.1.4. or 10.1.5. (as applicable) and 10.1.6., the place of production of the *aquatic animals* or ~~and~~ *aquatic animal products* is a country, *zone* or *compartment* declared free from infection with EHNV.

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* ~~*commodities*~~ listed ~~referred to~~ in point 1 of Article 10.1.3.

Article 10.1.8.

Importation of aquatic animals for aquaculture from a country, zone or compartment not declared free from infection with EHNV ~~the epizootic haematopoietic necrosis virus~~

When importing, for *aquaculture*, *aquatic animals* of a species referred to in Article 10.1.2. from a country, *zone* or *compartment* not declared free from infection with EHNV, 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) the treatment of all transport water, equipment, effluent and waste materials to inactive EHNV 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 EHNV.

   b) In the *importing country*:

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

      ii) test the F-0 population for EHNV 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 conductive to the clinical expression of infection with EHNV, ~~(as described in Chapter 2.3.1. of the *Aquatic Manual*)~~ and sample and test for EHNV in accordance with Chapter 1.4. of the *Aquatic Code* and ~~(as described in~~ Chapter 2.3.1. of the *Aquatic Manual*~~)~~;

      v) if EHNV is not detected in the F-1 population, it may be defined as free from infection with EHNV

and may be released from *quarantine*;

vi) if EHNV 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 10.1.9.

Importation of aquatic animals ~~and~~ or aquatic animal products for processing for human consumption from a country, zone or compartment not declared free from infection with EHNV ~~the epizootic haematopoietic necrosis virus~~

When importing, for processing for human consumption, *aquatic animals* ~~or *aquatic animal products*~~ of a species referred to in Article 10.1.2., or *aquatic animal products* derived thereof, from a country, *zone* or *compartment* not declared free from infection with EHNV, 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 10.1.3.~~, or products described~~ or in point 1 of Article 10.1.~~11~~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 EHNV 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 EHNV or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

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

Article 10.1.10.

Importation of aquatic animals or aquatic animal products intended for uses other than human consumption, including ~~use in~~ animal feed~~, or for~~ and agricultural, industrial, research or pharmaceutical use, from a country, zone or compartment not declared free from infection with EHNV ~~the epizootic haematopoietic necrosis virus~~

When importing *aquatic animals* of a species referred to in Article 9.5.2., or *aquatic animal products* derived thereof, intended for uses other than human consumption, including ~~use in~~ animal *feed* ~~or for~~ and agricultural, industrial, research or pharmaceutical use, ~~*aquatic animals* of species referred to in Article 10.1.2.~~ from a country, *zone* or *compartment* not declared free from infection with EHNV, the *Competent Authority* of the *importing country* should require that:

1) the consignment is delivered directly to, and held in, ~~in~~ *quarantine* or containment facilities until processed into one of the products referred to in point 1 of Article 10.1.3. or other ~~facilities for slaughter and processing into~~ 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 EHNV or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and ~~water used in transport and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of EHNV~~

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

~~This article does not apply to *commodities* referred to in point 1 of Article 10.1.3.~~

Article 10.1.11.

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

When importing, for use in laboratories or zoos, *aquatic animals* of species referred to in Article 10.1.2. from a country, *zone* or *compartment* not declared free from infection with EHNV, 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

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2) all water (including ice), equipment, *containers* and packaging material used in transport are treated to ensure inactivation of EHNV 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 EHNV 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 10.1.1~~1~~2.

Importation (or transit) of ~~aquatic animals and~~ aquatic animal products for retail trade for human consumption regardless of the infection with EHNV status of the exporting ~~from a~~ country, zone or compartment ~~not declared free from infection with the epizootic haematopoietic necrosis virus~~

1) *Competent Authorities* should not require any conditions related to EHNV, regardless of the infection with EHNV status of the *exporting country*, *zone* or *compartment*, when authorising the importation (or transit) of fish fillets or steaks (chilled or frozen) that ~~which~~ have been prepared and packaged for retail trade and ~~which~~ 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* ~~*commodities*~~ Member Countries may wish to consider introducing internal measures to address the *risks* associated with the *aquatic animal product* ~~*commodity*~~ being used for any purpose other than for human consumption.

2) When importing ~~*aquatic animals* or~~ *aquatic animal products*, other than those referred to in point 1 above, derived from a ~~of~~ species referred to in Article 10.1.2. from a country, *zone* or *compartment* not declared free from infection with EHNV, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

Annex 13

CHAPTER 10.2.

INFECTION WITH *APHANOMYCES INVADANS* (EPIZOOTIC ULCERATIVE SYNDROME)

EU position

The EU supports the adoption of this modified chapter.

Article 10.2.1.

For the purposes of the *Aquatic Code*, infection with *Aphanomyces invadans* means all *infections* caused by the pathogenic agent *Aphanomyces invadans* (syn. *A. piscicida*). The *disease* was previously referred to as epizootic ulcerative syndrome.

~~Standards for diagnostic tests are described~~ 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)*, bagrid catfishes *(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)*, tank goby *(Glossogobius giuris)*, marble goby *(Oxyeleotris marmoratus)*, gobies *(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)*, 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 gouramy *(Trichogaster trichopterus)*. These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

Article 10.2.3.

Importation or transit of ~~aquatic animals and~~ aquatic animal products for any purpose regardless of the infection with *A. invadans* status of the exporting country, zone or compartment

1) *Competent Authorities* should not require any conditions related to ~~infection with~~ *A. invadans*, regardless of the infection with *A. invadans* status of the *exporting country*, *zone* or *compartment*, when authorising the importation or transit of the following ~~*aquatic animals* and~~ *aquatic animal products* derived from ~~the~~ a species referred to in Article 10.2.2. that ~~which~~ are intended for any purpose and ~~which~~ 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 *A. invadans*);

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

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

   d) fish oil;

   e) fish *meal*;

f) frozen eviscerated fish;

g) frozen fish fillets or steaks.

2) When authorising the importation or transit of ~~*aquatic animals* and~~ *aquatic animal products* ~~of~~ derived from a species referred to in Article 10.2.2., other than those referred to in point 1 of Article 10.2.3., *Competent Authorities* should require the conditions prescribed in Articles 10.2.7. to 10.2.12~~1~~. relevant to infection with *A. invadans* status of the *exporting country*, *zone* or *compartment*.

3) When considering the importation or transit of ~~*aquatic animals* and *aquatic animal products* from an *exporting country*, *zone* or *compartment*~~ not declared free from infection with ~~*A. invadans* of~~ derived from a species not ~~covered~~ referred to in Article 10.2.2. but which could reasonably be expected to pose a *risk* of transmission ~~spread~~ of ~~infection with~~ *A. invadans*, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *exporting country* should be informed of the outcome of this analysis ~~assessment~~.

Article 10.2.4.

Country free from infection with *A. invadans*

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from infection with *A. invadans* if all the areas covered by the shared water bodies are declared countries or *zones* free from infection with *A. invadans* (see Article 10.2.5.).

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

1) a country where there has been no ~~observed~~ occurrence of infection with *A. invadans* 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*, may make a *self-declaration of freedom* from infection with *A. invadans* when *basic biosecurity conditions* have been continuously met in the country for at least the last ten years;

OR

2) the infection with *A. invadans* ~~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 two years; and

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

OR

3) it previously made a *self-declaration of freedom* from infection with *A. invadans* and subsequently lost its ~~*disease*~~ free status due to the detection of ~~infection with~~ *A. invadans* but the following conditions have been met:

   a) on detection of *A. invadans* ~~the *disease*~~, 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 ~~have been destroyed or removed from the *infected zone*~~ by means that minimise the likelihood ~~*risk*~~ of further transmission ~~spread~~ of *A. invadans* ~~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 infection with *A. invadans* ~~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 ~~infection with~~ *A. invadans*.

In the meantime, part or all of the unaffected ~~non-affected~~ area may be declared a free *zone* provided that such a part meets the conditions in point 2 of Article 10.2.5.

Article 10.2.5.

Zone or compartment free from infection with *A. invadans*

If a *zone* or *compartment* extends over more than one country, it can only be declared a *zone* or *compartment* free from infection with *A. invadans* 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 *A. invadans* may be declared free by the *Competent Authority~~(ies)~~* of the country~~(ies)~~ concerned if:

1) a *zone* or *compartment* where the species referred to in Article 10.2.2. are present but there has been no ~~observed~~ occurrence of infection with *A. invadans* ~~the disease~~ 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*, may be declared free from infection wiith *A. invadans* when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the last ten years;

OR

2) the infection with *A. invadans* ~~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 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 ~~infection with~~ *A. invadans*;

OR

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

   a) on detection of *A. invadans* ~~the *disease*~~, 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 ~~have been destroyed or removed from the *infected zone*~~ by means that minimise the likelihood ~~*risk*~~ of further transmission ~~spread~~ of *A. invadans* ~~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 infection with *A. invadans* ~~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 ~~infection with~~ *A. invadans*.

Article 10.2.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from infection with *A. invadans* following the provisions of point 1 of Articles 10.2.4. or 10.2.5. (as relevant) may maintain its status as free from infection with *A. invadans* provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from infection with *A. invadans* following the provisions of point 2 of Articles 10.2.4. or 10.2.5. (as relevant) may discontinue *targeted surveillance* and maintain its free status ~~as free from infection with *A. invadans*~~ provided that conditions that are conducive to clinical expression of infection with *A. invadans*, as described in the corresponding chapter of the *Aquatic Manual*, ~~exist~~ and *basic biosecurity conditions* are continuously maintained.

Annex 13 (contd)

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

Article 10.2.7.

Importation of aquatic animals ~~and~~ or aquatic animal products from a country, zone or compartment declared free from infection with *A. invadans*

When importing *aquatic animals* of a species referred to in Article 10.2.2., or ~~and~~ *aquatic animal products* ~~of species referred to in Article 10.2.2.~~ derived thereof, from a country, *zone* or *compartment* declared free from infection with *A. invadans*, 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 ~~certifying that~~, on the basis of the procedures described in Articles 10.2.4. or 10.2.5. (as applicable) and 10.2.6., the place of production of the *aquatic animals* or ~~and~~ *aquatic animal products* is a country, *zone* or *compartment* declared free from infection with *A. invadans*.

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 ~~*commodities* referred to~~ in point 1 of Article 10.2.3.

Article 10.2.8.

Importation of aquatic animals for aquaculture from a country, zone or compartment not declared free from infection with *A. invadans*

When importing, for *aquaculture*, *aquatic animals* of a species referred to in Article 10.2.2. from a country, *zone* or *compartment* not declared free from infection with *A. invadans*, 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) the treatment of all transport water, equipment, effluent and waste materials to inactive *A. invadans* 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 *A. invadans*.

   b) In the *importing country*:

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

      ii) test the F-0 population for *A. invadans* 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 *A. invadans*, ~~(as described in Chapter 2.3.2. of the *Aquatic Manual*)~~ and sample and test for *A. invadans* in accordance with Chapter 1.4. of the *Aquatic Code* and Chapter 2.3.2. of the *Aquatic Manual*;

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

vi) if *A. invadans* 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 10.2.9.

Importation of aquatic animals ~~and~~ or aquatic animal products for processing for human consumption from a country, zone or compartment not declared free from infection with *A. invadans*

When importing, for processing for human consumption, *aquatic animals* ~~or *aquatic animal products*~~ of a species referred to in Article 10.2.2, or *aquatic animal products* derived thereof, from a country, *zone* or *compartment* not declared free from infection with *A. invadans*, 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 10.2.3.~~,~~ or ~~products described~~ in point 1 of Article 10.2.12., 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 *A. invadans* or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and~~and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of *A. invadans* or is disposed in a manner that prevents contact of waste with *susceptible species*.~~

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

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

Article 10.2.10.

Importation of aquatic animals or aquatic animal products intended for uses other than human consumption, including ~~use in~~ animal feed~~, or for~~ and agricultural, industrial, research or pharmaceutical use, from a country, zone or compartment not declared free from infection with *A. invadans*

When importing *aquatic animals* of a species referred to in Article 10.2.2., or *aquatic animal products* derived thereof, intended for uses other than human consumption, including ~~use~~ ~~in~~ animal *feed* ~~or for~~ and agricultural, industrial, research or pharmaceutical use, ~~*aquatic animals* of species referred to in Article 10.2.2~~, from a country, *zone* or *compartment* not declared free from infection with *A. invadans*, 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 10.2.3. or other ~~facilities for slaughter and processing into~~ 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 *A. invadans* or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and~~ and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of *A. invadans* or is disposed in a manner that prevents contact of waste with *susceptible*~~

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

~~This Article does not apply to *commodities* referred to in point 1 of Article 10.2.3.~~

Annex 13 (contd)

Article 10.2.11.

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

When importing, for use in laboratories or zoos, *aquatic animals* of a species referred to in Article 10.2.2. from a country, *zone* or *compartment* not declared free from infection with *A. invadans*, 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 *A. invadans* 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 *A. invadans* 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 10.2.12~~1.~~

Importation (or transit) of ~~aquatic animals and~~ aquatic animal products for retail trade for human consumption regardless of the infection with *A. invadans* status of the exporting ~~from a~~ country, zone or compartment ~~not declared free from infection with *A. invadans*~~

1) *Competent Authorities* should not require any conditions related to ~~infection with~~ *A. invadans*, regardless of the infection with *A. invadans* status of the *exporting country*, *zone* or *compartment*, when authorising the importation (or transit) of fish fillets or steaks (chilled) that ~~which~~ have been prepared and packaged for retail trade and ~~which~~ 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* ~~*commodities*~~ Member Countries may wish to consider introducing internal measures to address the *risks* associated with the *aquatic animal product* ~~*commodity*~~ being used for any purpose other than for human consumption.

2) When importing ~~*aquatic animals* or~~ *aquatic animal products*, other than those referred to in point 1 above, derived from a ~~of~~ species referred to in Article 10.2.2. from a country, *zone* or *compartment* not declared free from infection with *A. invadans*, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

Annex 14

CHAPTER 10.3.

INFECTION WITH *GYRODACTYLUS SALARIS*

EU position
The EU notes that none of its previous comments (available here https://ec.europa.eu/food/sites/food/files/safety/docs/ia_standards_oie_eu_position_aahsc-report_201709.pdf) were taken into account.
We in general support all of the horizontal changes to the Fish chapters that are proposed by the OIE for adoption in this round, and agree that the changes should be made simultaneously for consistency across these chapters.
However, as pointed out in our previous comments, point 2 in Articles 10.3.4. and 10.3.5. as currently worded 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 symptoms and therefore would require active surveillance. We also note that the OIE AAC response as given in their February meeting report indicates that the sentence "conditions that are conducive to its clinical expression (as described in the corresponding chapter of the Aquatic Manual)" effectively addresses this. However, the meaning and application of this sentence is not always made sufficiently clear by reference to the Aquatic Manual chapters alone which leaves the phrase open to differing interpretation. Furthermore, if this phrase is not intended by the OIE to include requirement for susceptible species that express clinical disease as part of the "conducive conditions for clinical expression" then it is not an effective criteria for self-assessment of freedom for *G. salaris*, for the reasons we already presented previously. Considering that this is an important condition for self-declaration of freedom, and to ensure consistency and effective (safe) decision making, we would request the OIE consider:
1) deletion of point 2 in articles 10.3.4 and 10.3.5 as previously requested for this chapter, and/ or
2) overall clarification of the meaning of the sentence "conditions conducive to clinical expression" to ensure this is consistently understood and applied for all self-declarations going forwards, irrelevant of the disease in question. For example, we are aware ourselves of various interpretations as to how this is applied. Indeed, some consider this applies to environmental factors only, others to environmental factors and to the presence of susceptible species presenting with clinical or non-clinical infection, and others still to environmental factors and the presence of susceptible species presenting with clinical infection only. As this is such an important criteria, with serious consequences for disease spread if misunderstood, we request that the OIE gives consideration to either providing a clear definition in the glossary, or perhaps inserting a specific summary section in the corresponding Aquatic Manual chapters to make this absolutely clear (reference is made to the EUcomments on the new Manual chapter format at Annex 39). Also to note, Manual chapters do not consistently describe the susceptible species that present with clinical and non-clinical infections, which is valuable information for effective disease control and surveillance – again, please also refer to the EU comments on the new Manual chapter format at Annex 39.

Further comments are inserted in the text below.

Article 10.3.1.

For the purposes of the *Aquatic Code*, ~~gyrodactylosis~~ infection with *Gyrodactylus salaris* means *infection* with the *pathogenic agent* *Gyrodactylus salaris, a* viviparous freshwater ectoparasite, ~~*Gyrodactylus salaris* of the Genus *Gyrodactylus*~~ the Family Gyrodactylidae and (~~Phylum *Platyhelminthes*;~~ Class ~~Monogenea~~ Monogenea).

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

Article 10.3.2.

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.: Arctic char *(Salvelinus alpinus),* Atlantic salmon *(Salmo salar)*, brown trout *(Salmo trutta),* ~~rainbow trout *(Oncorhynchus mykiss)*, Arctic char *(Salvelinus alpinus)*, North American brook trout *(Salvelinus fontinalis)*~~, grayling *(Thymallus thymallus)*, ~~North American lake trout (*Salvelinus namaycush*),~~ and North American brook trout *(Salvelinus fontinalis)* and rainbow trout (*Oncorhynchus mykiss*) ~~brown trout *(Salmo trutta)*~~. ~~The recommendations also apply to other fish species in waters where the parasite is present, because these species may carry the parasite and act as *vectors*.~~

Article 10.3.3.

Importation or transit of ~~aquatic animals and~~ aquatic animal products for any purpose regardless of the infection with *G. salaris* status of the exporting country, zone or compartment

1) *Competent Authorities* should not require any ~~related~~ conditions related to ~~infection with~~ *G. salaris*, regardless of the infection with *G. salaris* status of the *exporting country*, *zone* or *compartment*, when authorising the importation or transit of the following ~~*aquatic animals* and~~ *aquatic animal products* derived from a ~~the~~ species referred to in Article 10.3.2. that ~~which~~ are intended for any purpose and ~~which~~ 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 *G. salaris*);

   b) pasteurised fish products that have been subjected to a heat treatment at 63˚C for at least 30 minutes (or any time/temperature equivalent ~~which~~ that has been demonstrated to inactivate *G. salaris*);

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

   d) naturally dried~~,~~ eviscerated fish (i.e. sun-dried or wind-dried);

   e) frozen eviscerated fish that have been subjected to minus 18°C or lower temperatures;

   f) frozen fish fillets or steaks that have been subjected to minus 18°C or lower temperatures;

   g) chilled eviscerated fish that have been harvested from seawater with a salinity of at least 25 parts per thousand (ppt);

   h) chilled fish fillets or steaks derived from fish that have been harvested from seawater with a salinity of at least 25 ppt;

   i) chilled fish products from which the skin, fins and gills have been removed;

   j) non-viable fish roe;

   k) fish oil;

   l) fish *meal*;

   m) fish skin leather.

2) When authorising the importation or transit of ~~*aquatic animals* and~~ *aquatic animal products* ~~of~~ derived from a species referred to in Article 10.3.2., other than those referred to in point 1 of Article 10.3.3., *Competent Authorities* should require the conditions prescribed in Articles 10.3.7. to 10.3.~~11.~~12. relevant to the infection with *G. salaris* status of the *exporting country*, *zone* or *compartment*.

3) When considering the importation or transit of ~~*aquatic animals* and~~ *aquatic animal products* ~~of~~ derived from a species not referred to ~~covered~~ in Article 10.3.2. but which could reasonably be expected to pose a *risk* of ~~spread~~ transmission of ~~infection with~~ *G. salaris*, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this ~~assessment~~ analysis.

Article 10.3.4.

Country free from infection with *G. salaris*

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from infection with *G. salaris* if all the areas covered by the shared water bodies ~~watercourse(s)~~ are declared countries or *zones* free from infection with *G. salaris* (see Article 10.3.5.).

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

1) none of the *susceptible species* referred to in Article 10.3.2. 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 10.3.2. are present and the following conditions have been met:

   a) there has been no ~~observed~~ occurrence of ~~the~~ infection with *G. salaris* ~~*disease*~~ 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 *G. salaris* ~~*disease*~~ status prior to *targeted surveillance* is unknown but the following conditions have been met:

EU comment

The EU suggests slightly amending the point above for readability, as follows:

"3) the status for infection with *G. salaris* ~~status~~ prior to [...]".

   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 ~~infection with~~ *G. salaris*;

OR

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

   a) on detection of ~~the~~ *G. salaris* ~~*disease*~~, 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 ~~have been destroyed or removed from the *infected zone*~~ by means that minimise the likelihood ~~*risk*~~ of further ~~spread~~ transmission of ~~the~~ *G. salaris* ~~*disease*~~, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed, or the waters containing the infected fish have been treated by chemicals that kill the parasite; and

EU comment

The EU suggests amending the text of point 4 b) above to accommodate successful eradication strategies for *G. salaris* that have used a combination of methods, including natural migration of infected fish to sea combined with physical barriers, as follows:

"b) infected populations within the infected zone have been killed and disposed of, removed or permitted to migrate from infected systems by means that minimise [...]"

Indeed, the currently proposed text does not account for the natural migration of infected wild fish to sea which together with the use of impassable barriers can form part of a successful eradication strategy as has been demonstrated by Norway

This comment is valid also for point point 5 b) of Article 10.3.5. below.

c) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place since eradication of ~~the~~ infection with *G. salaris* ~~*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 ~~infection with~~ *G. salaris*.

In the meantime, part or all of the unaffected ~~non-affected~~ area may be declared a free *zone* provided that such a part meets the conditions in point 3 of Article 10.3.5.

Article 10.3.5.

Zone or compartment free from infection with *G. salaris*

If a *zone* or *compartment* extends over more than one country, it can only be declared ~~for~~ a *zone* or *compartment* free from infection with *G. salaris* 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 *G. salaris* 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 10.3.2. 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 10.3.2. are present in the *zone* or *compartment* and the following conditions have been met:

a) there has been no ~~observed~~ occurrence of ~~the~~ infection with *G. salaris* ~~*disease*~~ 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 five years;

OR

3) a *zone* or *compartment* supplied with seawater with a salinity of at least 25 ppt may be declared free from infection with *G. salaris* provided that no *aquatic animal products* of a species referred to in Article 10.3.2. are introduced from a site of a lesser health status for infection with *G. salaris* during the 14 days prior to any ~~live~~ fish transfers from the *zone* or *compartment*;

OR

4) the infection with *G. salaris* ~~*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 ten 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 ~~infection with~~ *G. salaris*;

OR

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

a) on detection of ~~the~~ *G. salaris* ~~*disease*~~, 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 ~~have been destroyed or removed from the *infected zone*~~ by means that minimise the likelihood ~~*risk*~~ of further ~~spread~~ transmission of ~~the~~ *G. salaris* ~~*disease*~~, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed, or the waters containing the infected fish have been treated by chemicals that kill the parasite; 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 *G. salaris* ~~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 ~~infection with~~ *G. salaris*.

Article 10.3.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from infection with *G. salaris* following the provisions of points 1 or 2 of Articles 10.3.4. or 10.3.5. (as relevant) may maintain its status as free from infection with *G. salaris* provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from infection with *G. salaris* following the provisions of point 3 of Article 10.3.4. or point 4 of 10.3.5. (as relevant) may discontinue *targeted surveillance* and maintain its free status ~~as free from infection with *G. salaris*~~ provided that conditions that are conducive to clinical expression of infection with *G. salaris*, as described in the corresponding chapter of the *Aquatic Manual*, ~~exist,~~ and *basic biosecurity conditions* are continuously maintained.

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

Article 10.3.7.

Importation of aquatic animals ~~and~~ or aquatic animal products from a country, zone or compartment declared free from infection with *G. salaris*

When importing *aquatic animals* ~~and *aquatic animal products*~~ of a species referred to in Article 10.3.2., or *aquatic animal products* derived thereof, from a country, *zone* or *compartment* declared free from infection with *G. salaris*, 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, ~~certifying that,~~, on the basis of the procedures described in Articles 10.3.4. or 10.3.5. (as applicable) and 10.3.6., the place of production of the *aquatic animals* or ~~and~~ *aquatic animal products* is a country, *zone* or *compartment* declared free from infection with *G. salaris*.

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 ~~*commodities* referred to~~ in point 1 of Article 10.3.3.

Article 10.3.8.

Importation of aquatic animals for aquaculture from a country, zone or compartment not declared free from infection with *G. salaris*

When importing, for *aquaculture*, *aquatic animals* of a species referred to in Article 10.3.2. from a country, *zone* or *compartment* not declared free from infection with *G. salaris*, the *Competent Authority* of the *importing country* should assess the *risk* in accordance with Chapter 2.1. and consider the *risk* mitigation measures in the points ~~1 and 2~~ below.

1) *a)* the direct delivery to and lifelong holding of the imported *aquatic animals* in a *quarantine* facility;

and

b) the treatment of all transport water, equipment, effluent and waste materials to inactive *G. salaris* in accordance with Chapters 4.3., 4.7. and 5.5.;

OR

2) immediately prior to movement, the *aquatic animals* have been for a continuous period of at least 14 days:

a) held in water with a salinity of at least 25 parts per thousand;

and

b) had no contact with other *aquatic animals* of the species referred to in Article 10.3.2.;

OR

3) in the case of eggs, they have been disinfected by a method demonstrated to be effective against *G. salaris* and following *disinfection* do not come into contact with anything which may affect their health status.

~~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)* the treatment of all transport water, equipment, effluent and waste materials to inactive *G. salaris* in accordance with Chapters 4.3., 4.7. and 5.5.~~

1) ~~When importing, for *aquaculture*,~~ live ~~*aquatic animals* of a species referred to in Article 10.3.2. from a country, *zone* or *compartment* not declared free from infection with *G. salaris*, the *Competent Authority* of the *importing country* should:~~

~~*a)* require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* attesting that:~~

~~*i)* the *aquatic animals* have been held, immediately prior to export, in water with a salinity of at least 25 parts per thousand for a continuous period of at least 14 days; and~~

~~*ii)* no other *aquatic animals* of the species referred to in Article 10.3.2. have been introduced during that period;~~

~~OR~~

~~*iii)* in the case of eyed eggs, the eggs have been disinfected by a method demonstrated to be effective against *G. salaris*;~~

~~OR~~

~~*b)* assess the *risk* and apply *risk* mitigation measures such as:~~

~~*i)* the direct delivery to and lifelong holding of the imported *aquatic animals* in a *quarantine* facility; and~~

~~*ii)* if breeding from the imported fish, *disinfection* of the fertilised eggs by a method demonstrated to be effective against *G. salaris*, and complete separation of the hatched progeny from the imported animals;~~

~~*iii)* the treatment of all transport water, equipment, effluent and waste materials to inactive *G. salaris* 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 *G. salaris*.~~

Annex 14 (contd)

~~*b)* In the *importing country*:~~

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

~~*ii)* test the F-0 population for *G. salaris* 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 conductive to the clinical expression of *G. salaris*, (as described in Chapter 2.3.3. of the *Aquatic Manual*) and sample and test for *G. salaris* in accordance with Chapter 1.4. of the *Aquatic Code* and (as described in Chapter 2.3.3. of the *Aquatic Manual*);~~

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

~~*vi)* if *G. salaris* 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.~~

Article 10.3.9.

Importation of aquatic animals ~~and~~ or aquatic animal products for processing for human consumption from a country, zone or compartment not declared free from infection with *G. salaris*

When importing, for processing for human consumption, *aquatic animals* ~~or *aquatic animal products*~~ of a species referred to in Article 10.3.2., or *aquatic animal products* derived thereof, from a country, *zone* or *compartment* not declared free from infection with *G. salaris*, 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 10.3.3.~~,~~ ~~, or products described~~ or in point 1 of Article 10.3.~~11.~~12., or other products authorised by the *Competent Authority*; and

2) all water (including ice), equipment, *containers* and packaging material used are treated to ensure inactivation of *G. salaris* or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and ~~or is disposed in a manner that prevents contact of waste with *susceptible species*~~

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

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

Article 10.3.10.

Importation of aquatic animals or aquatic animal products intended for uses other than human consumption, including ~~use in~~ animal feed~~, or for~~ and agricultural, industrial, research or pharmaceutical use, from a country, zone or compartment not declared free from infection with *G. salaris*

When importing *aquatic animals* of a species referred to in Article 10.3.2., or *aquatic animal products* derived thereof, intended for ~~use~~ uses other than human consumption, including ~~in~~ animal *feed*~~, or for~~ and agricultural, industrial, research or pharmaceutical use, ~~*aquatic animals* of species referred to in Article 10.3.2.~~ from a country, *zone* or *compartment* not declared free from infection with *G. salaris*, the *Competent Authority* of the *importing country* should require that:

1) ~~require~~ an *international aquatic animal health certificate* is issued by the *Competent Authority* of the *exporting country* attesting that the *aquatic animals* have been held, immediately prior to export, in water with a salinity of at least 25 ppt for a continuous period of at least 14 days, and no other *aquatic animals* of ~~the~~ a species referred to in Article 10.3.2. have been introduced during that period;

OR

2) ~~require that~~ the consignment is ~~be~~ delivered directly to, and held in, ~~in~~ *quarantine* or containment facilities until processed into one of the products referred to in point 1 of Article 10.3.3. ~~facilities for slaughter and processing into~~ or other products authorised by the *Competent Authority;* and ~~water used in transport and all effluent and waste materials be treated in a manner that ensures inactivation of *G. salaris*~~.

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

4) all effluent and waste materials from the *quarantine* facilities are treated to ensure inactivation of *G. salaris* or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

~~This article does not apply to *commodities* referred to in point 1 of Article 10.3.3.~~

Article 10.3.11.

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

When importing, for use in laboratories or zoos, *aquatic animals* of a species referred to in Article 10.3.2. from a country, *zone* or *compartment* not declared free from infection with *G. salaris*, 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 *G. salaris* 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 *G. salaris* 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 10.3.1~~1~~2.

Importation (or transit) of ~~aquatic animals and~~ aquatic animal products for retail trade for human consumption regardless of the infection with *G. salaris* status of the exporting ~~from a~~ country, zone or compartment ~~not declared free from infection with *G. salaris*~~

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

   – no *aquatic animal products* listed.

2) When importing ~~*aquatic animals* or~~ *aquatic animal products*, other than those referred to in point 1 above, derived from a ~~of~~ species referred to in Article 10.3.2. from a country, *zone* or *compartment* not declared free from infection with *G. salaris*, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

Annex 15

CHAPTER 10.4.

INFECTION WITH INFECTIOUS SALMON ANAEMIA VIRUS

EU position

The EU thanks the OIE and supports the adoption of this modified chapter.

Article 10.4.1.

For the purposes of the *Aquatic Code*, infection with infectious salmon anaemia virus ~~(ISAV)~~ means *infection* with the *pathogenic agent* ~~HPR0 (non-deleted highly polymorphic region) or~~ highly polymorphic region (HPR)-deleted infectious salmon anaemia virus (ISAV) ~~)~~, or the non-pathogenic HPR0 (non-deleted highly polymorphic region) ISAV, of the Genus ~~genus~~ *Isavirus* ~~of~~ and ~~the family~~ Family ~~Orthomyxoviridae~~ *Orthomyxoviridae*. Both genotypes should be notified in accordance with Chapter 1.1. of the *Aquatic Code*.

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

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

1) HPR0 ISAV and HPR-deleted ISAV free;

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

3) HPR0 ISAV and HPR-deleted ISAV endemic.

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

Article 10.4.2.

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

Article 10.4.3.

Importation or transit of ~~aquatic animals and~~ aquatic animal products for any purpose regardless of the ~~infection with~~ ISAV ~~infectious salmon anaemia virus~~ status of the exporting country, zone or compartment

In this article, all statements referring to ISAV include~~s~~ HPR deleted ISAV and HPR0 ISAV.

1) *Competent Authorities* should not require any conditions related to ~~infection with~~ ISAV, regardless of the infection with ISAV status of the *exporting country*, *zone* or *compartment*, when authorising the importation or transit of the following *aquatic animal products* derived from a ~~the~~ species referred to in Article 10.4.2. that ~~which~~ are intended for any purpose and ~~which~~ 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 ISAV);

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

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

   d) fish oil;

e) fish *meal*;

f) fish skin leather.

2) When authorising the importation or transit of ~~*aquatic animals* and~~ *aquatic animal products* ~~of~~ derived from a species referred to in Article 10.4.2., other than those referred to in point 1 of Article 10.4.3., *Competent Authorities* should require the conditions prescribed in Articles 10.4.10. to 10.4.~~16.~~17. relevant to the infection with ISAV status of the *exporting country*, *zone* or *compartment*.

3) When considering the importation or transit of ~~*aquatic animals* and~~ *aquatic animal products* ~~of~~ derived from a species not referred to ~~covered~~ in Article 10.4.2. but which could reasonably be expected to pose a *risk* of ~~spread~~ transmission of ~~infection with~~ ISAV, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this ~~assessment~~ analysis.

Article 10.4.4.

Country free from infection with ISAV ~~infectious salmon anaemia virus~~

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

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from infection with ISAV if all the areas covered by the shared water bodies are declared countries or *zones* free from infection with ISAV (see Article 10.4.6.)

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

1) none of the *susceptible species* referred to in Article 10.4.2. are present and *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

2) the ~~disease~~ infection with ISAV 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 ~~infection with~~ ISAV;

OR

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

a) on detection of ~~the~~ ISAV ~~*disease*~~, 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 ~~have been destroyed or removed from the *infected zone*~~ by means that minimise the likelihood ~~*risk*~~ of further ~~spread~~ transmission of ISAV t~~he *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 infection with ISAV ~~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 ~~infection with~~ ISAV.

In the meantime, part or all of the unaffected ~~non-affected~~ area may be declared a free *zone* provided that such a part meets the conditions in point 3 of Article 10.4.6.

The pathway for *self-declaration of freedom* from infection with ISAV HPR0 based on absence of clinical ~~disease~~ expression of infection with ISAV (referred to as historical freedom in Article 1.4.6.) cannot be achieved because infection with ISAV HPR0 is unlikely to cause any clinical signs.

Article 10.4.5.

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

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

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from infection with HPR-deleted ISAV if all the areas covered by the shared water bodies are declared countries or *zones* free from infection with HPR-deleted ISAV (see Article 10.4.7.)

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

1) any of the *susceptible species* referred to in Article 10.4.2. are present and the following conditions have been met:

   a) there has been no ~~observed~~ occurrence of infection with HPR-deleted ISAV 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

2) the infection with HPR-deleted ISAV ~~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 two years; and

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

OR

3) it previously made a *self-declaration of freedom* from infection with HPR-deleted ISAV and subsequently lost its ~~*disease*~~ free status due to the detection of ~~infection with~~ HPR-deleted ISAV but the following conditions have been met:

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

   b) infected populations within the *infected zone* have been killed and disposed of ~~been destroyed or removed from the *infected zone*~~ by means that minimise the likelihood ~~*risk*~~ of further ~~spread~~ transmission of HPR-deleted ISAV, 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~~ infection with HPR-deleted ISAV ~~*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 ~~infection with~~ HPR-deleted ISAV.

In the meantime, part or all of the unaffected ~~non-affected~~ area may be declared a free *zone* provided that such a part meets the conditions in point 3 of Article 10.4.7.

Article 10.4.6.

Zone or compartment free from infection with ISAV ~~infectious salmon anaemia virus~~

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

If a *zone* or *compartment* extends over more than one country, it can only be declared a *zone* or *compartment* free from infection with ISAV 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 ISAV 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 10.4.2. are present in the *zone* or *compartment* and *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

2) the infection with ISAV ~~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 two years; and

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

OR

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

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

   b) infected populations within the *infected zone* have been killed and disposed of ~~have been destroyed or removed from the *infected zone*~~ by means that minimise the likelihood ~~*risk*~~ of further ~~spread~~ transmission of the ISAV ~~*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~~ infection with ISAV ~~*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 ~~infection with~~ ISAV.

Article 10.4.7.

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

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

If a *zone* or *compartment* extends over more than one country, it can only be declared a *zone* or *compartment* free from infection with HPR-deleted ISAV 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 HPR-deleted ISAV may be declared free by the *Competent Authority~~(ies)~~* of the country~~(ies)~~ concerned if:

1) any of the *susceptible species* referred to in Article 10.4.2. are present in the *zone* or *compartment* and the following conditions have been met:

   a) there has been no ~~observed~~ occurrence of infection with HPR-deleted ISAV 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

2) the infection with HPR-deleted ISAV~~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 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 ~~infection with~~ HPR-deleted ISAV;

OR

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

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

b) infected populations within the *infected zone* have been killed and disposed of ~~have been destroyed or removed from the *infected zone*~~ by means that minimise the likelihood ~~*risk*~~ of further ~~spread~~ transmission of the ISAV ~~*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~~ infection with HPR-deleted ISAV ~~*disease*~~; and

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

Article 10.4.8.

Maintenance of free status for infection with ISAV ~~infectious salmon anaemia virus~~

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

A country, *zone* or *compartment* that is declared free from infection with ISAV following the provisions of point 1 of Articles 10.4.4. or 10.4.6. (as relevant) may maintain its status as free from infection with ISAV provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from infection with ISAV following the provisions of point 2 of Articles 10.4.4. or 10.4.6. (as relevant) may maintain its status as free from infection with ISAV provided that *targeted surveillance* is continued at a level determined by the *Aquatic Animal Health Service* on the basis of the likelihood of *infection*, and *basic biosecurity conditions* are continuously maintained.

Annex 15 (contd)

Article 10.4.9.

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

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

A country, *zone* or *compartment* that is declared free from infection with HPR-deleted ISAV following the provisions of points 1 or 2 of Articles 10.4.5. or 10.4.7. (as relevant) may maintain its status as free from infection with HPR-deleted ISAV provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from infection with HPR-deleted ISAV following the provisions of point 3 of Articles 10.4.5. or 10.4.7. (as relevant) may discontinue *targeted surveillance* and maintain its free status provided that conditions that are conducive to clinical expression of infection with HPR-deleted ISAV, as described in the corresponding chapter of the *Aquatic Manual*, ~~exist~~ and *basic biosecurity conditions* are continuously maintained.

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

Article 10.4.10.

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

In this article, all statements referring to a country, *zone* or *compartment* free from infection with ISAV include~~s~~ HPR-deleted ISAV and HPR0 ISAV.

When importing *aquatic animals* ~~and *aquatic animal products*~~ of a species referred to in Article 10.4.2., or *aquatic animal products* derived thereof, from a country, *zone* or *compartment* declared free from infection with ISAV, 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, ~~certifying that,~~ on the basis of the procedures described in Articles 10.4.4. or 10.4.6. (as applicable) and 10.4.8., the place of production of the *aquatic animals* ~~and~~ or *aquatic animal products* is a country, *zone* or *compartment* declared free from infection with ISAV.

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* ~~*commodities* referred to~~ listed in point 1 of Article 10.4.3.

Article 10.4.11.

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

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

When importing *aquatic animals* ~~and *aquatic animal products*~~ of a species referred to in Article 10.4.2., or *aquatic animal products* derived thereof, from a country, *zone* or *compartment* declared free from infection with HPR-deleted ISAV, 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*~~. The *international aquatic animal health certificate* should state that, ~~certifying that,~~ on the basis of the procedures described in Articles 10.4.5. or 10.4.7. (as applicable) and 10.4.9., the place of production of the *aquatic animals* ~~and~~ or *aquatic animal products* is a country, *zone* or *compartment* declared free from infection with HPR-deleted ISAV.

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* ~~*commodities* referred to~~ listed in point 1 of Article 10.4.3.

Article 10.4.12.

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

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

When importing, for *aquaculture*, *aquatic animals* ~~or *aquatic animal products*~~ of a species referred to in Article 10.4.2. from a country, *zone* or *compartment* not declared free from infection with ISAV, the *Competent Authority* of the *importing country* should assess the *risk* 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) the treatment of all transport water, equipment, effluent and waste materials to inactive ISAV 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 ISAV.

   b) In the *importing country*:

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

      ii) test the F-0 population for ISAV 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 conductive to the clinical expression of infection with ISAV, ~~(as described in Chapter 2.3.5. of the *Aquatic Manual*)~~ and sample and test for ISAV in accordance with Chapter 1.4. of the *Aquatic Code* and ~~(as described in~~ Chapter 2.3.5. of the *Aquatic Manual*~~)~~;

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

      vi) if ISAV 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 10.4.13.

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

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

When importing, for processing for human consumption, *aquatic animals* ~~or *aquatic animal products*~~ of a species referred to in Article 10.1.2., or *aquatic animal products* derived thereof, from a country, *zone* or *compartment* not declared free from infection with ISAV, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, require that:

1) the consignment is delivered directly to, and held in, *quarantine* or containment facilities until processing into one of the products referred to in point 1 of Article 10.4.3., or ~~products described~~ in point 1 of Article 10.4.~~15.~~16., 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 ISAV or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; ~~and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of ISAV or is disposed in a manner that prevents contact of waste with *susceptible species*~~; and

3) all effluent and waste materials are treated to ensure inactivation of ISAV or disposed of in a biosecure

manner in accordance with Chapters 4.3. and 4.7.

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

Article 10.4.14.

Importation of aquatic animals or aquatic animal products intended for ~~use~~ uses other than human consumption, including ~~in~~ animal feed, ~~or for~~ and agricultural, industrial, research or pharmaceutical use, from a country, zone or compartment not declared free from infection with ISAV ~~infectious salmon anaemia virus~~

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

When importing *aquatic animals* of a species referred to in Article 10.4.2., or *aquatic animal products* derived thereof, intended for ~~use~~ uses other than human consumption, including ~~in~~ animal *feed*, ~~or for~~ and agricultural, industrial, research or pharmaceutical use, ~~*aquatic animals* of species referred to in Article 10.4.2.~~ from a country, *zone* or *compartment* not declared free from infection with ISAV, the *Competent Authority* of the *importing country* should require that:

1) the consignment is delivered directly to, and held in, *quarantine* or containment facilities until processed into one of the products referred to in point 1 of Article 10.4.3. or other ~~for slaughter and processing into~~ products authorised by the *Competent Authority*; and

2) all water (including ice), equipment, *containers* and packaging material used are treated to ensure inactivation of ISAV or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and~~water used in transport and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of ISAV.~~

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

~~This article does not apply to *commodities* referred to in point 1 of Article 10.4.3.~~

Article 10.4.15.

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

When importing, for use in laboratories or zoos, *aquatic animals* of species referred to in Article 10.4.2. from a country, *zone* or *compartment* not declared free from infection with ISAV, 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 ISAV 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 ISAV 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 10.4.1~~5~~6.

Importation (or transit) of ~~aquatic animals and~~ aquatic animal products for retail trade for human consumption regardless of the infection with ISAV status of the exporting ~~from a~~ country, zone or compartment ~~not declared free from infection with infectious salmon anaemia virus~~

In this article, all statements referring to infection with ISAV includes HPR deleted ISAV and HPR0 ISAV.

1) *Competent Authorities* should not require any conditions related to infection with ISAV, regardless of the infection with ISAV status of the *exporting country*, *zone* or *compartment*, when authorising the importation (or transit) of fish fillets or steaks (frozen or chilled) ~~which~~ that have been prepared and packaged for retail trade and ~~which~~ 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 ~~*commodities*~~ *aquatic animal products* Member Countries may wish to consider introducing internal measures to address the *risks* associated with the ~~*commodity*~~ *aquatic animal product* being used for any purpose other than for human consumption.

2) When importing ~~*aquatic animals or*~~ *aquatic animal products*, other than those referred to in point 1 above, ~~of~~ derived from a species referred to in Article 10.4.2. from a country, *zone* or *compartment* not declared free from infection with ISAV, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

Article 10.4.1~~6~~7.

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

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

1) When importing disinfected eggs of the species referred to in Article 10.4.2. for *aquaculture*, from a country, *zone* or *compartment* not declared free from infection with ISAV, the *Competent Authority* of the *importing country* should assess the *risk* associated with at least:

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

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

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

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

   a) the eggs should be disinfected prior to importing, 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 10.4.2. for *aquaculture*, from a country, *zone* or *compartment* not declared free from infection with ISAV, the *Competent Authority* of the *importing country* should require 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*~~ certifying that the procedures described in point 2 of this article have been fulfilled.

Annex 16

CHAPTER 10.5.

INFECTION WITH SALMONID ALPHAVIRUS

EU position

The EU supports the adoption of this modified chapter.

Article 10.5.1.

General provisions

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

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

Article 10.5.2.

Scope

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

Article 10.5.3.

Importation or transit of ~~aquatic animals and~~ aquatic animal products for any purpose regardless of the infection with SAV ~~salmonid alphavirus~~ status of the exporting country, zone or compartment

1) *Competent Authorities* should not require any conditions related to ~~infection with~~ SAV, regardless of the infection with SAV status of the *exporting country*, *zone* or *compartment,* when authorising the importation or transit of the following *aquatic animal products* derived from ~~the~~ a species referred to in Article 10.5.2. that ~~which~~ are intended for any purpose and comply~~ing~~ 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 SAV);

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

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

   d) fish oil;

   e) fish *meal*;

   f) fish skin leather.

2) When authorising the importation or transit of ~~*aquatic animals* and~~ *aquatic animal products* ~~of~~ derived from a species referred to in Article 10.5.2., other than those referred to in point 1 of Article 10.5.3., *Competent Authorities* should require the conditions prescribed in Articles 10.5.7. to 10.5.13~~2~~. relevant to the infection with SAV status of the *exporting country*, *zone* or *compartment*.

3) When considering the importation or transit of ~~*aquatic animals* and~~ *aquatic animal products* ~~of~~ derived from a

species not ~~covered~~ referred to in Article 10.5.2. but which could reasonably be expected to pose a *risk* of transmission ~~spread~~ of ~~infection with~~ SAV, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this analysis ~~assessment~~.

Article 10.5.4.

Country free from infection with SAV ~~salmonid alphavirus~~

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from infection with SAV if all the areas covered by the shared water bodies are declared countries or *zones* free from infection with SAV (see Article 10.5.5.).

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

1) none of the *susceptible species* referred to in Article 10.5.2. 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 10.5.2. are present and the following conditions have been met:

   a) there has been no ~~observed~~ occurrence of infection with SAV ~~the~~ ~~*disease*~~ for at least the last ten years despite conditions that are conducive to 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 ~~past~~ last ten years;

OR

3) the ~~disease~~ infection with SAV 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 ~~infection with~~ SAV;

OR

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

   a) on detection of SAV ~~the~~ ~~*disease*~~, 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 ~~have been destroyed or removed from the *infected zone*~~ by means that minimise the likelihood ~~*risk*~~ of further transmission ~~spread~~ of SAV ~~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 infection with SAV ~~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 ~~infection with~~ SAV.

   In the meantime, part or all of the unaffected ~~non affected~~ area may be declared a free *zone* provided that such a part meets the conditions in point 3 of Article 10.5.5.

Article 10.5.5.

Zone or compartment free from infection with SAV ~~salmonid alphavirus~~

If a *zone* or *compartment* extends over more than one country, it can only be declared a *zone* or *compartment* free from infection with SAV 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 SAV 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 10.5.2. 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 10.5.2. are present in the *zone* or *compartment* and the following conditions have been met:

   a) there has been no ~~observed~~ occurrence of infection with SAV ~~the *disease*~~ 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 SAV ~~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 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 ~~infection with~~ SAV;

OR

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

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

   b) infected populations within the *infected zone* have been killed and disposed of ~~have been destroyed or removed from the *infected zone*~~ by means that minimise the likelihood ~~*risk*~~ of further transmission ~~spread~~ of SAV ~~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 infection with SAV ~~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 ~~infection with~~ SAV.

Annex 16 (contd)

Article 10.5.6.

Maintenance of free status ~~for infection with salmonid alphavirus~~

A country, *zone* or *compartment* that is declared free from infection with SAV following the provisions of points 1 or 2 of Articles 10.5.4. or 10.5.5. (as relevant) may maintain its status as free from infection with SAV provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from infection with SAV following the provisions of point 3 of Articles 10.5.4. or 10.5.5. (as relevant) may discontinue *targeted surveillance* and maintain its free status ~~as free from infection with SAV~~ provided that conditions that are conducive to clinical expression of infection with SAV, as described in the corresponding chapter of the *Aquatic Manual*, ~~exist~~ and *basic biosecurity conditions* are continuously maintained.

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

Article 10.5.7.

Importation of aquatic animals ~~and~~ or aquatic animal products from a country, zone or compartment declared free from infection with SAV ~~salmonid alphavirus~~

When importing *aquatic animals* of a species referred to in Article 10.5.2., or ~~and~~ *aquatic animal products* ~~of species referred to in Article 10.2.2.~~ derived thereof, from a country, *zone* or *compartment* declared free from infection with SAV, 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 ~~certifying that~~, on the basis of the procedures described in Articles 10.5.4. or 10.5.5. (as applicable) and 10.5.6., the place of production of the *aquatic animals* or ~~and~~ *aquatic animal products* is a country, *zone* or *compartment* declared free from infection with SAV.

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 ~~*commodities* referred to~~ in point 1 of Article 10.5.3.

Article 10.5.8.

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

When importing, for *aquaculture*, *aquatic animals* of a species referred to in Article 10.5.2. from a country, *zone* or *compartment* not declared free from infection with SAV, the *Competent Authority* of the *importing country* should assess the *risk* 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) the treatment of all transport water, equipment, effluent and waste materials to inactive SAV 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 SAV.

b) In the *importing country*:

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

ii) test the F-0 population for SAV 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 SAV, ~~(as described in Chapter 2.3.6. of the *Aquatic Manual*)~~ and sample and test for SAV in accordance with Chapter 1.4. of the *Aquatic Code* and Chapter 2.3.6. of the *Aquatic Manual*;

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

vi) if SAV 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 10.5.9.

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

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

1) the consignment is delivered directly to, and held, in *quarantine* or containment facilities until processing into one of the products referred to in point 1 of Article 10.5.3.~~,~~ or ~~products described~~ in point 1 of Article 10.5.12., 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 SAV or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and ~~and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of SAV or is disposed in a manner that prevents contact of waste with *susceptible species*.~~

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

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

Article 10.5.10.

Importation of aquatic animals or aquatic animal products intended for uses other than human consumption, including ~~use in~~ animal feed~~, or for~~ and agricultural, industrial, research or pharmaceutical use, from a country, zone or compartment not declared free from infection with SAV ~~salmonid alphavirus~~

When importing *aquatic animals* of a species referred to in Article 10.5.2., or *aquatic animal products* derived thereof, intended for uses other than human consumption, including ~~use~~ ~~in~~ animal *feed* ~~or for~~ and agricultural, industrial, research or pharmaceutical use, ~~*aquatic animals* of species referred to in Article 10.5.2.~~ from a country, *zone* or *compartment* not declared free from infection with SAV, the *Competent Authority* of the *importing country* should require that:

1) the consignment is delivered directly to, and held in, *quarantine* or containment facilities until processed into one of the products referred to in point 1 of Article 10.5.3. or other ~~facilities for slaughter and processing into~~ 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 SAV or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and ~~and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of SAV or is disposed in a manner that prevents contact of waste with *susceptible species*.~~

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

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

Article 10.5.11.

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

When importing, for use in laboratories or zoos, *aquatic animals* of a species referred to in Article 10.5.2. from a country, *zone* or *compartment* not declared free from infection with SAV, 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 SAV 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 SAV 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 10.5.12~~1.~~

Importation (or transit) of ~~aquatic animals and~~ aquatic animal products for retail trade for human consumption regardless of the infection with SAV status of the ~~exporting from a~~ country, zone or compartment ~~not declared free from infection with SAV salmonid alphavirus~~

1) *Competent Authorities* should not require any conditions related to ~~infection with~~ SAV, regardless of the infection with SAV status of the *exporting country*, *zone* or *compartment*, when authorising the importation (or transit) of fish fillets or steaks (chilled) ~~which~~ that have been prepared and packaged for retail trade and ~~which~~ 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* ~~*commodities*~~ Member Countries may wish to consider introducing internal measures to address the *risks* associated with the *aquatic animal product* ~~*commodity*~~ being used for any purpose other than for human consumption.

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

Annex 16 (contd)

Article 10.5.13~~2~~.

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

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

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

   b) the ~~level~~ prevalence of infection with SAV in broodstock; 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 10.5.2. for *aquaculture*, from a country, *zone* or *compartment* not declared free from infection with SAV, the *Competent Authority* of the *importing country* should require 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*~~ certifying that the procedures described in point 2 of this article have been fulfilled.

Annex 17

CHAPTER 10.6.

INFECTION WITH INFECTIOUS HAEMATOPOIETIC NECROSIS VIRUS

EU position

The EU in general supports the adoption of this modified chapter.

Comments are inserted in the text below.

Article 10.6.1.

For the purposes of the *Aquatic Code*, infection with infectious haematopoietic necrosis virus ~~(IHN)~~ means *infection* with the *pathogenic agent* infectious haematopoietic necrosis virus (IHNV), of the ~~g~~Genus *Novirhabdovirus* ~~of~~ and ~~the f~~Family ~~Rhabdoviridae~~ *Rhabdoviridae*.

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

Article 10.6.2.

Scope

The recommendations in this chapter apply to: 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 Atlantic salmon *(Salmo salar)*. These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

EU comment

The EU suggests adding the following species as susceptible:

Pike (*Esox lucius*), Pacific herring (*Clupea pallasii*), Atlantic cod (*Gadus morhua*), ayu (*Plecoglossus altivelis*), shiner perch (*Cymatogaster aggregate*), tube-snout (*Aulorhynchus flavidus*), European eel (*Anguilla anguilla*), char (*Salvelinus* spp.).

References:

References are included in the Table in the Appendix.

We however understand that the OIE *ad hoc* group on Susceptibility of fish species to infection with OIE listed diseases is currently in the process of evaluating the susceptibility of fish species to this disease. The EU therefore can await the results of that evaluation by the *ad hoc* Group, before any changes to Article 10.6.2 are made.

Article 10.6.3.

Importation or transit of ~~aquatic animals and~~ aquatic animal products for any purpose regardless of the infection with IHNV ~~infectious haematopoietic necrosis~~ 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 ~~the~~ species referred to in Article 10.6.2. that ~~which~~ are intended for any purpose and ~~which~~ 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 ~~which~~ 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 ~~which~~ 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 animals* and~~ *aquatic animal products* ~~of~~ 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~~2~~. relevant to the infection with IHNV status of the *exporting country*, *zone* or *compartment*.

3) When considering the importation or transit of ~~*aquatic animals* and~~ *aquatic animal products* ~~of~~ derived from a species not ~~covered~~ referred to in Article 10.6.2. but which could reasonably be expected to pose a *risk* of transmission ~~spread~~ 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 ~~assessment~~.

Article 10.6.4.

Country free from infection with IHNV ~~infectious haematopoietic necrosis~~

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 ~~observed~~ occurrence of infection with IHNV ~~the *disease*~~ 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 ~~disease~~ 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 ~~*disease*~~ free status due to the detection of IHNV but the following conditions have been met:

a) on detection of IHNV ~~the *disease*~~, 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 ~~have been destroyed or removed from the *infected zone*~~ by means that minimise the likelihood ~~*risk*~~ of further transmission ~~spread~~ of IHNV ~~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 infection with IHNV ~~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 IHNV.

In the meantime, part or all of the unaffected ~~non-affected~~ 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 ~~infectious haematopoietic necrosis~~

If a *zone* or *compartment* extends over more than one country, it can only be declared ~~an IHN~~ a ~~free~~ *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~~(ies)~~* of the country~~(ies)~~ 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 ~~observed~~ occurrence of infection with IHNV ~~the *disease*~~ 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 ~~disease~~ 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 ~~*disease*~~ free status due to the detection of IHNV in the *zone* but the following conditions have been met:

a) on detection of IHNV~~the *disease*~~, 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 ~~have been destroyed or removed from the *infected zone*~~ by means that minimise the likelihood ~~*risk*~~ of further transmission ~~spread~~ of IHNV ~~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 infection with IHNV ~~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 IHNV.

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 ~~as free from IHN~~ provided that conditions that are conducive to clinical expression of infection with IHNV, as described in the corresponding chapter of the *Aquatic Manual*, ~~exist~~ and *basic biosecurity conditions* are continuously maintained.

However, for declared free *zones* or *compartments* in infected countries and in all cases where conditions are not conducive to clinical expression of infection with IHNV, *targeted surveillance* ~~needs to~~ 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 ~~and~~ or aquatic animal products from a country, zone or compartment declared free from infection with IHNV ~~infectious haematopoietic necrosis~~

When importing *aquatic animals* of a species referred to in Article 10.6.2., or ~~and~~ *aquatic animal products* ~~of species referred to in Article 10.2.2.~~ 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* ~~or a *certifying official* approved by the *importing country*~~. The *international aquatic animal health certificate* should state that ~~certifying 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 ~~and~~ *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 ~~*commodities* referred to~~ 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 ~~infectious haematopoietic necrosis~~

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) 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.

EU comment

It is not clear what is meant by "high health status for infection with IHNV". This should preferably by clarified.

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. ~~(as described in Chapter 2.3.4. of the *Aquatic Manual*)~~ 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 ~~and~~ or aquatic animal products for processing for human consumption from a country, zone or compartment not declared free from infection with IHNV ~~infectious haematopoietic necrosis~~

When importing, for processing for human consumption, *aquatic animals* ~~or *aquatic animal products*~~ 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 ~~products described~~ 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 ~~and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of IHNV or is disposed in a manner that prevents contact of waste with *susceptible species*.~~

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* ~~*commodities*~~ Member Countries may wish to consider introducing internal measures to address the *risks* associated with the *aquatic animal* or *aquatic animal product* ~~*commodity*~~ 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 ~~use in~~ animal feed~~, or for~~ and agricultural, industrial, research or pharmaceutical use, from a country, zone or compartment not declared free from infection with IHNV ~~infectious haematopoietic necrosis~~

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 ~~use~~ ~~in~~ animal *feed* ~~or for~~ and agricultural, industrial, research or pharmaceutical use, ~~*aquatic animals* of 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 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 ~~facilities for slaughter and processing into~~ 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 ~~and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of IHNV or is disposed in a manner that prevents contact of waste with *susceptible*~~

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.

EU comment

We note that the point 4) seems to be missing from the article above ("the carcasses are disposed of in accordance with Chapter 4.7.", cf. Article 10.6.11. below).

~~This article does not apply to *commodities* referred to in point 1 of Article 10.6.3.~~

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 ~~SAV~~ 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~~1~~.

Importation (or transit) of ~~aquatic animals and~~ aquatic animal products for retail trade for human consumption regardless of the infection with IHNV status of the exporting ~~from a~~ country, zone or compartment ~~not declared free from infection with IHNV infectious haematopoietic necrosis~~

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 ~~which~~ have been prepared and packaged for retail trade and ~~which~~ 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* ~~*commodities*~~ Member Countries may wish to consider introducing internal measures to address the *risks* associated with the *aquatic animal product* ~~*commodity*~~ being used for any purpose other than for human consumption.

2) When importing ~~*aquatic animals* or~~ *aquatic animal products*, other than those referred to in point 1 above, derived from a ~~of~~ 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~~2~~.

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

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 ~~IHN virus~~ IHNV status of the water to be used during the *disinfection* of the eggs;

   b) the prevalence of infection with IHNV ~~virus~~ 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 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* ~~or a *certifying official* approved by the *importing country* ~~certifying that the procedures described in point 2 of ~~Article 10.6.12.~~ of this article have been fulfilled.

Annex 18

CHAPTER 10.7.

INFECTION WITH KOI HERPESVIRUS ~~DISEASE~~

EU position

The EU in general supports the adoption of this modified chapter.

A comment is inserted in the text below.

Article 10.7.1.

For the purposes of the *Aquatic Code*, infection with koi herpesvirus ~~disease (KHVD)~~ means *infection* with the *pathogenic agent* ~~viral species~~ koi herpesvirus (KHV) ~~tentatively placed~~ in the ~~sub-family~~ Genus ~~*Cyprinid Cyprinivirus herpesvirus* of the~~ and ~~f~~Family *Alloherpesviridae*.

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

Article 10.7.2.

Scope

The recommendations in this chapter apply to: common carp (*Cyprinus carpio carpio*), ghost carp *(Cyprinus carpio goi)*, 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.

EU comment

The EU notes that there are inconsistencies in the French translation of the common names of the various carp species, and would invite the OIE to review the French version of the paragraph above. (This comment is also valid for the corresponding Aquatic Manual chapter.)

Indeed, the French name of *Cyprinus carpio* is "Carpe commune" and not to "carpe courante". The name "carpe commune" is also used when referring to hybrids, as are discussed in the paragraph above.

Article 10.7.3.

Importation or transit of ~~aquatic animals and~~ aquatic animal products for any purpose regardless of the infection with KHV ~~koi herpesvirus disease~~ status of the exporting country, zone or compartment

1) *Competent Authorities* should not require any conditions related to KHV~~D~~, regardless of the infection with KHV~~D~~ status of the *exporting country*, *zone* or *compartment*, when authorising the importation or transit of the following *aquatic animal products* derived from a ~~the~~ species referred to in Article 10.7.2. that ~~which~~ are intended for any purpose and ~~which~~ 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 KHV);

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

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

   d) fish oil;

e) fish *meal*.

2) When authorising the importation or transit of ~~*aquatic animals* and~~ *aquatic animal products* ~~of~~ derived from a species referred to in Article 10.7.2., other than those referred to in point 1 of Article 10.7.3., *Competent Authorities* should require the conditions prescribed in Articles 10.7.7. to 10.7.12~~1~~. relevant to the infection with KHV~~D~~ status of the *exporting country*, *zone* or *compartment*.

3) When considering the importation or transit of ~~*aquatic animals* and~~ *aquatic animal products* ~~of~~ derived from a species not ~~covered~~ referred to in Article 10.7.2. but which could reasonably be expected to pose a *risk* of transmission ~~spread~~ of KHV~~D~~, 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 ~~assessment~~.

Article 10.7.4.

Country free from infection with KHV ~~koi herpesvirus disease~~

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from infection with KHV~~D~~ if all the areas covered by the shared water bodies are declared countries or *zones* free from infection with KHV~~D~~ (see Article 10.7.5.).

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

1) none of the *susceptible species* referred to in Article 10.7.2. 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 10.7.2. are present and the following conditions have been met:

   a) there has been no ~~observed~~ occurrence of infection with KHV ~~the *disease*~~ 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 for at least the last ten years;

OR

3) the ~~disease~~ infection with KHV 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 KHV~~D~~;

OR

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

   a) on detection of ~~the~~ KHV ~~*disease*~~, 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 ~~have been destroyed or removed from the *infected zone*~~ by means that minimise the likelihood ~~*risk*~~ of further transmission ~~spread~~ of KHV ~~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~~ infection with KHV ~~*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 KHV~~D~~.

   In the meantime, part or all of the unaffected ~~non-affected~~ area may be declared a free *zone* provided that such a part meets the conditions in point 3 of Article 10.7.5.

Article 10.7.5.

Zone or compartment free from infection with KHV ~~koi herpesvirus disease~~

If a *zone* or *compartment* extends over more than one country, it can only be declared a ~~KHVD free~~ *zone* or *compartment* free from infection with KHV 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 KHV~~D~~ 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 10.7.2. 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 10.7.2. are present in the *zone* or *compartment* and the following conditions have been met:

   a) there has been no ~~observed~~ occurrence of infection with KHV ~~the *disease*~~ 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 ~~disease~~ infection with KHV 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 KHV~~D~~;

OR

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

   a) on detection of KHV ~~the *disease*~~, 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 ~~have been destroyed or removed from the *infected zone*~~ by means that minimise the likelihood ~~*risk*~~ of further transmission ~~spread~~ of KHV ~~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 infection with KHV ~~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 KHV~~D~~.

Article 10.7.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from infection with KHV~~D~~ following the provisions of points 1 or 2 of Articles 10.7.4. or 10.7.5. (as relevant) may maintain its status as free from infection with KHV~~D~~ provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from infection with KHV~~D~~ following the provisions of point 3 of Articles 10.7.4. or 10.7.5. (as relevant) may discontinue *targeted surveillance* and maintain its free status ~~as free from KHVD~~ provided that conditions that are conducive to clinical expression of infection with KHV~~D~~, as

described in the corresponding chapter of the *Aquatic Manual*, ~~exist,~~ and *basic biosecurity conditions* are continuously maintained.

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

Article 10.7.7.

Importation of aquatic animals ~~and~~ or aquatic animal products from a country, zone or compartment declared free from infection with KHV ~~koi herpesvirus disease~~

When importing *aquatic animals* of a species referred to in Article 10.7.2., or ~~and~~ *aquatic animal products* ~~of species referred to in Article 10.2.2.~~ derived thereof, from a country, *zone* or *compartment* declared free from infection with KHV~~D~~, 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 ~~certifying that~~, on the basis of the procedures described in Articles 10.7.4. or 10.7.5. (as applicable) and 10.7.6., the place of production of *aquatic animals* or ~~and~~ *aquatic animal products* is a country, *zone* or *compartment* declared free from infection with KHV~~D~~.

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 ~~*commodities* referred to~~ in point 1 of Article 10.7.3.

Article 10.7.8.

Importation of aquatic animals for aquaculture from a country, zone or compartment not declared free from infection with KHV ~~koi herpesvirus disease~~

When importing, for *aquaculture*, *aquatic animals* of a species referred to in Article 10.7.2. from a country, *zone* or *compartment* not declared free from infection with KHV~~D~~., 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) the treatment of all transport water, equipment, effluent and waste materials to inactive KHV 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 KHV~~D~~.

   b) In the *importing country*:

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

      ii) test the F-0 population for KHV 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 KHV~~D~~, ~~(as described in Chapter 2.3.7. of the *Aquatic Manual*)~~ and sample and test for KHV in accordance with Chapter 1.4. of the *Aquatic Code* and Chapter 2.3.7. of the *Aquatic Manual*;

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

vi) if KHV 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 10.7.9.

Importation of aquatic animals ~~and~~ or aquatic animal products for processing for human consumption from a country, zone or compartment not declared free from infection with KHV ~~koi herpesvirus disease~~

When importing, for processing for human consumption, *aquatic animals* ~~or *aquatic animal products*~~ of a species referred to in Article 10.7.2., or *aquatic animal products* derived thereof, from a country, *zone* or *compartment* not declared free from infection with KHV~~D~~, 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 10.7.3.~~,~~ or ~~products described~~ products described in point 1 of Article 10.7.12., 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 KHV or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and ~~and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of KVH or is disposed in a manner that prevents contact of waste with *susceptible species*.~~

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

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

Article 10.7.10.

Importation of aquatic animals or aquatic animal products intended for uses other than human consumption, including ~~use in~~ animal feed~~, or for~~ and agricultural, industrial, research or pharmaceutical use, from a country, zone or compartment not declared free from infection with KHV ~~koi herpesvirus disease~~

When importing *aquatic animals* of a species referred to in Article 10.7.2., or *aquatic animal products* derived thereof, intended for uses other than human consumption, including ~~use~~ ~~in~~ animal *feed* ~~or for~~ and agricultural, industrial, research or pharmaceutical use, ~~*aquatic animals* of species referred to in Article 10.2.2.~~ from a country, *zone* or *compartment* not declared free from infection with KHV~~D~~, 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 10.7.3. or other ~~facilities for slaughter and processing into~~ 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 KHV or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and ~~and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of KHV or is disposed in a manner that prevents contact of waste with *susceptible*~~

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

~~This article does not apply to *commodities* referred to in point 1 of Article 10.7.3.~~

Annex 18 (contd)

Article 10.7.11.

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

When importing, for use in laboratories or zoos, *aquatic animals* of a species referred to in Article 10.7.2. from a country, *zone* or *compartment* not declared free from infection with KHV, 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 KHV 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 KHV 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 10.7.12~~1~~.

Importation (or transit) of ~~aquatic animals and~~ aquatic animal products for retail trade for human consumption regardless of the infection with KHV status of the ~~exporting from a~~ country, zone or compartment ~~not declared free from infection with KHV koi herpesvirus disease~~

1) *Competent Authorities* should not require any conditions related to KHV~~D~~, regardless of the infection with KHV~~D~~ status of the *exporting country*, *zone* or *compartment*, when authorising the importation (or transit) of fish fillets or steaks (chilled) that ~~which~~ have been prepared and packaged for retail trade and ~~which~~ 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* ~~*commodities*~~ Member Countries may wish to consider introducing internal measures to address the *risks* associated with the *aquatic animal product* ~~*commodity*~~ being used for any purpose other than for human consumption.

2) When importing ~~*aquatic animals* or~~ *aquatic animal products*, other than those referred to in point 1 above, derived from a ~~of~~ species referred to in Article 10.7.2. from a country, *zone* or *compartment* not declared free from ~~the~~ infection with KHV~~D~~, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

Annex 19

CHAPTER 10.8.

INFECTION WITH RED SEA BREAM IRIDOVIRUS ~~IRIDOVIRAL DISEASE~~

EU position

The EU supports the adoption of this modified chapter.

Article 10.8.1.

For the purposes of the *Aquatic Code*, infection with red sea bream iridovirus ~~iridoviral disease (RSIVD)~~ means *infection* with the *pathogenic agent* red sea bream iridovirus (RSIV) of the Genus *Megalocytivirus* and ~~f~~Family ~~Iridoviridae~~ *Iridoviridae*.

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

Article 10.8.2.

Scope

The recommendations in this chapter apply to: red sea bream *(Pagrus major)*, yellowtail *(Seriola quinqueradiata)*, amberjack *(Seriola dumerili)*, sea bass (*Lateolabrax* sp. and *Lates calcarifer*), Albacore *(Thunnus thynnus)*, Japanese parrotfish *(Oplegnathus fasciatus)*, striped jack *(Caranx delicatissimus)*, mandarin fish *(Siniperca chuatsi)*, red drum *(Sciaenops ocellatus)*, mullet *(Mugil cephalus)* and groupers (*Epinephelus* spp.). These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

Article 10.8.3.

Importation or transit of ~~aquatic animals and~~ aquatic animal products for any purpose regardless of the infection with RSIV ~~red sea bream iridoviral disease~~ status of the exporting country, zone or compartment

1) *Competent Authorities* should not require any conditions related to RSIV~~D~~, regardless of the infection with RSIV~~D~~ status of the *exporting country*, *zone* or *compartment*, when authorising the importation or transit of the following *aquatic animal products* derived from ~~the~~ a species referred to in Article 10.8.2. that ~~which~~ are intended for any purpose and ~~which~~ comply with Article 5.4.1.:

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

   b) pasteurised fish products that have been subjected to heat treatment at 90ºC for at least ten minutes (or any time/temperature equivalent ~~which~~ that has been demonstrated to inactivate RSIV);

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

   d) fish oil;

   e) fish *meal*;

   f) fish skin leather.

2) When authorising the importation or transit of ~~*aquatic animals* and~~ *aquatic animal products* ~~of~~ derived from a species referred to in Article 10.8.2., other than those referred to in point 1 of Article 10.8.3., *Competent Authorities* should require the conditions prescribed in Articles 10.8.7. to 10.8.12~~1~~. relevant to the infection with RSIV~~D~~ status of the *exporting country*, *zone* or *compartment*.

3) When considering the importation or transit of ~~*aquatic animals* and~~ *aquatic animal products* ~~of~~ derived from a species not ~~covered~~ referred to in Article 10.8.2. but which could reasonably be expected to pose a *risk* of transmission ~~spread~~ of RSIV~~D~~, 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 ~~assessment~~.

Article 10.8.4.

~~Red sea bream iridovirus free~~ ~~c~~Country free from infection with RSIV

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from infection with RSIV~~D~~ if all the areas covered by the shared water bodies are declared countries or *zones* free from infection with RSIV~~D~~ (see Article 10.8.5.).

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

1) none of the *susceptible species* referred to in Article 10.8.2. 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 10.8.2. are present and the following conditions have been met:

   a) there has been no ~~observed~~ occurrence of infection with RSIV ~~the *disease*~~ 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 ~~disease~~ infection with RSIV 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 RSIV;

OR

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

   a) on detection of RSIV ~~the *disease*~~, 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 ~~have been destroyed or removed from the *infected zone*~~ by means that minimise the likelihood ~~*risk*~~ of further transmission ~~spread~~ of RSIV ~~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 infection with RSIV ~~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 RSIV.

   In the meantime, part or all of the unaffected ~~non affected~~ area may be declared a free *zone* provided that such a part meets the conditions in point 3 of Article 10.8.5.

Article 10.8.5.

~~Red sea bream iridoviral diseases free~~ ~~z~~Zone or ~~free~~ compartment free from infection with RSIV

If a *zone* or *compartment* extends over more than one country, it can only be declared a ~~RSIVD free~~ *zone* or *compartment* free from infection with RSIV 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 RSIV~~D~~ 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 10.8.2. 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 10.8.2. are present in the *zone* or *compartment* and the following conditions have been met:

   a) there has been no ~~observed~~ occurrence of infection with RSIV ~~the *disease*~~ 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 ~~disease~~ infection with RSIV 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 RSIV;

OR

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

   a) on detection of RSIV~~the *disease*~~, 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 ~~have been destroyed or removed from the *infected zone*~~ by means that minimise the likelihood ~~*risk*~~ of further transmission ~~spread~~ of RSIV~~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*~~ infection with RSIV~~D~~; and

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

Article 10.8.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from infection with RSIV~~D~~ following the provisions of points 1 or 2 of Articles 10.8.4. or 10.8.5. (as relevant) may maintain its status as free from infection with RSIV~~D~~ provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from infection with RSIV~~D~~ following the provisions of point 3 of Articles 10.8.4. or 10.8.5. (as relevant) may discontinue *targeted surveillance* and maintain its free status ~~as free from RSIVD~~ provided that conditions that are conducive to clinical expression of infection with RSIV~~D~~, as described in the corresponding chapter of the *Aquatic Manual*, ~~exist~~ and *basic biosecurity conditions* are continuously maintained.

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

Article 10.8.7.

Importation of aquatic animals ~~and~~ or aquatic animal products from a country, zone or compartment declared free from infection with RSIV ~~red sea bream iridoviral disease~~

When importing *aquatic animals* of a species referred to in Article 10.8.2., or ~~and~~ *aquatic animal products* ~~of species referred to in Article 10.2.2.~~ derived thereof, from a country, *zone* or *compartment* declared free from infection with RSIV~~D~~, 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 ~~certifying that~~, on the basis of the procedures described in Articles 10.8.4. or 10.8.5. (as applicable) and 10.8.6., the place of production of the *aquatic animals* or ~~and~~ *aquatic animal products* is a country, *zone* or *compartment* declared free from infection with RSIV~~D.~~

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 ~~*commodities* referred to~~ in point 1 of Article 10.8.3.

Article 10.8.8.

Importation of aquatic animals for aquaculture from a country, zone or compartment not declared free from infection with RSIV ~~red sea bream iridoviral disease~~

When importing, for *aquaculture*, *aquatic animals* of a species referred to in Article 10.8.2. from a country, *zone* or *compartment* not declared free from infection with RSIV~~D~~, 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) the treatment of all transport water, equipment, effluent and waste materials to inactive RSIV 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 RSIV~~D.~~.

   b) In the *importing country*:

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

      ii) test the F-0 population for RSIV 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 RSIV~~D~~, ~~(as described in Chapter 2.3.8. of the *Aquatic Manual*)~~ and sample and test for RSIV in accordance with Chapter 1.4. of the *Aquatic Code* and Chapter 2.3.8. of the *Aquatic Manual*;

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

vi) if RSIV 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 10.8.9.

Importation of aquatic animals ~~and~~ or aquatic animal products for processing for human consumption from a country, zone or compartment not declared free from infection with RSIV ~~red sea bream iridoviral disease~~

When importing, for processing for human consumption, *aquatic animals* ~~or *aquatic animal products*~~ of a species referred to in Article 10.8.2., or *aquatic animal products* derived thereof, from a country, *zone* or *compartment* not declared free from RSIV~~D~~, 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 10.8.3., ~~or~~ ~~products described~~ in point 1 of Article 10.8.12., 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 RSIV or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and~~and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of RSIV or is disposed in a manner that prevents contact of waste with *susceptible species*.~~

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

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

Article 10.8.10.

Importation of aquatic animals or aquatic animal products intended for uses other than human consumption, including ~~use in~~ animal feed~~, or for~~ and agricultural, industrial, research or pharmaceutical use, from a country, zone or compartment not declared free from infection with RSIV ~~red sea bream iridoviral disease~~

When importing *aquatic animals* of a species referred to in Article 10.8.2., or *aquatic animal products* derived thereof, intended for uses other than human consumption, including ~~use~~ ~~in~~ animal *feed* ~~or for~~ and agricultural, industrial, research or pharmaceutical use, ~~*aquatic animals* of species referred to in Article 10.2.2.~~, from a country, *zone* or *compartment* not declared free from infection with RSIV~~D~~, 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 10.8.3. or other ~~facilities for slaughter and processing into~~ 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 RSIV or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and ~~and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of RSIV or is disposed in a manner that prevents contact of waste with *susceptible*~~

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

~~This article does not apply to *commodities* referred to in point 1 of Article 10.8.3.~~

Annex 19 (contd)

Article 10.8.11.

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

When importing, for use in laboratories and zoos, *aquatic animals* of a species referred to in Article 10.8.2. from a country, *zone* or *compartment* not declared free from infection with RSIV, 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~~ RSIV 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 RSIV 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 10.8.12~~1~~.

Importation (or transit) of ~~aquatic animals and~~ aquatic animal products for retail trade for human consumption regardless of the infection with RSIV status of the ~~exporting from a~~ country, zone or compartment ~~not declared free from~~ infection with ~~RSIV red sea bream iridoviral disease~~

1) Competent Authorities should not require any conditions related to RSIV~~D~~, regardless of the infection with RSIV~~D~~ status of the exporting country, *zone* or *compartment*, when authorising the importation (or transit) of fish fillets or steaks (chilled) that ~~which~~ have been prepared and packaged for retail trade and ~~which~~ 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* ~~*commodities*~~ Member Countries may wish to consider introducing internal measures to address the *risks* associated with the *aquatic animal product* ~~*commodity*~~ being used for any purpose other than for human consumption.

2) When importing ~~*aquatic animals or*~~ *aquatic animal products*, other than those referred to in point 1 above, derived from a ~~of~~ species referred to in Article 10.8.2. from a country, *zone* or *compartment* not declared free from infection with RSIV~~D~~, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

Annex 20

CHAPTER 10.9.

INFECTION WITH SPRING VIRAEMIA OF CARP VIRUS

EU position

The EU in general supports the adoption of this modified chapter.

A comment is inserted in the text below.

Article 10.9.1.

For the purposes of the *Aquatic Code*, infection with spring viraemia of carp virus ~~(SVC)~~ means *infection* with the *pathogenic agent* ~~viral species SVC~~ spring viraemia of carp virus (SVCV) ~~tentatively placed~~ in the ~~g~~Genus ~~*Vesiculovirus*~~ *Sprivivirus* ~~of the~~ and ~~f~~Family ~~Rhabdoviridae~~ *Rhabdoviridae*.

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

Article 10.9.2.

Scope

The recommendations in this chapter apply to: common carp *(Cyprinus carpio carpio)* and koi carp *(Cyprinus carpio koi)*, crucian carp *(Carassius carassius)*, sheatfish (also known as European 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 tench *(Tinca tinca)*. These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

EU comment

We note that the scientific name for "grass carp" should be "*Ctenopharyngodon idella*", instead of "*Ctenopharyngodon idellus*". In addition, to avoid any confusion with the first parenthesis after "grass carp", we would also suggest inserting the words "also called" before "white amur", as we understand this is an alternative common name used in some countries.

Furthermore, the EU notes that there are inconsistencies in the French translation of the common names of the various carp species, and would invite the OIE to review the French version of the paragraph above. (This comment is also valid for the corresponding Aquatic Manual chapter.)

Indeed, the French name of *Cyprinus carpio* is "Carpe commune" and not to "carpe courante". The name "carpe commune" is also used when referring to hybrids, as are discussed in the paragraph above. In addition, the French name of *Ctenopharyngodon idella* is "carpe herbivore" or "amour blanc".

Article 10.9.3.

Importation or transit of ~~aquatic animals and~~ aquatic animal products for any purpose regardless of the infection with SVCV ~~spring viraemia of carp~~ status of the exporting country, zone or compartment

1) *Competent Authorities* should not require any conditions related to SVCV, regardless of the infection with SVCV status of the *exporting country*, *zone* or *compartment*, when authorising the importation or transit of the following *aquatic animal products* derived from ~~the~~ a species referred to in Article 10.9.2. that ~~which~~ are

intended for any purpose and ~~which~~ 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 SVCV);

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

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

d) fish oil;

e) fish *meal*.

2) When authorising the importation or transit of ~~*aquatic animals* and~~ *aquatic animal products* ~~of~~ derived from a species referred to in Article 10.9.2., other than those referred to in point 1 of Article 10.9.3., *Competent Authorities* should require the conditions prescribed in Articles 10.9.7. to 10.9.12~~1~~. relevant to the infection with SVCV status of the *exporting country*, *zone* or *compartment*.

3) When considering the importation or transit of ~~*aquatic animals* and~~ *aquatic animal products* ~~of~~ derived from a species not ~~covered~~ referred to in Article 10.9.2. but which could reasonably be expected to pose a *risk* of transmission ~~spread~~ of SVCV, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this analysis ~~assessment~~.

Article 10.9.4.

Country free from infection with SVCV ~~spring viraemia of carp~~

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from infection with SVCV if all the areas covered by the shared water bodies are declared countries or *zones* free from infection with SVCV (see Article 10.9.5.).

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

1) none of the *susceptible species* referred to in Article 10.9.2. 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 10.9.2. are present and the following conditions have been met:

a) there has been no ~~observed~~ occurrence of infection with SVCV ~~the *disease*~~ 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 ~~disease~~ infection with SVCV 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 SVCV;

OR

4) it previously made a *self-declaration of freedom* from infection with SVCV and subsequently lost its ~~*disease*~~

free status due to the detection of SVCV but the following conditions have been met:

a) on detection of SVCV ~~the *disease*~~, 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 ~~have been destroyed or removed from the *infected zone*~~ by means that minimise the likelihood ~~*risk*~~ of further transmission ~~spread~~ of SVCV ~~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 infection with SVCV ~~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 SVCV.

In the meantime, part or all of the unaffected ~~non-affected~~ area may be declared a free *zone* provided that such a part meets the conditions in point 3 of Article 10.9.5.

Article 10.9.5.

Zone or compartment free from infection with SVCV ~~spring viraemia of carp~~

If a *zone* or *compartment* extends over more than one country, it can only be declared a ~~an SVC free~~ *zone* or *compartment* free from infection with SVCV 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 SVCV 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 10.9.2. 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 10.9.2. are present in the *zone* or *compartment* and the following conditions have been met:

   a) there has been no ~~observed~~ occurrence of infection with SVCV ~~the *disease*~~ 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 ~~disease~~ infection with SVCV 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 SVCV;

OR

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

   a) on detection of SVCV ~~the *disease*~~, 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 ~~have been destroyed or removed from the *infected zone*~~ by means that minimise the likelihood ~~*risk*~~ of further transmission ~~spread~~ of SVCV ~~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 infection with SVCV ~~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 SVCV.

Article 10.9.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from infection with SVCV following the provisions of points 1 or 2 of Articles 10.9.4. or 10.9.5. (as relevant) may maintain its status as free from infection with SVCV provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from infection with SVCV following the provisions of point 3 of Articles 10.9.4. or 10.9.5. (as relevant) may discontinue *targeted surveillance* and maintain its free status ~~as free from SVC~~ provided that conditions that are conducive to clinical expression of infection with SVCV, as described in the corresponding chapter of the *Aquatic Manual*, ~~exist~~ and *basic biosecurity conditions* are continuously maintained.

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

Article 10.9.7.

Importation of aquatic animals ~~and~~ or aquatic animal products from a country, zone or compartment declared free from infection with SVCV ~~spring viraemia of carp~~

When importing *aquatic animals* of a species referred to in Article 10.9.2., or ~~and~~ *aquatic animal products* ~~of species referred to in Article 10.2.2.~~ derived thereof, from a country, *zone* or *compartment* declared free from infection with SVCV, 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 ~~certifying that~~, on the basis of the procedures described in Articles 10.9.4. or 10.9.5. (as applicable) and 10.9.6., the place of production of the *aquatic animals* or ~~and~~ *aquatic animal products* is a country, *zone* or *compartment* declared free from infection with SVCV.

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 ~~*commodities* referred to~~ in point 1 of Article 10.9.3.

Article 10.9.8.

Importation of aquatic animals for aquaculture from a country, zone or compartment not declared free from infection with SVCV ~~spring viraemia of carp~~

When importing, for *aquaculture*, *aquatic animals* of a species referred to in Article 10.9.2. from a country, *zone* or *compartment* not declared free from infection with SVCV, 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) the treatment of all transport water, equipment, effluent and waste materials to inactive SVCV 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 SVCV.

b) In the *importing country:*

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

ii) test the F-0 population for SVCV 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 SVCV, ~~(as described in Chapter 2.3.9. of the *Aquatic Manual*)~~ and sample and test for SVCV in accordance with Chapter 1.4. of the *Aquatic Code* and Chapter 2.3.9. of the *Aquatic Manual*;

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

vi) if SVCV 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 10.9.9.

Importation of aquatic animals ~~and~~ or aquatic animal products for processing for human consumption from a country, zone or compartment not declared free from infection with SVCV ~~spring viraemia of carp~~

When importing, for processing for human consumption, *aquatic animals* ~~or *aquatic animal products*~~ of a species referred to in Article 10.9.2., or *aquatic animal products* derived thereof, from a country, *zone* or *compartment* not declared free from infection with SVCV, 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 10.9.3., or ~~products described~~ in point 1 of Article 10.9.12, 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 SVCV or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and ~~and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of SVCV or is disposed in a manner that prevents contact of waste with *susceptible species*.~~

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

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

Annex 20 (contd)

Article 10.9.10.

Importation of aquatic animals or aquatic animal products intended for uses other than human consumption, including ~~use in~~ animal feed~~, or for~~ and agricultural, industrial, research or pharmaceutical use, from a country, zone or compartment not declared free from infection with SVCV ~~spring viraemia of carp~~

When importing *aquatic animals* of a species referred to in Article 10.9.2., or *aquatic animal products* derived thereof, intended for uses other than human consumption, including ~~use~~ ~~in~~ animal *feed* ~~or for~~ and agricultural, industrial, research or pharmaceutical use, ~~*aquatic animals* of species referred to in Article 10.2.2.~~ from a country, *zone* or *compartment* not declared free from infection with SVCV, 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 10.9.3. or other ~~facilities for slaughter and processing into~~ 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 SVCV or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and ~~and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of SVCV or is disposed in a manner that prevents contact of waste with *susceptible*~~

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

~~This article does not apply to *commodities* referred to in point 1 of Article 10.9.3.~~

Article 10.9.11.

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

When importing, for use in laboratories or zoos, *aquatic animals* of a species referred to in Article 10.11.2. from a country, *zone* or *compartment* not declared free from infection with SVCV, 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 SVCV 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 SVCV 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 10.9.12~~1~~.

Importation (or transit) of ~~aquatic animals and~~ aquatic animal products for retail trade for human consumption regardless of the infection with SVCV status of the exporting ~~from a~~ country, zone or compartment ~~not declared free from~~ infection with SVCV ~~spring viraemia of carp~~

1) *Competent Authorities* should not require any conditions related to SVCV, regardless of the infection with SVCV status of the *exporting country*, *zone* or *compartment*, when authorising the importation or (or transit) of fish fillets or steaks (chilled) that ~~which~~ have been prepared and packaged for retail trade and ~~which~~ 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* ~~*commodities*~~ Member Countries may wish to consider introducing internal measures to address the *risks* associated with the *aquatic animal product* ~~*commodity*~~ being used for any purpose other than for human consumption.

2) When importing ~~*aquatic animals or*~~ *aquatic animal products*, other than those referred to in point 1 above, derived from a ~~of~~ of species referred to in Article 10.9.2. from a country, *zone* or *compartment* not declared free from infection with SVCV, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

Annex 21

CHAPTER 10.10.

INFECTION WITH VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUS

EU position

The EU in general supports the adoption of this modified chapter.

A comment is inserted in the text below.

Article 10.10.1.

For the purposes of the *Aquatic Code*, infection with viral haemorrhagic septicaemia virus ~~(VHS)~~ means *infection* with the *pathogenic agent* ~~VHS~~ viral haemorrhagic septicaemia virus (VHSV~~, synonym: Egtved virus~~), of the ~~g~~Genus *Novirhabdovirus* and ~~f~~Family ~~Rhabdoviridae~~ *Rhabdoviridae*.

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

Article 10.10.2.

Scope

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

EU comment

The EU notes that there are inconsistencies in the French translation of the common names of the various trout species, and would invite the OIE to review the French version of the paragraph above. (This comment is also valid for the corresponding Aquatic Manual chapter.)

Indeed, *Salmo trutta* comprises two subspecies: "truite de mer" (migratory, called "sea trout" in English) and "truite de rivière", also called "truite fario" (sedentary, in fresh water which is called "brown trout" in English). The latter is also called "truite brune" in French in North America. Concerning VHS susceptible species, the French version uses the name "truite de mer" as a susceptible species for this VHS virus, whereas in the English version, reference is made to "brown trout". Therefore, the EU suggests translating *Salmo trutta* (brown trout in English) in "truite brune ou truite fario" in the French version of both the relevant Aquatic Code and Manual chapters.

Article 10.10.3.

Importation or transit of ~~aquatic animals and~~ aquatic animal products for any purpose regardless of the infection with VHSV ~~viral haemorrhagic septicaemia~~ status of the exporting country, zone or compartment

1) *Competent Authorities* should not require any conditions related to VHSV, regardless of the infection with VHSV status of the *exporting country*, *zone* or *compartment*, when authorising the importation or transit of the following *aquatic animal products* derived from ~~the~~ a species referred to in Article 10.10.2. that ~~which~~ are intended for any purpose and ~~which~~ 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 VHSV);

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

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

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

e) fish oil;

f) fish *meal*;

g) fish skin leather.

2) When authorising the importation or transit of ~~*aquatic animals* and~~ *aquatic animal products* ~~of~~ derived from a species referred to in Article 10.10.2., other than those referred to in point 1 of Article 10.10.3., *Competent Authorities* should require the conditions prescribed in Articles 10.10.7. to 10.10.13~~2~~. relevant to the infection with VHSV status of the *exporting country*, *zone* or *compartment*.

3) When considering the importation or transit of ~~*aquatic animals* and~~ *aquatic animal products* ~~of~~ derived from a species not ~~covered~~ referred to in Article 10.10.2. but which could reasonably be expected to pose a *risk* of transmission ~~spread~~ of VHSV, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this analysis ~~assessment~~.

Article 10.10.4.

Country free from infection with VHSV ~~viral haemorrhagic septicaemia~~

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from infection with VHSV if all the areas covered by the shared water bodies are declared countries or *zones* free from infection with VHSV (see Article 10.10.5.).

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

1) a country where the species referred to in Article 10.10.2. are present but there has been no ~~observed~~ occurrence of infection with VHSV ~~the *disease*~~ 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*, may make a *self-declaration of freedom* from infection with VHSV when *basic biosecurity conditions* have been continuously met in the country for at least the last ten years;

OR

2) the ~~disease~~ infection with VHSV 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 VHSV;

OR

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

a) on detection of VHSV ~~the *disease*~~, 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 ~~have been destroyed or removed from the *infected zone*~~ by means that minimise the likelihood ~~*risk*~~ of further transmission ~~spread~~ of VHSV ~~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 infection with VHSV ~~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 VHSV.

In the meantime, part or all of the unaffected ~~non-affected~~ area may be declared a free *zone* provided that such a part meets the conditions in point 2 of Article 10.10.5.

Article 10.10.5.

Zone or compartment free from infection with VHSV ~~viral haemorrhagic septicaemia~~

If a *zone* or *compartment* extends over more than one country, it can only be declared a ~~VHS free~~ *zone* or *compartment* free from infection with VHSV 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 VHSV may be declared free by the *Competent Authority~~(ies)~~* of the country~~(ies)~~ concerned if:

1) a *zone* or *compartment* where the species referred to in Article 10.10.2. are present but there has been no ~~observed~~ occurrence of infection with VHSV ~~the *disease*~~ 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*, may be declared free from infection with VHSV when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the last ten years;

OR

2) the ~~disease~~ infection with VHSV 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 VHSV;

OR

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

   a) on detection of VHSV ~~the *disease*~~, 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 ~~have been destroyed or removed from the *infected zone*~~ by means that minimise the likelihood ~~*risk*~~ of further transmission ~~spread~~ of VHSV ~~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 infection with VHSV ~~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 VHSV.

Article 10.10.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from infection with VHSV following the provisions of point 1 of Articles 10.10.4. or 10.10.5. (as relevant) may maintain its status as free from infection with VHSV provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from infection with VHSV following the provisions of point 2 of Articles 10.10.4. or 10.10.5. (as relevant) may discontinue *targeted surveillance* and maintain its free status ~~as free from VHS~~ provided that conditions that are conducive to clinical expression of infection with VHSV, as described in the corresponding chapter of the *Aquatic Manual*, ~~exist~~ and *basic biosecurity conditions* are continuously maintained.

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

Article 10.10.7.

Importation of aquatic animals and aquatic animal products from a country, zone or compartment declared free from infection with VHSV ~~viral haemorrhagic septicaemia~~

When importing *aquatic animals* of a species referred to in Article 10.10.2., or ~~and~~ *aquatic animal products* ~~of species referred to in Article 10.2.2.~~ derived thereof, from a country, *zone* or *compartment* declared free from infection with VHSV, 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 ~~certifying that~~, on the basis of the procedures described in Articles 10.10.4. or 10.10.5. (as applicable) and 10.10.6., the place of production of the *aquatic animals* or ~~and~~ *aquatic animal products* is a country, *zone* or *compartment* declared free from infection with VHSV.

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 ~~*commodities* referred to~~ in point 1 of Article 10.10.3.

Article 10.10.8.

Importation of aquatic animals for aquaculture from a country, zone or compartment not declared free from infection with VHSV ~~viral haemorrhagic septicaemia~~

When importing, for *aquaculture*, *aquatic animals* of a species referred to in Article 10.10.2. from a country, *zone* or *compartment* not declared free from infection with VHSV, 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) the treatment of all transport water, equipment, effluent and waste materials to inactive ~~KHV~~ VHSV 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 VHSV.

   b) In the *importing country*:

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

      ii) test the F-0 population for VHSV 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 VHSV, ~~(as described in Chapter 2.3.10. of the *Aquatic Manual*)~~ and sample and test for VHSV in accordance with Chapter 1.4. of the *Aquatic Code* and Chapter 2.3.10. of the *Aquatic Manual*;

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

      vi) if VHSV 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 10.10.9.

Importation of aquatic animals ~~and~~ or aquatic animal products for processing for human consumption from a country, zone or compartment not declared free from infection with VHSV ~~viral haemorrhagic septicaemia~~

When importing, for processing for human consumption, *aquatic animals* ~~or *aquatic animal products*~~ of a species referred to in Article 10.10.2., or *aquatic animal products* derived thereof, from a country, *zone* or *compartment* not declared free from infection with VHSV, 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 10.10.3., or ~~products described~~ in point 1 of Article 10.10.12., 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 VHSV or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and ~~and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of VHSV or is disposed in a manner that prevents contact of waste with *susceptible species*.~~

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

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

Article 10.10.10.

Importation of aquatic animals or aquatic animal products intended for uses other than human consumption, including ~~use in~~ animal feed~~, or for~~ and agricultural, industrial, research or pharmaceutical use, from a country, zone or compartment not declared free from infection with VHSV ~~viral haemorrhagic septicaemia~~

When importing *aquatic animals* of a species referred to in Article 10.10.2., or *aquatic animal products* derived thereof, intended for uses other than human consumption, including ~~use~~ ~~in~~ animal *feed* ~~or for~~ and agricultural, industrial, research or pharmaceutical use, ~~*aquatic animals* of species referred to in Article 10.10.2.~~, from a country, *zone* or *compartment* not declared free from infection with VHSV, 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 10.10.3. or other ~~facilities for slaughter and processing into~~ 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 VHSV or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and ~~and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of VHSV or is disposed in a manner that prevents contact of waste with *susceptible*~~

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

~~This article does not apply to *commodities* referred to in point 1 of Article 10.10.3.~~

Article 10.10.11.

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

When importing, for use in laboratories or zoos, *aquatic animals* of a species referred to in Article 10.10.2. from a country, *zone* or *compartment* not declared free from infection with VHSV, 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 VHSV 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 VHSV 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 10.10.12~~1~~.

Importation (or transit) of ~~aquatic animals and~~ aquatic animal products for retail trade for human consumption regardless of the infection with VHSV status of the exporting ~~from a~~ country, zone or compartment ~~not declared free from infection with VHSV viral haemorrhagic septicaemia~~

1) *Competent Authorities* should not require any conditions related to VHSV, regardless of the infection with VHSV, status of the *exporting country*, *zone* or *compartment*, when authorising the importation or (or transit) of fish fillets or steaks (chilled) that ~~which~~ have been prepared and packaged for retail trade and ~~which~~ 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* ~~*commodities*~~ Member Countries may wish to consider introducing internal measures to address the *risks* associated with the *aquatic animal product* ~~*commodity*~~ being used for any purpose other than for human consumption.

2) When importing ~~*aquatic animals or*~~ *aquatic animal products*, other than those referred to in point 1 above, derived from a ~~of~~ species referred to in Article 10.10.2. from a country, *zone* or *compartment* not declared free from infection with VHSV, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

Article 10.10.13~~2~~.

Importation of disinfected eggs for aquaculture from a country, zone or compartment not declared free from infection with VHSV ~~viral haemorrhagic septicaemia~~

1) When importing disinfected eggs of the species referred to in Article 10.10.2. for *aquaculture*, from a country, *zone* or *compartment* not declared free from infection with VHSV, the *Competent Authority* of the *importing country* should assess the *risk* associated with at least:

   a) the ~~VHS virus~~ VHSV status of the water to be used during the *disinfection* of the eggs;

   b) the prevalence of infection with VHSV ~~virus~~ 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 10.10.2. for *aquaculture*, from a country, *zone* or *compartment* not declared free from infection with VHSV, 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*~~ certifying that the procedures described in point 2 of this article ~~Article 10.10.12.~~ have been fulfilled.

Annex 22

Model Articles X.X.8., X.X.9., X.X.10. and X.X.11.

EU position

The EU in geneal supports the adoption of these modified model articles.

A comment is inserted in the text below.

Article X.X.8.

[…]

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 pathogenic agent X.

b) In the *importing country*:

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

ii) test the F-0 population for pathogenic agent 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 conductive to the clinical expression of infection with pathogenic agent X, ~~(as described in Chapter X.X.X. of the *Aquatic Manual*)~~ and sample and test for pathogenic agent X in accordance with Chapter 1.4. of the *Aquatic Code* and ~~(as described in~~ Chapter X.X.X. of the *Aquatic Manual*~~)~~;

EU comment

In point IV) above, please replace "conductive" with "conducive" (typographical error).

In addition, we make reference here to our comment on this paragraph inserted in Annex 14.

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

vi) if pathogenic agent 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.

Article X.X.9.

Importation of aquatic animals ~~and~~ or aquatic animal products for processing for human consumption from a country, zone or compartment not declared free from infection with pathogenic agent X

When importing, for processing for human consumption, *aquatic animals* ~~or *aquatic animal products*~~ of a species referred to in Article X.X.2., or *aquatic animal products* derived thereof, from a country, *zone* or *compartment* not declared free from infection with pathogenic agent X, the *Competent Authority* of the *importing country* should assess the *risk* 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 X.X.3., ~~or products described~~ or in point 1 of Article X.X.12./13, 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 pathogenic agent X or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and ~~and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of pathogenic agent X or is disposed in a manner that prevents contact of waste with *susceptible species*.~~

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

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

Article X.X.10.

Importation of aquatic animals or aquatic animal products intended for uses other than human consumption, including ~~use in~~ animal feed~~, or for~~ and agricultural, industrial, research or pharmaceutical use, from a country, zone or compartment not declared free from infection with pathogenic agent X

When importing *aquatic animals* of a species referred to in Article X.X.2., or *aquatic animal products* derived thereof, intended for uses other than human consumption, including ~~for use in~~ animal *feed* ~~or for~~ and agricultural, industrial, research or pharmaceutical use, ~~*aquatic animals* of a species referred to in Article X.X.2. or *aquatic animal products* derived thereof,~~ from a country, *zone* or *compartment* not declared free from infection with pathogenic agent X, the *Competent Authority* of the *importing country* should require that:

1) the consignment is delivered directly to, and held in, ~~in~~ *quarantine* or containment facilities until processed into one of the products referred to in point 1 of Article X.X.3. or other ~~facilities for slaughter and processing into~~ 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 pathogenic agent X or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and ~~and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of pathogenic agent X or is disposed in a manner that prevents contact of waste with *susceptible species*.~~

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

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

Article X.X.11.

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

When importing, for use in laboratories and zoos, *aquatic animals* of a species referred to in Article X.X.2. from a country, *zone* or *compartment* not declared free from infection with pathogenic agent X, 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 pathogenic agent X 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 pathogenic agent X 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.

Annex 23

CHAPTER 2.2.7.

INFECTION WITH WHITE SPOT SYNDROME VIRUS ~~DISEASE~~

EU position

The EU thanks the OIE and supports the adoption of this modified chapter.

1. Scope

~~For the purpose of this chapter,~~ Infection with ~~disease (WSD) is considered to be infection with~~ white spot syndrome virus ~~(WSSV)~~ means infection with the pathogenic agent white spot syndrome virus (WSSV), Genus *Whispovirus,* Family *Nimaviridae*.

2. Disease information

2.1. Agent factors

~~Various WSSV isolates with small genetic polymorphisms have been identified (variants). It should be realised, however, that as the *Nimaviridae* is a newly recognised family, the species concept will be subject to change after existing and new isolates have been studied in more detail.~~

2.1.1. Aetiological agent, agent strains

WSSV was assigned by the International Committee on Taxonomy of Viruses (ICTV) as the only member of the genus *Whispovirus* within the *Nimaviridae* family. Virions of WSSV are ovoid or ellipsoid to bacilliform in shape, have a regular symmetry, and measure 80–120 nm in diameter and 250–380 nm in length. ~~Most notable is the thread- or~~ A flagella-like extension (appendage) may be observed at one end of the virion. Today, although various geographical isolates with genotypic variability have been identified, they are all classified as a single species (white spot syndrome virus) within the genus *Whispovirus* (Lo *et al.,* 2012).

2.1.2. Survival outside the host

The agent is viable for at least 30 days at 30°C in seawater under laboratory conditions (Momoyama *et al.*, 1998); and is viable in ponds for at least 3–4 days (Nakano *et al.*, 1998). Laboratory emulations of drainable and non-drainable ponds suggest that the virus is no longer infective after 21 days of sun-drying or after 40 days in waterlogged pond sediment (Satheesh Kumar *et al.*, 2013).

2.1.3. Stability of the agent (effective inactivation methods)

The agent is inactivated in <120 minutes at 50°C and <1 minute at 60°C (Nakano *et al.*, 1998).

In laboratory studies, WSSV was inactivated under following conditions:

Heat: 55°C for 90 minutes, 70°C for 5 minutes (Chang *et al.*, 1998); 50°C for 60 minutes; 60°C for 1 minute; 70°C for 0.2 minutes (Nakano *et al.*, 1998).

Desiccation: WSSV adsorbed onto the filter paper and allowed to dry subsequently was inactivated in 1 hour at 30°C and in 3 hours at 26°C (Maeda *et al.,* 1998, Nakano *et al.,* 1998).

pH: pH 3 for 60 minutes; pH 12 for 10 minutes (Chang *et al.*, 1998, Balasubramanian *et al.*, 2006).

Ultraviolet light: Total dose of 9.30×10^5 µWs/cm^2 (Chang *et al.,* 1998).

Ozone: Total residual oxidants concentration of 0.5 µg ml^{-1} for 10 minutes (Chang *et al.,* 1998).

Sodium hypochlorite: Total free chlorine concentration of 100 ppm for 10 minutes (Chang *et al.,* 1998).

Benzalkonium chloride: 100 ppm for 10 minutes (Balasubramanian *et al.,* 2006).

Iodophore: Total free iodine concentration of 100 ppm for 10 minutes (Chang *et al.,* 1998).

2.1.4. Life cycle

In-vitro studies with primary cell culture and *in-vivo* studies with postlarvae (PL) show that the replication cycle is approximately 20 hours at 25°C (Chang *et al.*, 1996; Chen *et al.*, 2011; Wang *et al.*, 2000).

2.2. Host factors

~~WSSV has an extremely wide host range.~~ The virus can infect a wide range of aquatic crustaceans especially decapods, including marine, brackish and freshwater prawns, crabs, crayfish and lobsters (Maeda *et al.*, 2000).

2.2.1. Susceptible host species

Of all of the species that have been tested to date, no decapod (order Decapoda) crustacean from marine and brackish or freshwater sources has been reported to be refractory ~~resistant~~ to infection with WSSV (Flegel, 1997; Lightner, 1996; Lo & Kou, 1998; Maeda *et al.*, 2000; Stentiford *et al.,* 2009).

2.2.2. Susceptible stages of the host

All life stages are potentially susceptible, from eggs to broodstock (Lightner, 1996; Venegas *et al.*, 1999). WSSV genetic material has been detected in reproductive organs (Lo *et al.,* 1997), but susceptibility of the gametes to WSSV infection has not been determined definitively.

2.2.3. Species or subpopulation predilection (probability of detection)

The best life stages of crustaceans for detection of ~~infection with~~ WSSV are late PL stages, juveniles and adults. Probability of detection can be increased by exposure to stressful conditions (e.g. eye-stalk ablation, spawning, moulting, changes in salinity, temperature or pH, and during plankton blooms).

2.2.4. Target organs and infected tissue

The major target~~s~~ tissues of ~~infection with~~ WSSV ~~infection~~ are ~~tissues~~ of ectodermal and mesodermal embryonic origin, especially the cuticular epithelium and subcuticular connective tissues (Momoyama *et al.*, 1994; Wongteerasupaya *et al.*, 1995). Although WSSV infects the underlying connective tissue in the ~~shrimp~~ crustacean hepatopancreas and midgut, the tubular epithelial cells of these two organs are of endodermal origin, and they do not become infected.

2.2.5. Persistent infection ~~with lifelong carriers~~

Many decapod species have been shown to be subclinically infected with WSSV and are thought to be carriers ~~of disease~~ ~~Persistent infection occurs commonly and lifelong infection has been shown~~ (Lo & Kou, 1998). ~~Viral loads during persistent infection can be extremely low and are very hard to detect even by sensitive methods such as real-time and nested PCR.~~

2.2.6. Vectors

The virus can be transmitted directly from host to host ~~and does not need~~ without the need for a biological vector.

2.2.7. Known or suspected wild aquatic animal carriers

Wild decapods known to be resevoirs of infection with WSSV include *Mysis* sp. (Huang *et al.,* 1995a), *Acetes* sp., *Alpheus* sp., *Callianassa* sp., *Exopalaemon* sp., *Helice* sp., *Hemigrapsus* sp. *Macrophthalmus* sp., *Macrophthel* sp., *Metaplax* sp., *Orithyia* sp., *Palaemonoidea* sp., *Scylla* sp,. *Sesarma* sp., *Stomatopoda* sp. and (He & Zhou, 1996; Lei *et al.,* 2002). These species can ~~be easily infected by WSSV and may~~ express the disease under suitable environmental conditions. However, non-decapodal crustaceans, such as copepods (Huang *et al.,* 1995a), rotifers (Yan *et al.,* 2004), ~~*Artemia salina* (Chang *et al.,* 2002)~~, *Balanus* sp. (Lei *et al.,* 2002), and *Tachypleidue* sp. (He & Zhou, 1996) may be apparently healthy carrier animals ~~become wild aquatic animal carriers by latent infection without disease~~. There is inconclusive evidence for the WSSV carrier status of *Artemia salina* (Chang *et al.,* 2002). Other marine molluscs, polychaete worms (Vijayan *et al.,* 2005), as well as non-crustacean aquatic arthropods such as sea slaters (*Isopoda*) and Euphydradae insect larvae can mechanically carry the virus without evidence of infection (Lo & Kou, 1998).

2.3. Disease pattern

Infection with WSSV sometimes causes clinical disease ~~and sometimes not~~ (Tsai *et al.*, 1999), depending on factors as yet poorly understood but related to species tolerance and environmental triggers. With an appropriate infection dose to allow sufficient time before mortality, animals susceptible to disease show large numbers of virions circulating in the haemolymph (Lo *et al.*, 1997), but this may also occur for tolerant species that show no mortality. Thus, high viral loads *per se* do not cause disease or mortality for all susceptible species.

2.3.1. Transmission mechanisms

The infection with WSSV can be transmitted ~~vertically (trans ovum),~~ horizontally by consumption of infected tissue (e.g. cannibalism, predation, etc.), and by water-borne routes. Transmission of ~~infection with~~ WSSV can occur from apparently healthy animals in the absence of disease. Dead and moribund animals can be a source of disease transmission (Lo & Kou, 1998).

True vertical transmission (intra-ovum) of WSSV to the progeny has not been demonstrated.

2.3.2. Prevalence

Prevalence of infection with WSSV is highly variable, from <1% in infected wild populations to up to 100% in captive populations (Lo & Kou, 1998).

2.3.3. Geographical distribution

~~WSD~~ Infection with WSSV has been identified from crustaceans in China (People's Rep. of), Japan, Korea (Rep. of), South-East Asia, South Asia, the Indian Continent, the Mediterranean (Stentiford & Lightner, 2011), the Middle East, and the Americas. ~~WSD free~~ Zones and compartments free from infection with WSSV are known within these regions (Lo *et al.,* 2012).

2.3.4. Mortality and morbidity

All penaeid shrimp species are highly susceptible to infection with WSSV, often resulting in high mortality. Crabs, crayfish, freshwater prawns, spiny lobsters and clawed lobsters are susceptible to infection with WSSV, but morbidity and mortality as a consequence of infection are highly variable (Lo & Kou, 1998). High level infections with WSSV are known in some decapods in the absence of clinical disease.

2.3.5. Environmental factors

Disease outbreaks may be induced by stressors, such as rapid changes in salinity. Water temperature has a profound effect on disease expression, with average water temperatures of between 18 and 30°C being conducive to ~~WSD~~ WSSV outbreaks (Song *et al.*, 1996; Vidal *et al.*, 2001). Under experimental challenge condition, WSSV-induced mortality in shrimp is reduced when the temperature increases above 32°C (Vidal *et al.,* 2001).

2.4. Control and prevention

Although the underlying mechanism remains unknown, laboratory experiments have shown that 'vaccinated' shrimp and crayfish have better survival rates after WSSV challenge. It was first shown that *Penaeus japonicus* shrimp that survived natural and experimental WSSV infections displayed resistance to subsequent challenge with WSSV (Venegas *et al.*, 2000). Later studies showed that intramuscular injection of inactivated WSSV virions or recombinant structural protein, (VP28), provided shrimp with some protection against experimental WSSV infection. Furthermore, shrimp fed with food pellets coated with inactivated bacteria over-expressing VP28 showed better survival rates after WSSV challenge (Witteveldt *et al.*, 2004). However, although these results seemed promising, the protection was effective only when the shrimp were infected with a low dosage of WSSV. Also, the effect usually lasted for only a few days, or in the case of crayfish, for about 20 days. Another potential means of protecting shrimp against infection with WSSV ~~infection~~ is to use RNA interference (RNAi). WSSV gene-specific double-stranded (ds) RNAs produced strong anti-WSSV activity, protecting the shrimp against infection with WSSV ~~infection~~, but the same study showed that long dsRNA induced both sequence-dependent and independent anti-viral responses in shrimp (Robalino *et al.*, 2005). A more recent study ~~even~~ showed that even oral administration of bacterially expressed VP28 dsRNA could protect shrimp against infection with WSSV ~~infection~~ (Sarathi *et al.*, 2008). ~~To date,~~ However, although dsRNA technology continues to be explored, there are still no field trial data for either the vaccination or the RNAi approach.

2.4.1. Vaccination

No consistently effective vaccination methods have been developed for infection with WSSV.

2.4.2. Chemotherapy

~~No scientifically confirmed reports for infection with WSSV.~~ No published or validated methods.

2.4.3. Immunostimulation

Several reports have shown that beta-glucan, vitamin C, seaweed extracts (fucoidan) and other immunostimulants may improve resistance to infection with WSSV ~~WSD~~ (Chang *et al.*, 2003; Chotigeat *et al.*, 2004).

2.4.4. Resistance breeding

~~No significant Improvements~~ Progress in breeding *P. vannamei* for resistance to infections with WSSV ~~have~~ has been reported ~~for infections with WSSV~~ (Cuéllar-Anjel *et al.,* 2012; Huang *et al.,* 2012).

2.4.5. Restocking with resistant species

Not applicable for infection with WSSV ~~WSD~~.

2.4.6. Blocking agents

There are no efficient blocking agents that can be recommended at this time. rVP28 has an effect, but it cannot yet be used as a practical blocking agent.

2.4.7. Disinfection of eggs and larvae

For transovum transmission, disinfection of egg is likely to be effective (Lo & Kou, 1998), but this has not yet been confirmed in formal scientific trials.

2.4.8. General husbandry practices

A number of husbandry practices have been used successfully to manage infection with WSSV ~~WSD~~, such as avoiding stocking in the cold season, use of specific pathogen free (SPF) or polymerase chain reaction (PCR)-negative seed stocks, ~~and~~ use of biosecure water and culture systems (Withyachumnarnkul, 1999) and polyculture of shrimp and fish (He *et al.*, unpublished data).

3. Sampling

3.1. Selection of individual specimens

Samples of moribund shrimp or shrimp that show clinical signs (see Section 4.1.1) or exhibit behavioural changes (Section 4.1.2) should be selected for ~~WSSV~~ detection of ~~infection with~~ WSSV.

3.2. Preservation of samples for submission

See Chapter 2.2.0 *General information* (for diseases of crustaceans) for guidance on preservation of samples for the intended test method.

3.3. Pooling of samples

~~Samples taken for molecular or antibody based test methods for WSD may be combined as pooled samples of no more than five specimens per pooled sample of juveniles or subadults. However, for eggs, larvae and PL, pooling of larger numbers (e.g. ~150 or more eggs or larvae or 50 to 150 PL depending on their size/age) may be necessary to obtain sufficient sample material. See also chapter 2.2.0.~~

The effect of pooling on diagnostic sensitivity has not been evaluated, therefore larger life stages should be processed and tested individually. However, it may be necessary to pool small life stages, ~~especially~~ for example PL, ~~or specimens up to 0.5 g, can be pooled~~ to obtain ~~enough~~ sufficient material for nucleic acid extraction and molecular testing.

3.4. Best organs or tissues

Tissue tropism analysis from both experimentally infected shrimp and wild-captured brooders shows that tissues originating from the ectoderm and mesoderm, especially the cuticular epithelium and subcuticular connective tissues, as well as other target tissues (e.g. antennal gland, haematopoietic organ, etc.), are the main target tissues for infection with WSSV. Samples of or from the pleopods, gills, haemolymph, stomach or abdominal muscle are recommended for submission (Lo *et al.*, 1997).

For ~~non-destructive~~ non-lethal sampling and screening by PCR, ~~it is recommended to submit (a small piece of)~~ gill, ~~(a small aliquot of)~~ haemolymph or ~~(a small piece of)~~ pleopod are suitable tissues. ~~There is also some evidence to suggest that an ablated eyestalk would be a good alternative, provided that the compound eye is removed prior to submission.~~

Please see section 4.3.1.2.4.1 for details of the sample procedure.

3.5. Samples/tissues that are not suitable

Although WSSV infects the underlying connective tissue in the shrimp hepatopancreas and midgut, the columnar epithelial cells of these two organs are of endodermal embryonic origin (Lo *et al.*, 1997), and they are not appropriate tissues for detection. The compound eye may contain a PCR inhibitor (Lo *et al.*, 1997) and it is therefore not suitable for PCR-based diagnosis.

4. Diagnostic methods

4.1. Field diagnostic methods

4.1.1. Clinical signs

White spots embedded within the exoskeleton are the most commonly observed clinical sign. In most shrimp, these spots range from barely visible to 3 mm in diameter, and they sometimes coalesce into larger plates. However, it should be noted that environmental stress factors, such as high alkalinity, or bacterial disease can also cause white spots on the carapace of shrimp, and that moribund shrimp with infection with WSSV ~~WSD~~ may ~~in fact~~ have few, if any, white spots. Therefore, the appearance of white spots is ~~absolutely~~ not a ~~good~~ reliable diagnostic sign of infection with WSSV infection. ~~Furthermore, other crustaceans, such as most crayfish, are often reported to show no sign of white spots when infected with WSSV.~~ High degrees of colour variation with a predominance of reddish or pinkish discoloured shrimp are seen in diseased populations.

WSSV infections can be subclinical or manifest as clinical disease. Penaeid shrimp in aquaculture will generally show clinical signs associated with high morbidity and mortality. Some animals may die without showing any clinical signs. Non-penaeid species (e.g. crab, lobster) generally have subclinical infections under natural conditions.

4.1.2. Behavioural changes

The affected animals can show lethargy, decreased or absent feed consumption and abnormal swimming behaviour – slow swimming, swimming on side, swimming near water surface and gathering around edges of rearing units (Corbel *et al.,* 2001, Sahul Hameed *et al.,* 1998, 2001). ~~The presence of white spots does not always mean that the condition is terminal. For instance, under non-stressful conditions, infected shrimp that have white spots may survive indefinitely.~~ A very high mortality rate in the shrimp population can be expected within a few days of the onset of behavioural signs. ~~However, if the shrimp also appear lethargic, if their colour changes to pink or reddish brown, if they gather around the edges of ponds/tanks at the water surface, or if there is a rapid reduction in food consumption, then a very high mortality rate in the shrimp population can be expected within a few hours to a few days of the onset of these signs.~~

Annex 23 (contd)

4.2. Clinical methods

4.2.1. Gross pathology

~~See~~ In addition to the clinical and behavioural signs in Section 4.1.1 and 4.1.2 above, the following gross pathology has been reported in clinically affected penaeid shrimp: loosened attachment of the carapace with underlying cuticular epithelium (Sánchez-Paz, 2010), so carapace can be easily removed (Wen-Bin Zhan, 1998); empty gastro-intestinal tract due to anorexia (Escobedo-Bonilla, 2008); delayed clotting of haemolymph (Heidarieh, 2013); excessive fouling of gills (Wu *et al.*, 2013) and exoskeleton.

4.2.2. Clinical chemistry

Haemolymph withdrawn from WSSV-infected shrimp always has a delayed (or sometimes completely absent) clotting reaction.

4.2.3. Microscopic pathology

4.2.3.1. Wet mounts

Demonstration of hypertrophied nuclei in squash preparations of the gills and/or cuticular epithelium, which may be stained or unstained.

4.2.3.1.1 T-E staining

A T-E staining solution may be prepared from Trypan blue 0.6%, Eosin Y 0.2%, NaCl 0.5%, phenol 0.5%, and glycerol 20% (Huang & Yu, 1995).and used as follows:

i) Place a piece of lesion tissue (e.g. a piece of gill or stomach epithelium without the cuticle) on a slide and mince with a scalpel.

ii) Add 1–2 drops of the T-E staining solution to the minced tissue, mix and allow to stain for 3–5 minutes.

iii) Lay a cover glass over the stained tissue and cover with several pieces of absorbent paper. Use a thumb to squash the mince into a single layer of cells.

If the sample was taken from a heavily infected shrimp, it should be easy to see the hypertrophied nuclei and intranuclear eosinophilic or vacuolation-like inclusion bodies under a 400–1000× light microscope.

4.2.3.2. Smears

Demonstration of aggregates of WSSV virions in unstained smear preparations of haemolymph by dark-field microscopy.

NOTE: This is the simplest of the microscopic techniques and is recommended for people with limited expertise in diagnosing infection with WSSV. The aggregates appear as small reflective spots of 0.5 µm in diameter (Momoyama *et al.*, 1995).

4.2.3.3. Fixed sections

Histological changes commonly reported ~~with WSSV infection~~ in susceptible species include: Hypertrophied nuclei with marginated chromatin material in virus-infected cells; eosinophilic to pale basophilic (with haematoxylin & eosin stain) stained intranuclear viral inclusions within hypertrophied nuclei and multifocal necrosis associated with pyknotic and karyorrhectic nuclei in affected tissues of ectodermal and mesodermal origin. The infection with infectious hypodermal and hematopoietic necrosis virus, another DNA virus, produces similar inclusions that need to be differentiated from those of WSSV. ~~Histological demonstration of pathognomonic inclusion bodies in target tissues.~~

4.2.3.4. *In-situ* hybridisation

Use of WSSV-specific DNA probes with histological sections to demonstrate the presence of WSSV nuclei acid in infected cells.

4.2.3.5. Immunohistochemistry

Use of WSSV-specific antibodies with histological sections or wet mounts to demonstrate the presence of WSSV antigen in infected cells.

4.2.4. Electron microscopy/cytopathology

Demonstration of the virus in tissue sections or in semi-purified negatively stained virus preparations (e.g. from haemolymph). See Section 2.1.1 for virion morphology.

4.3. Agent detection and identification methods

4.3.1. Direct detection methods

Not reported.

4.3.1.1. Microscopic methods

See Section 4.2.3 above.

4.3.1.1.1. Wet mounts

See Section 4.2.4 above.

4.3.1.1.2. Smears

See Section 4.2.5 above.

4.3.1.1.3. Fixed sections

See Section 4.2.3 above.

4.3.1.2. Agent isolation and identification

4.3.1.2.1. Bioassay method

If SPF shrimp are available, the following bioassay method is based on Nunan *et al.* (1998) and Durand *et al*. (2000), is suitable for WSSV diagnosis.

i) For bioassay, remove the pleopods from shrimp suspected of being infected with WSSV ~~infection~~ and homogenise in TN buffer (0.02 M Tris/HCl, 0.4 M NaCl, pH 7.4).

ii) Following centrifugation at 1000 ***g*** for 10 minutes, dilute the supernatant fluid 1/10 with 2% NaCl and filter (0.2 µm filter)

iii) Inject 0.2 ml of inoculum into the dorso-lateral aspect of the fourth abdominal segment of indicator shrimp (e.g. SPF *P. vannamei* at the juvenile stage), injecting between the tergal plates into the muscle of the third abdominal segment.

iv) Examine moribund shrimp grossly or by using the methods described above. If at 3–5 days after inoculation there are still no moribund shrimp and all test results are negative, then it is safe to conclude that the bioassay results are negative.

4.3.1.2.2. Cell culture/artificial media

WSSV can be isolated from primary cultures of lymphoid or ovary cells, However, it is NOT recommended to use cell culture as a routine isolation method because of: 1) the high risk of contamination, and, 2) the composition of the medium varies depending on the tissue type, host species and experimental purpose; that is, to date there is no standard or recognised medium that can be recommended. As primary cell culture is so difficult to initiate and maintain for virus isolation purposes, bioassay should be the primary means for virus propagation

4.3.1.2.3. Antibody-based antigen detection methods

Both polyclonal and monoclonal antibodies raised against either the virus or a recombinant viral structural protein have been used in various immunological assays including western blot analysis, immunodot assay, indirect fluorescent antibody test (IFAT), immunohistochemistry (IHC) or enzyme-linked immunosorbent assay (ELISA) to detect WSSV (Huang *et al*., 1995a; Poulos *et al.*, 2001; Sithigorngul *et al*., 2006; Yoganandhan *et al*., 2004). Antibody-based methods can be fast, convenient and applicable to field use, but as they have only about the same sensitivity as 1-step PCR, they are recommended only to confirm acute infection with WSSV ~~WSD~~.

4.3.1.2.4. Molecular techniques

4.3.1.2.4.1 Polymerase chain reaction (PCR)

The PCR protocol described here is from Lo *et al.* 1996a and b, and uses sampling methods from Lo *et al.* 1997. It is recommended for all situations where infection with WSSV diagnosis is required. A positive result in the first step of this standard protocol implies a serious infection with WSSV ~~infection~~, whereas, when a positive result is obtained in the second amplification step only, a latent or carrier-state infection is indicated. Alternative PCR assays have also been developed (e.g. Numan & Lightner, 2011), but before use they should first be compared with the protocol described here.

PCR commercial kits are available for WSSV detection ~~diagnosis~~ and are acceptable provided they have been validated as fit for such purpose. Please consult the OIE Register for kits that have been certified by the OIE (http://www.oie.int/en/our-scientific-expertise/certification-of-diagnostic-tests/the-register-of-diagnostic-tests/).

DNA extraction

i) Collect 100–200 mg shrimp tissue (pleopod of live juvenile to subadult shrimp, postlarvae 11 upwards [PL11 up] with removed heads, or whole PL10, or use 100 µl haemolymph) in a 1.5 ml microfuge tube with 600 µl lysis solution (100 mM NaCl, 10 mM Tris/HCl, pH 8, 25 mM EDTA [ethylene diamine tetra-acetic acid], 0.5% SLS [sodium N-laurylsarcosinate] or 2% SDS [sodium dodecyl sulphate], and 0.5 mg ml^{-1} proteinase K added just before use). For non-destructive screening, pleopods can be removed using red-hot forceps. For this procedure, the animal should be wrapped in a wet towel such that only the organ to be excised is left exposed.

ii) Using a disposable stick, homogenise the tissue in the tube thoroughly.

iii) After homogenisation, incubate at 65°C for 1 hour.

iv) Add 5 M NaCl to a final concentration of 0.7 M. Next, slowly add 1/10 volume of N-cetyl N,N,Ntrimethylammonium bromide (CTAB)/NaCl solution (10% CTAB in 0.7 M NaCl) and mix thoroughly.

NOTE: In addition to the CTAB extraction method described here, commercial extraction kits are often used as part of normal surveillance activities.

v) Incubate at 65°C for 10 minutes, and then, at room temperature, add an equal volume of chloroform/isoamyl alcohol (24/1) and mix gently. Centrifuge at 13,000 *g* for 5 minutes and then transfer the aqueous solution (upper layer) to a fresh 1.5 ml tube and add an equal volume of phenol.

vi) Mix gently and centrifuge at 13,000 *g* for 5 minutes. Collect the upper layer solution and repeat the phenol extraction process once or twice.

vii) Transfer the final upper layer to a new tube, mix gently with two volumes of chloroform/isoamyl alcohol (24/1) and centrifuge at 13,000 ***g*** for 5 minutes.

viii) Transfer the upper layer to a new tube and precipitate DNA by adding two volumes of 95% or absolute ethanol followed by standing at –20°C for 30 minutes or –80° C for 15 minutes.

ix) Centrifuge at 13,000 *g* for 30 minutes and discard the ethanol. Wash the DNA pellet with 70% ethanol, dry and resuspend in 100 µl sterilised double-distilled water at 65°C for 15 minutes.

x) Use 1 µl of this DNA solution for one PCR.

Note: the following nested PCR procedures are well established and provide reliable diagnostic results under the specified conditions. Care should be taken, however, to ensure that DNA samples are prepared from the recommended organs, and that the PCR temperature is accurately applied (particularly for annealing, the recommended temperature is 55 ~~62~~°C). To prevent the possibility of false positive results, it is important to adhere to the specified procedures, especially when they are used to test new candidate hosts such as *Cherax quadricarinatus* (Claydon *et al.*, 2004), as well as *Procambarus clarkii* (red swamp crayfish) and *Procambarus zonangulus* (Southern white river crayfish). For diagnosed incidences of infection with WSSV in a new host or in a previously free zone, DNA sequencing should be used to confirm the positive results.

First-step PCR

i) Add 1 µl DNA template solution (containing about 0.1–0.3 µg DNA) to a PCR tube containing 100 µl of reaction mixture (10 mM Tris/HCl, pH 8.8, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 200 µM of each dNTP, 100 pmol of each primer, 2 units of heat-stable DNA polymerase).

ii) The outer primer sequences are 146F1, 5'-ACT-ACT-AAC-TTC-AGC-CTA-TCTAG-3' and 146R1, 5'-TAA-TGC-GGG-TGT-AAT-GTT-CTT-ACG-A-3'.

iii) The PCR profile is one cycle of 94°C for 4 minutes, 55°C for 1 minute, and 72°C for 2 minutes, followed by 39 cycles of 94°C for 1 minute, 55°C for 1 minute, and 72°C for 2 minutes and a final 5-minute extension at 72°C. The WSSV-specific amplicon from this reaction is 1447 bp. The sensitivity is approximately 20,000 copies of a plasmid template.

Second step of the (nested) PCR

This second step is necessary for the detection of ~~infection with~~ WSSV in shrimp at the carrier stage.

i) Add 10 µl of the first-step PCR product to 90 µl of a PCR cocktail with the same composition as above except that it contains the second (inner) primer pair: 146F2 (5'-GTA-ACT-GCC-CCT-TCC-ATC-TCC-A-3') and 146R2 (5'-TAC-GGC-AGC-TGC-TGC-ACC-TTG-T-3').

ii) Use the same PCR amplification protocol as above. The WSSV-specific amplicon from this reaction is 941 bp. The overall sensitivity of both steps is approximately 20 copies of a WSSV plasmid template.

iii) To visualise, electrophorese 10 µl PCR products on 1% agarose gels containing ethidium bromide at a concentration of 0.5 µg ml^{-1}.

iv) Decapod-specific primers (143F 5'-TGC-CTT-ATC-AGCTNT-CGA-TTG-TAG-3' and 145R 5'-TTC-AGN-TTT-GCA-ACC-ATA-CTT-CCC-3' yielding an 848 bp amplicon; N represents G, A, T, or C) should be used in control reactions to verify the quality of the extracted DNA and the integrity of the PCR. In the penaeid shrimp *P. aztecus,* the PCR product generated by this decapod-specific primer pair corresponds to nucleotide sequence 352–1200 of the 18s rRNA. The decapod 18s RNA sequence is highly conserved and produces a similar sized PCR product in almost all decapods. A positive control (WSSV DNA template) and negative controls (no template and shrimp DNA template) should be included in every assay.

4.3.1.2.4.2 DNA sequencing of PCR products

~~For confirmation of suspected new host of WSSV, the DNA fragment amplified~~ The amplicon from the two-step nested diagnostic PCR should be sequenced. The cloning and sequencing protocols described here are according to Claydon *et al.* (2004).

~~Note: to save time and money, it is acceptable to sequence the PCR amplicon directly. If a positive result is obtained, then go to step iv below. In the event that only band[s] of unexpected size are obtained, then the sample should be tested again using the cloning and sequencing procedures described below.~~

i) Excise the DNA fragments selected for further analysis from the agarose gels and purify them using any of the commercially available PCR clean up kits.

ii) Faint ~~Ligate~~ amplicons can be cloned into vector plasmids ~~and clone~~ prior to sequencing if required ~~the construct~~. Amplify and purify the recombinant plasmid for DNA sequencing.

Annex 23 (contd)

iii) Use suitable primers to ~~amplify~~ sequence the ~~inserted~~ amplicon~~, and then subject the amplified product to DNA sequencing~~.

iv) Compare the sequences obtained with available databases using the Basic Local Alignment Search Tool (BLAST) to determine approximate phylogenetic affiliations.

4.3.1.2.4.3 Taqman real-time PCR method

The protocol described here is from Durand & Lightner (2002). This detection method is highly specific to WSSV, is extremely sensitive (four copies) and has a wide dynamic range (seven logs).

Construction of positive control vector and preparation of standard curve

The DNA fragment of 69 bp amplified by the forward and reverse primers (indicated below) is cloned in pGEM-T easy or other suitable vectors, and then confirmed by sequencing. The plasmid DNA is purified by any commercial plasmid extraction kits and the concentration is determined by using a spectrophotometer or other methods. The gene copy number is determined according to the molar mass derived from the plasmid DNA containing the 69 bp insert. The plasmid DNAs are then serially diluted tenfold to generate standard curves ranging from ~~10^2~~-10^1 to 10^7 copies.

DNA extraction

DNA extraction should be performed according to the above protocol described for PCR (4.3.1.2.4.1) or by using a commercial kit. The concentration of purified DNA can be determined by spectrophotometer or by other methods.

Real-time PCR

The TaqMan assay is carried out using the TaqMan Universal PCR Master Mix, which contains AmpliTaq Gold DNA polymerase, AmpErase UNG, dNTPs with dUTP and optimised buffer components (PE Applied Biosystems, Foster City, CA, USA[1]). Primer sequences are WSS1011F: 5'-TGG-TCC-CGT-CCT-CAT-CTC-AG-3', WSS1079R: 5'-GCT-GCC-TTG-CCG-GAA-ATT-A-3', Taqman Probe: 5'-AGC-CAT-GAA-GAA-TGC-CGT-CTA-TCA-CAC-A-3'.

i) Add a sample of 10–50 ng of DNA to set up a 25 µl reaction mixture containing 0.3 µM of each primer and 0.15 µM of TaqMan probe.

ii) The PCR profile is one cycle of 50°C for 2 minutes for AmpErase uracil-N-glycosylase (UNG) and 95°C for 10 minutes for activation of AmpliTaq, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute.

iii) To determine the WSSV copy number of the extracted DNA samples, the samples are subjected to PCR reaction alongside the serially diluted plasmid DNA standard. After reaction, the software accompanying the PCR system automatically determines the Ct value for each PCR sample. Based on the Ct values, the software calculates the standard curve for standard dilution and determines the WSSV copy number for the DNA samples by extrapolating values from the standard curve.

4.3.1.2.4.4. In-situ *hybridisation* (*ISH*) *method*

The protocol described here is based on that developed by Nunan & Lightner (1997).

i) Fix moribund shrimp with Davidson's AFA fixative for 24–48 hours.

ii) Embed the tissues in paraffin and cut into 5 µm sections. Place sections on to positively charged microscope slides.

iii) Heat the slide on a hot plate at 65°C for 30 minutes.

iv) Deparaffinise, rehydrate and then treat for 2–30 minutes (depending on tissue type) with 100 µg ml^{-1} proteinase K in Tris/NaCl/EDTA (TNE) buffer at 37°C.

[1] Reference to specific commercial products as examples does not imply their endorsement by the OIE. This applies to all commercial products referred to in this *Aquatic Manual*.

v) Post-fix the slides by chilling in pre-cooled 0.4% formaldehyde for 5 minutes at 4°C and wash the slides in 2 × standard saline citrate (SSC; 1 × SSC = 150 mM NaCl, 15 mM tri-sodium citrate, pH 7.0) at room temperature.

vi) Pre-hybridise the slides with pre-hybridisation solution (50% formamide, 0.2% Ficoll 400, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, 5 × SSC, 1 mM EDTA, 50 mM Tris/HCl, pH 8) for 30 minutes at 42°C.

vii) Follow with hybridisation with the 1447 bp WSSV-specific PCR amplicon (or with any other WSSV-specific PCR amplicon; see Section 4.3.1.2.3.1 “First-step PCR” above) that has been labelled with digoxigenin. It is recommended that the probe be labelled by incorporating DIG-dNTP by the PCR method. Optimum concentration should be determined by testing and adjusting until a high specific signal is obtained against a low background.

viii) For hybridisation, boil the probe for 10 minutes and immediately place on ice. Dilute the probe to 30–50 ng ml^{-1} in pre-hybridisation solution and apply 500 µl to each slide.

ix) Put the slide on a hotplate at 85–95°C for 6–10 minutes (make sure that it does not reach boiling point), quench slides on ice for 5 minutes and then transfer to a humid chamber for 16–20 hours at 42°C.

x) After hybridisation, wash the slides twice for 15 minutes each time with 2 × SSC at room temperature, twice for 5 minutes with 1 × SSC at 37°C, and twice for 5 minutes with 0.5 × SSC at 37°C.

xi) For hybridisation detection, wash slides with maleic acid buffer (100 mM maleic acid, 150 mM NaCl, pH 7.5) for 5 minutes at room temperature.

xii) Block the slides with blocking solution (2% normal goat serum and 0.3% Triton X-100 in maleic acid buffer) for 30 minutes at 37°C.

xiii) Add 250 µl anti-DIG alkaline phosphatase (AP)-conjugated antibody solution (1 µl ml^{-1} anti-DIG/AP-Fab fragment in maleic acid buffer containing 1% normal goat serum and 0.3% Triton X-100) to each slide, and incubate at 37°C for 30 minutes.

xiv) Wash the slides twice with maleic acid buffer for 10 minutes each and once with detection buffer (100 mM Tris/HCl, 100 mM NaCl, pH 9.5) at room temperature.

xv) Add 500 µl development solution (prepare immediately before use by adding 45 µl NBT salt solution [75 mg ml^{-1} in 70% dimethyformamide], 35 µl 5-bromo-4-chloro-3-indoyl phosphate, toluidinum salt [X-phosphate] solution [50 mg ml^{-1} in dimethylformamide] and 1 ml 10% PVA to 9 ml of detection buffer) to each slide and incubate in the dark in a humid chamber for 1–3 hours.

xvi) Stop the reaction by washing the slides in TE buffer (10 mM Tri/HCl, 1 mM EDTA, pH 8.0) for 15 minutes at room temperature. Wash the slides in distilled water for ten dips, counterstain the slides in 0.5% aqueous Bismarck Brown Y for approximately 5 minutes and then rinse with water. Wet mount using aqueous mounting media for observation immediately or dehydrate the slides and mount with mounting media for long-term preservation.

xvii) Mount the slides with cover-slips and examine with a bright field microscope. Positive hybridisation appears as a dark blue to black precipitate against the yellow to brown counterstain.

4.3.1.2.4.5. Loop-mediated isothermal amplification (LAMP) method

The protocol described here is from Kono *et al.* (2004). The LAMP method is sensitive and rapid, and it amplifies the target nucleic acids under isothermal conditions, therefore needing no sophisticated machine for thermal cycling.

DNA extraction

DNA extraction could be performed according to the above protocol described for PCR (4.3.1.2.4.1) or by other suitable methods or by commercial kits.

Annex 23 (contd)

LAMP reaction

i) Add DNA to a tube to set up a 25 µl reaction mixture (20 mM Tris/HCl, pH 8.8, 10 mM KCl, 8 mM $MgSO_4$, 10 mM $(NH_4)_2SO_4$, 0.1% Tween 20, 0.8M Betaine, 1.4 mM of each dNTP, 40 pmol of WSSV-FIP and -BIP primers, 5 pmol of WSSV-F3 and -B3 primers).

ii) The primer sequences are WSSV-FIP: 5'-GGG-TCG-TCG-AAT-GTT-GCC-CAT-TTT-GCC-TAC- GCA-CCA-ATC-TGT-G-3', WSSV-BIP: 5'-AAA-GGA-CAA-TCC-CTC-TCC-TGC-GTT-TTA-GAA-CGG-AAG-AAA-CTG-CC-TT-3', WSSV-F3: ACG-GAC-GGA-GGA-CCC-AAA-TCG-A-3', WSSV-B3: 5'-GCC-TCT-GCA-ACA-TCC-TTT-CC-3'.

iii) Heat the mixture at 50°C for 5 minutes and at 95°C for 5 minutes, then chill on ice, and add 1 µl (8 U) of *Bst* DNA polymerase.

iv) Incubate the mixture at 65°C for 60 minutes, and then terminate the reaction at 80°C for 10 minutes.

v) To visualise, electrophorese 2 µl LAMP reaction products on 2% agarose gels containing ethidium bromide at a concentration of 0.5 µg ml^{-1}. This reaction produces WSSV-specific LAMP products with multiple bands of various sizes from approximately 200 bp to the loading well.

Reliable LAMP commercial kits may be an alternative for WSSV diagnosis.

4.3.1.2.5. Agent purification

The WSSV virion can be purified as described previously with slight modifications (Xie *et al.*, 2005). Briefly, collect five or six moribund crayfish or shrimp (20–25 g each) at 3 days to 1 week post-infection. Homogenise all tissues excluding the hepatopancreas for 2 minutes using a mechanical homogeniser in 1200 ml TNE buffer (50 mM Tris/HCl, 400 mM NaCl, 5 mM EDTA, pH 8.5) containing protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, and 1 mM $Na_2S_2O_5$). Centrifuge at 3500 ***g*** for 5 minutes. Save the supernatant and rehomogenise the pellet in 1200 ml TNE buffer. Filter the pooled supernatant through a nylon net (400 mesh) and centrifuge at 30,000 ***g*** for 30 minutes. Discard the supernatant and carefully rinse out the upper loose layer (pink) of the pellet using a Pasteur pipette. Resuspend the lower compact layer (grey) in 10 ml TM buffer (50 mM Tris/HCl, 10 mM $MgCl_2$, pH 7.5). Pool the crude virus suspension and centrifuge at 3000 ***g*** for 5 minutes. Centrifuge the supernatant again at 30,000 ***g*** for 20 minutes. Remove the supernatant and pink loose layer and resuspend the white pellet in 1.2 ml TM buffer containing 0.1% NaN3. Transfer to a 1.5-ml Eppendorf tube. Centrifuge the suspension three to five times at 650 ***g*** for 5 minutes each time to remove pink impurities. Finally, store the milk-like pure virus suspension at 4°C until use.

4.3.2. Serological methods

None developed.

5. Rating of tests against purpose of use

The methods currently available for targeted surveillance and diagnosis of infection with WSSV 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
	Larvae	PLs	Juveniles	Adults		
Gross signs	d	d	c	c	c	d
Bioassay	d	d	d	d	c	b
~~Direct LM~~ Wet mounts and smears	d	d	c	c	c	~~c~~ d
Histopathology	d	c	c	c	a	c
Transmission EM	d	d	d	d	d	a
Antibody-based assays	d	d	c	c	a	b
In-situ DNA probes	d	d	c	c	a	a
Real-time PCR	~~d~~ c	b	a	a	a	a
Conventional PCR	d	c	b	b	b	a
Sequence	d	d	d	d	d	a
LAMP	d	d	a	a	a	a

PLs = postlarvae; LM = light microscopy; EM = electron microscopy;
PCR = polymerase chain reaction; LAMP = loop-mediated isothermal amplification.

6. Test(s) recommended for targeted surveillance to declare freedom from infection with white spot syndrome virus ~~disease~~

~~Two-step PCR and sequencing are the recommended methods for declaring freedom, only for juveniles and adults and possibly PLs. Two-step PCR negative results are required. Where a two-step PCR positive result cannot be confirmed as infection with WSSV by sequencing, this also counts as a negative result.~~

Real-time PCR is the recommended test for targeted surveillance to declare freedom from infection with white spot ~~disease~~ syndrome virus.

Annex 23 (contd)

7. Corroborative diagnostic criteria

7.1. Definition of suspect case

~~*For juvenile and adult shrimp:* gross signs of WSD (See Sections 4.1.1 and 4.1.2 above).~~

~~*For shrimp at any life stage* (*larva to adult*): mortality.~~

~~*For shrimp and crab at any life stage* (*larva to adult*): hypertrophied nuclei in squash preparations of gill and/or cuticular epithelium; unusual aggregates in haemolymph by dark-field microscopy; inclusion bodies in histological sections in target tissues.~~

Infection with WSSV is suspected if at least one of the following criteria is met:

1. Gross pathology consistent with infection with WSSV
2. Histopathology consistent with infection with WSSV
3. Positive conventional PCR result
4. Positive real-time PCR result
5. Positive LAMP result

7.2. Definition of confirmed case

~~Suspect cases should first be checked by PCR or LAMP. If in a previously WSSV free country/zone/compartment, where PCR results are positive, they should be confirmed by sequencing. Histopathology, probes and electron microscopy also can be used to confirm the case.~~

Infection with WSSV is considered to be confirmed if one or more of the following criteria are met:

1. Histopathology consistent with WSSV and positive *in-situ* hybridisation test
2. Positive conventional PCR results ~~from~~ and ~~two~~ conventional PCR~~s and conventional PCR~~ targeting ~~a two~~ different region~~s~~ of the WSSV genome with sequence analysis consistent with WSSV
3. Positive real-time PCR results and conventional PCR targeting ~~a~~ different region of the WSSV genome with sequence analysis consistent with WSSV ~~and conventional PCR targeting a two different regions of the WSSV genome~~
4. Positive LAMP results and conventional PCR targeting ~~a~~ different region of the WSSV genome with sequence analysis consistent with WSSV ~~and conventional PCR targeting a two different regions different region of the WSSV genome~~

~~For confirmation of an index case in a previously free zone or country, sequence analysis of conventional PCR amplicons is required.~~

8. References

BALASUBRAMANIAN G., SUDHAKARAN R., SYED MUSTHAQ S., SARATHI M. & SAHUL HAMEED A.S. (2006). Studies on the inactivation of white spot syndrome virus of shrimp by physical and chemical treatments, and seaweed extracts tested in marine and freshwater animal models. *J. Fish Dis.*, **29**, 569–572.

CHANG C.-F., SU M.-S., CHEN H.-Y. & LIAO I.C. (2003). Dietary ~~[beta]~~ β-1,3-glucan effectively improves immunity and survival of *Penaeus monodon* challenged with white spot syndrome virus. *Fish Shellfish Immunol.*, **15**, 297–310.

CHANG P.S., CHEN H.C. & WANG Y.C. (1998). Detection of white spot syndrome associated baculovirus in experimentally infected wild shrimp, crab and lobsters by *in situ* hybridization. *Aquaculture*, **164**, 233–242.

CHANG P.S., LO C.F., WANG Y.C. & KOU G.H. (1996).Identification of white spot syndrome associated baculovirus (WSBV) target organs in the shrimp *Penaeus monodon* by ~~in situ~~ *in situ* hybridization. *Dis. Aquat. Org.,* **27**, 131–139.

CHANG Y.S., LO C.F., PENG S.E., LIU K.F., WANG C.H. & KOU G.H. (2002). White spot syndrome virus (WSSV) PCR-positive *Artemia* cysts yield PCR-negative nauplii that fail to transmit WSSV when fed to shrimp postlarvae. *Dis. Aquat. Org.,* **49**, 1–10.

CHEN I.T, AOKI T., HUANG Y.T., HIRONO I., CHEN T.C., HUANG J.Y., CHANG G.D., LO C.F., WANG H.C. (2011). White spot syndrome virus induces metabolic changes resembling the Warburg effect in shrimp hemocytes in the early stage of infection. *J. Virol.,* **85**, 12919–12928.

CHOTIGEAT W., TONGSUPA S., SUPAMATAYA K. & PHONGDARA A. (2004). Effect of fucoidan on disease resistance of black tiger shrimp. *Aquaculture*, **233**, 23–30.

CLAYDON K., CULLEN B. & OWENS L. (2004). OIE white spot syndrome virus PCR gives false-positive results in *Cherax quadricarinatus. Dis. Aquat. Org.,* **62**, 265–268.

CORBEL V., ZUPRIZAL Z., SHI C., HUANG, SUMARTONO, ARCIER J.-M. & BONAMI J.-R. (2001). Experimental infection of European crustaceans with white spot syndrome virus (WSSV). *J. Fish Dis.*, **24**, 377–382.

CUÉLLAR-ANJEL J., WHITE-NOBLE B., SCHOFIELD P., CHAMORRO R. & LIGHTNER D.V. (2012). Report of significant WSSV-r esistance in the Pacific white shrimp, *Litopenaeus vannamei*, from a Panamanian breeding program. *Aquaculture*, 368–369, 36–39.

DURAND S.V. & LIGHTNER D.V. (2002). Quantitative real time PCR for the measurement of white spot syndrome virus in shrimp. *J. Fish Dis.*, **25**, 381–389.

DURAND S.V., TANG K.F.J. & LIGHTNER D.V. (2000). Frozen commodity shrimp: potential avenue for introduction of white spot syndrome virus and yellow head virus. *J. Aquat. Anim. Health,* **12**, 128–135.

ESCOBEDO-BONILLA C. M., ALDAY-SANZ V., WILLE M., SORGELOOS P., PENSAERT M.B. & NAUWYNCK H.J. (2008). A review on the morphology, molecular characterization, morphogenesis and pathogenesis of white spot syndrome virus. *J. Fish Dis.*, **31**, 1–18.

FLEGEL T.W. (1997). Major viral diseases of the black tiger prawn (*Penaeus monodon*) in Thailand. *World J. Microbiol. Biotechnol.,* **13**, 433–442.

HE J. & ZHOU H. (1996). Infection route and host species of white spot syndrome baculovirus. *Acta Sci. Natur. Univ. Sunyatseni*, **38**, 65–69.

HEIDARIEH M., SOLTANI M., MOTAMEDI SEDEH F. & SHEIKHZADEH N. (2013). Low water temperature retards white spot syndrome virus replication in *Astacus leptodactylus* Crayfish. *Acta Sci. Vet.*, **41,** 1–6.

HUANG J. & YU J. (1995). A new staining method for on-site observation of viral inclusion bodies of penaeid shrimp. (*Chinese J.*). *Mar. Fish. Res.*, **16**, 31–39.

HUANG J., YU J., WANG X.-H., SONG X.-L., MA C.-S., ZHAO F.-Z. & YANG C.-H. (1995a). Survey on the pathogen and route of transmission of baculoviral hypodermal and hematopoietic necrosis in shrimp by ELISA of monoclone antibody. (*Chinese J.*). *Mar. Fish. Res.*, **16**, 40–50.

HUANG Y., YIN Z., WENG S., HE J. & LI S. (2012). Selective breeding and preliminary commercial performance of *Penaeus vannamei* for resistance to white spot syndrome virus (WSSV). *Aquaculture*, **364–365**, 111–117.

KONO T., SAVAN R., SAKAI M., &ITAMI T. (2004). Detection of white spot syndrome virus in shrimp by loop-mediated isothermal amplification. *J. Virol. Methods*, **115**, 59–65.

LEI Z.-W., HUANG J., SHI C.-Y., ZHANG L.-J. & YU K.-K. (2002). Investigation into the hosts of white spot syndrome virus (WSSV). *Oceanol. Limnol. Sin.*, **33**, 250–258.

LIGHTNER D.V. (1996). A handbook of pathology and diagnostic procedures for diseases of penaeid shrimp. Baton Rouge, Louisiana, USA: World Aquaculture Society, 1996.

Annex 23 (contd)

LO C.F., AOKI T., BONAMI J.R., FLEGEL T.W., LEU J.H., LIGHTNER D.V., STENTIFORD G., SÖDERHÄLL K., WALKER P.W. WANG H.C.., XUN X., YANG F. & VLAK J.M. (2012). *Nimaviridae*. *In:* Virus Taxonomy: Classification and Nomenclature of Viruses: Ninth Report of the International Committee on Taxonomy of Viruses, King A.M.Q., Adams M.J., Carstens E.B., & Lefkowitz E.J., eds. Elsevier Academic Press, San Diego, CA. USA, pp: 229–234.

LO C.F., HO C.H., CHEN C.H., LIU K.F., CHIU Y.L., YEH P.Y., PENG S.E., HSU H.C., LIU H.C., CHANG C.F., SU M.S., WANG C.H. & KOU G.H. (1997). Detection and tissue tropism of white spot syndrome baculovirus (WSBV) in captured brooders of *Penaeus monodon* with a special emphasis on reproductive organs. *Dis. Aquat. Org.,* **30**, 53–72.

LO C.F., HO C.H., PENG S.E., CHEN C.H., HSU H.C., CHIU Y.L., CHANG C.F., LIU K.F., SU M.S., WANG C.H. & KOU G.H. (1996b). White spot syndrome baculovirus (WSBV) detected in cultured and captured shrimp, ~~crab~~ crabs and other arthropods. *Dis. Aquat. Org.*, **27**, 215–225.

LO C.F. & KOU G.H. (1998). Virus-associated white spot syndrome of shrimp in Taiwan: a review. *Fish Pathol.,* **33**, 365–371.

LO C.F., LEU J.H., HO C.H., CHEN C.H., PENG S.E., CHEN Y.T., CHOU C.M., YEH P.Y., HUANG C.J., CHOU H.Y., WANG C.H. & KOU G.H. (1996a). Detection of baculovirus associated with white spot syndrome (WSBV) in penaeid shrimps using polymerase chain reaction. *Dis. Aquat .Org.*, **25**, 133–141. Annex 28 (contd)

MAEDA M., ITAMI T., MIZUKI E., TANAKA R., YOSHIZU Y., DOI K., YASUNAGA-AOKI C., TAKAHASHI Y. & KAWARABATA T. (2000). Red swamp crawfish (~~Procambarus clarkii~~ *Procambarus clarkii*): an alternative experimental host in the study of white spot syndrome virus. *Acta Virol.,* **44**, 371–374.

MAEDA M., KASORNCHANDRA J., ITAMI T., SUZUKI N., HENNIG O., KONDO M., ALBALADEJO J.D. & TAKAHASHI Y. (1998). Effect of Various Treatments on White Spot Syndrome Virus (WSSV) from *Penaeus japonicus* (Japan) and *P. monodon* (Thailand). *Fish Pathol.*, **33**, 381–387.

MOMOYAMA K., HIRAOKA M., INOUYE K., KIMURA T. & NAKANO H. (1995). Diagnostic techniques of the rod-shaped nuclear virus infection in the kuruma shrimp, *Penaeus japonicus. Fish Pathol.,* **30**, 263–269.

MOMOYAMA K., HIRAOKA M., NAKANO H., KOUBE H., INOUYE K. & OSEKO N. (1994). Mass mortalities of cultured kuruma shrimp, *Penaeus japonicus*, in Japan in 1993: Histopathological study. *Fish Pathol.,* **29**, 141–148.

MOMOYAMA K., HIRAOKA M., NAKANO H. & SAMESHIMA M. (1998). Cryopreservation of penaeid rod-shaped DNA virus (PRDV) and its survival in sea water at different temperatures. *Fish Pathol.,* **33**, 95–96.

NAKANO H., HIRAOKA M., SAMESHIMA M., KIMURA T. & MOMOYAMA K. (1998). Inactivation of penaeid rod-shaped DNA virus (PRDV), the causative agent of penaeid acute viraemia (PAV), by chemical and physical treatments. *Fish Pathol.,* **33**, 65–71.

NUNAN L.M. & LIGHTNER D.V. (1997). Development of a non-radioactive gene probe by PCR for detection of white spot syndrome virus (WSSV). *J. Virol. Methods,* **63**, 193–201.

NUNAN L.M. & LIGHTNER D.V. (2011). Optimized PCR assay for detection of white spot syndrome virus (WSSV). *J. Virol. Methods,* **171**, 318–321.

NUNAN L.M., POULOS B.T. & LIGHTNER D.V. (1998). The detection of white spot syndrome virus (WSSV) and yellow head virus (YHV) in imported commodity shrimp. *Aquaculture,* **160**, 19–30.

POULOS B.T., PANTOJA C.R., BRADLEY-DUNLOP D., AGUILAR J. & LIGHTNER D.V. (2001). Development and application of monoclonal antibodies for the detection of white spot syndrome virus of penaeid shrimp. *Dis. Aquat. Org.,* **47**, 13–23.

ROBALINO J., BARTLETT T., SHEPARD E., PRIOR S., JARAMILLO G., SCURA E., CHAPMAN R.W., GROSS P.S., BROWDY C.L. & WARR G.W. (2005). Double-stranded RNA induces sequence-specific antiviral silencing in addition to nonspecific immunity in a marine shrimp: convergence of RNA interference and innate immunity in the invertebrate antiviral response? *J Virol.,* **79**, 13561–13571.

SAHUL HAMEED A.S., ANILKUMAR M., STEPHEN RAJ M.L. & JAYARAMAN K. (1998). Studies on the pathogenicity of systemic ectodermal and mesodermal baculovirus and its detection in shrimp by immunological methods. *Aquaculture*, **160**, 31–45.

SAHUL HAMEED A.S., YOGANANDHAN K., SATHISH S., RASHEED M., MURUGAN V. & JAYARAMAN K. (2001). White spot syndrome virus (WSSV) in two species of freshwater crabs (Paratelphusa hydrodomous and P. pulvinata). *Aquaculture*, **201**, 179–186.

SÁNCHEZ-PAZ A. (2010). White spot syndrome virus: an overview on an emergent concern. *Vet. Res.*, **41**, 43.

SARATHI M., SIMON M.C., VENKATESAN C. & SAHUL HAMEED A.S. (2008). Oral administration of bacterially expressed VP28dsRNA to protect *Penaeus monodon* from white spot syndrome virus. *J. Mar. Biotechnol.*, **10**, 242–249.

SATHEESH KUMAR S., ANANDA BHARATHI R., RAJAN J.J.S., ALAVANDI S.V., POORNIMA M., BALASUBRAMANIAN C.P. & PONNIAH A.G. (2013). Viability of white spot syndrome virus (WSSV) in sediment during sun-drying (drainable pond) and under non-drainable pond conditions indicated by infectivity to shrimp. *Aquaculture*, **402–403**, 119–126.

SITHIGORNGUL W., RUKPRATANPORN S., PECHARABURANIN N., LONGYANT S., CHAIVISUTHANGKURA P. & SITHIGORNGUL P. (2006). A simple and rapid immunochromatographic test strip for detection of white spot syndrome virus (WSSV) of shrimp. *Dis. Aquat. Org.,* **72**, 101–106.

SONG X., HUANG J., WANG C., YU J., CHEN B. & YANG C. (1996). Artificial infection of brood shrimp of Penaeus chinensis with hypodermal and hematopoietic necrosis baculovirus. *J. Fish. China,* **20**, 374–378.

STENTIFORD G.D., BONAMI J.R. & ALDAY-SANZ V. (2009). A critical review of susceptibility of crustaceans to Taura Syndrome, yellowhead disease and white spot disease and implications of inclusion of these diseases in European legislation. *Aquaculture,* **291**, 1–17.

STENTIFORD G.D. & LIGHTNER D.V. (2011). Cases of white spot disease (WSD) in European shrimp farms. *Aquaculture,* **319**, 302–306.

TSAI M.F., KOU G.H., LIU H.C., LIU K.F., CHANG C.F., PENG S.E., HSU H.C., WANG C.H. & LO C.F. (1999). Long-term presence of white spot syndrome virus (WSSV) in a cultivated shrimp population without disease outbreaks. *Dis. Aquat. Org.,* **38**, 107–114.

VENEGAS C.A., NONAKA L., MUSHIAKE K., NISHIZAWA T. & MUROG K. (2000). Quasi-immune response of *Penaeus japonicus* to penaeid rod-shaped DNA virus (PRDV). *Dis. Aquat. Org.,* **42**, 83–89.

VENEGAS C.A., NONAKA L., MUSHIAKE K., SHIMIZU K., NISHIZAWA T. & MUROGA K. (1999). Pathogenicity of penaeid rod-shaped DNA virus (PRDV) to kuruma prawn in different developmental stages. *Fish Pathol.,* **34**, 19–23.

VIDAL O.M., GRANJA C.B., ARANGUREN F., BROCK J.A. & SALAZAR M. (2001). A profound effect of hyperthermia on survival of *Litopenaeus vannamei* juveniles infected with white spot syndrome virus. *J. World Aquac. Soc.,* **32**, 364–372.

VIJAYAN K.K., STALIN RAJ V., BALASUBRAMANIAN C.P., ALAVANDI S.V., THILLAI SEKHAR V. & SANTIAGO T.C. (2005). Polychaete worms – a vector for white spot syndrome virus (WSSV). *Dis. Aquat. Org.,* **63**, 107–111.

WANG C.H., YANG H.N., TANG C.Y., LU C.H., KOU G.H. & LO C.F. (2000). Ultrastructure of white spot syndrome virus development in primary lymphoid organ cell cultures. *Dis. Aquat. Org.*, **41**, 91–104.

WITHYACHUMNARNKUL B. (1999). Results from black tiger shrimp *Penaeus monodon* culture ponds stocked with postlarvae PCR-positive or -negative for white-spot syndrome virus (WSSV). *Dis. Aquat. Org.,* **39**, 21–27.

WITTEVELDT J., CIFUENTES C.C., VLAK J.M. & VAN HULTEN M.C. (2004). Protection of Penaeus monodon against white spot syndrome virus by oral vaccination. *J. Virol.,* **78**, 2057–2061.

Annex 23 (contd)

WONGTEERASUPAYA C., VICKERS J.E., SRIURAIRATANA S., NASH G.L., AKARAJAMORN A., BOONSAENG V., PANYIM S., TASSANAKAJON A., WITHYACHUMNARNKUL B. & FLEGEL T.W. (1995). A non-occluded, systemic baculovirus that occurs in cells of ectodermal and mesodermal origin and causes high mortality in the black tiger prawn *Penaeus monodon*. *Dis. Aquat. Org.,* **21**, 69–77.

WU W., WU B., YE T., HUANG H., DAI C., YUAN J. & WANG W. (2013). TCTP is a critical factor in shrimp immune response to virus infection. *PloS One, 8,* e74460.

XIE X., LI H., XU L. & YANG F. (2005). A simple and efficient method for purification of intact white spot syndrome virus (WSSV) viral particles. *Virus Res.,* **108**, 63–67.

YAN D.C., DONG S.L., HUANG J., YU X.M., FENG M.Y. & LIU X.Y. (2004). White spot syndrome virus (WSSV) detected by PCR in rotifers and rotifer resting eggs from shrimp pond sediments. *Dis. Aquat. Org.,* **59**, 69–73.

YOGANANDHAN K., SYED MUSTHAQ S., NARAYANAN R.B. & SAHUL HAMEED A.S. (2004). Production of polyclonal antiserum against recombinant VP28 protein and its application for the detection of white spot syndrome virus in crustaceans. *J. Fish Dis.,* **27**, 517–522.

ZHAN W.B., WANG Y.H., FRYER J.L., YU K.K., FUKUDA H. & MENG Q.X. (1998). White Spot Syndrome Virus Infection of Cultured Shrimp in China. *J. Aquat. Anim. Health*, **10**, 405–410.

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* *

NB: There is an OIE Reference Laboratory for infection with white spot syndrome virus ~~disease~~
(see Table at the end of this *Aquatic Manual* or consult the OIE web site for the most up-to-date list:
http://www.oie.int/en/our-scientific-expertise/reference-laboratories/list-of-laboratories/).
Please contact the OIE Reference Laboratories for any further information on for infection with white spot syndrome virus ~~disease~~

NB: FIRST ADOPTED IN 1997. MOST RECENT UPDATES ADOPTED IN 2012.

Annex 24

CHAPTER 2.3.1.

INFECTION WITH EPIZOOTIC HAEMATOPOIETIC NECROSIS VIRUS

EU position

The EU supports the adoption of this modified chapter.

1. Scope

~~For the purpose of this chapter,~~ Infection with epizootic haematopoietic necrosis virus means ~~is considered to be systemic clinical or subclinical~~ infection ~~of finfish~~ with the pathogenic agent epizootic haematopoietic necrosis virus (EHNV) of the Genus *Ranavirus*, Family *Iridoviridae*.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent, agent strains

EHNV is a member of the genus *Ranavirus* in the Family *Iridoviridae* with the type species Frog virus 3 (FV3) (Chinchar *et al.,* 2005). Other species include Bohle virus (BIV), European catfish virus (ECV), European sheatfish virus (ESV) and Santee-Cooper ranavirus. Caution should be taken when speaking of ECV and ESV as two separate viruses because the scientific literature (Hyatt *et al.*, 2000) indicates they are isolates of the same virus. There are many other tentative species in this Genus. Ranaviruses have been isolated from healthy or diseased frogs, salamanders and reptiles in America, Europe and Australia (Chinchar*.,* 2002; Drury *et al.,* 2002; Fijan *et al.,* 1991; Hyatt *et al.*, 2002; Speare & Smith, 1992; Whittington *et al.*, 2010; Wolf *et al*., 1968; Zupanovic *et al*., 1998). Ranaviruses have large (150–180 nm), icosahedral virions, a double-stranded DNA genome 150–170 kb, and replicate in both the nucleus and cytoplasm with cytoplasmic assembly (Chinchar *et al.,* 2005). They possess common antigens that can be detected by several techniques.

Since the recognition of disease due to EHNV in Australia in 1986, similar systemic necrotising iridovirus syndromes have been reported in farmed fish. These include catfish (*Ictalurus melas*) in France (ECV) (Pozet *et al*., 1992), sheatfish (*Silurus glanis*) in Germany (ESV) (Ahne *et al.,* 1989; 1990), turbot (*Scophthalmus maximus*) in Denmark (Bloch & Larsen, 2009) and others in Finland (Ariel *et al.,* 1999).

EHNV and ECV are distinct viruses that can be differentiated using genomic analysis (Ahne *et al.,* 1998; Holopainen *et al*., 2009; Hyatt *et al*., 2000; Mao *et al*., 1996; 1997; Marsh *et al*., 2002). This enables epidemiological separation of disease events in finfish in Australia (EHNV) and Europe (ECV), and differentiation of these from ranavirus occurrences in frogs (FV3 and BIV). However, many ranavirus isolates have not been characterised to this level.

2.1.2. Survival outside the host

EHNV is extremely resistant to drying and, in water, can survive for months (Langdon, 1989). It can persist in frozen fish tissues for more than 2 years (Langdon, 1989) and frozen fish carcases for at least a year (Whittington *et al.*, 1996). For these reasons, it is presumed that EHNV would persist for months to years on a fish farm in water and sediment, as well as on plants and equipment.

2.1.3. Stability of the agent (effective inactivation methods)

EHNV is susceptible to 70% ethanol, 200 mg litre^{-1} sodium hypochlorite or heating to 60°C for 15 minutes (Langdon, 1989). Data for the inactivation of an amphibian ranavirus may also be relevant: 150 mg/litre chlorhexidine and 200 mg/litre potassium peroxymonosulphate were effective after 1 minute contact time (Bryan *et al*., 2009). If it is first dried, EHNV in cell culture supernatant is resistant to heating (Whittington *et al.*, 2010).

2.1.4. Life cycle

The route of infection is unknown but fish are susceptible experimentally following bath exposure. The virus infects a range of cell types including hepatocytes, haematopoietic cells and endothelial cells in many organs (Reddacliff & Whittington, 1996). Virus is shed into water from infected tissues and carcasses as they disintegrate.

2.2. Host factors

2.2.1. Susceptible host species

~~Natural EHNV infections are known from only two teleost species, redfin perch (*Perca fluviatilis*) and rainbow trout (*Oncorhynchus mykiss*) (Langdon, 1989; Langdon *et al.*, 1986; 1987; 1988), however, a number of other finfish species are susceptible to EHNV experimentally. Individuals of the following species have died after bath inoculation:~~ Species that fulfil the criteria for listing as susceptible to infection with EHNV according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) include: black bullhead (*Ameiurus melas*), crimson spotted rainbow fish (*Melanotaenia fluviatilis*), eastern mosquito fish (*Gambusia holbrooki*), European perch (*Perca fluviatilis*), Macquarie perch (*Macquaria australasica*), mosquito fish (*Gambusia affinis*), mountain galaxias (*Galaxias olidus*), northern pike (*Esox lucius*), pike-perch (*Sander lucioperca*), rainbow trout (*Oncorhynchus mykiss*) and silver perch (*Bidyanus bidyanus*).

~~Macquarie perch (*Macquaria australasica*), silver perch (*Bidyanus bidyanus*), mosquito fish (*Gambusia affinis*) and mountain galaxias (*Galaxias olidus*). Some species, for example goldfish (*Carassius auratus*) and common carp (*Cyprinus carpio*), are resistant (Langdon, 1989). European studies have shown that black bullhead (*Ameirus melas*) and pike (*Esox lucius*) are susceptible to EHNV by bath exposure (Bang Jensen *et al.*, 2009; Gobbo *et al.*, 2010).~~

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: none known.

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following organisms, but an active infection has not been demonstrated: Atlantic salmon (*Salmo salar*), freshwater catfish (*Tandanus tandanus*), golden perch (*Macquaria ambigua*), Murray cod (*Maccullochella peelii*) and purple spotted gudgeon (*Mogurnda adspersa*).

2.2.3~~2~~. Susceptible stages of the host

Susceptible stages of the host are all age classes of rainbow trout and ~~redfin~~ European perch.

2.2.4~~3~~. Species or subpopulation predilection (probability of detection)

Clinical signs are usually more obvious in fingerlings and juvenile fish than adults of both rainbow trout and ~~redfin~~ European perch.

2.2.5~~4~~. Target organs and infected tissue

Target organs and tissues infected with the virus are~~:~~ ~~liver,~~ kidney, spleen and liver ~~other parenchymal tissues~~. It is not known if EHNV can be detected in gonadal tissues, ovarian fluid or milt or whether these tissues are suitable for surveillance of broodstock.

2.2.65. Persistent infection ~~with lifelong carriers~~

2.2.65.1. Rainbow trout

The high case fatality rate and low prevalence of infection with EHNV ~~infection~~ in natural infections in rainbow trout means that the recruitment rate of carriers is likely to be very low (<2%) (Whittington et al., 1994). ~~Persistent infection with very small numbers of infectious virions was detected in one clinically healthy rainbow trout fingerling 63 days after intraperitoneal inoculation (Whittington & Reddacliff, 1995), but the significance of this observation is unclear because of the artificial route of infection.~~ EHNV has been detected in ~~grower~~ growout fish, but as histopathological lesions consistent with infection with EHNV were also present there was active infection rather than a carrier state (Whittington et al., 1999). ~~Too few broodstock samples have been examined to be certain that broodstock are not infected (Whittington et al., 1994).~~ Anti-EHNV serum antibodies were not detected in ~~0+~~ fingerlings during or after an outbreak but were detected in a low proportion of ~~1+ to 2+ grower~~ growout fish, hence, it is uncertain whether these were survivors of the outbreak (Whittington et al., 1994; Whittington et al., 1999). There are data for European stocks of rainbow trout in experimental infections where potential carriers were identified (Ariel & Bang Jensen, 2009).

2.2.65.2. ~~Redfin~~ European perch

This species is extremely susceptible to infection with EHNV ~~and it seems unlikely that it is a suitable reservoir host in Australia~~ (Whittington & Reddacliff, 1995). ~~However, there is some conflicting evidence.~~ EHNV~~, or a related ranavirus,~~ was isolated from 2 of 40 apparently healthy adult ~~redfin~~ European perch during epizootics in juveniles in Victoria, Australia (Langdon & Humphrey, 1987), but as the incubation period extends for up to 28 days (Whittington & Reddacliff, 1995), these fish may have been in the preclinical phase. Several ranavirus isolates have been obtained from ~~redfin~~ European perch in Victoria at times when there was no obvious epizootic, and some apparently healthy ~~redfin~~ European perch in Victoria had serum antibodies against EHNV or a related virus (Whittington & Hyatt, unpublished data~~). Furthermore, there are data for European stocks of redfin European perch in experimental infections where the virulence of EHNV appeared to be lower than in Australia (Ariel & Bang Jensen, 2009)~~.

~~#### 2.2.65.3. Murray cod~~

~~This species may be a suitable carrier as infection without disease has occurred after bath inoculation (Langdon, 1989).~~

~~#### 2.2.65.4. Rainbow trout and Atlantic salmon~~

~~These species may be a suitable carrier as infection without disease has occurred after intraperitoneal or bath inoculation (Langdon, 1989).~~

~~#### 2.2.65.5. Pike~~

~~This species may be a suitable carrier based on limited trials with fry (Bang Jensen et al., 2009).~~

2.2.76. Vectors

~~Since EHNV is a resistant virus, it may be transferred on nets, boats and other equipment, or in fish used for bait by recreational fishers.~~ Birds are potential ~~mechanical~~ vectors for EHNV, it being carried in the gut, on feathers, feet and the bill. ~~Piscivorous birds feed on affected juvenile redfin perch and the gastrointestinal contents of these birds may contain EHNV (Whittington et al., 1994). However, the virus is likely to may be inactivated at typical avian body temperatures (40–44°C), Nevertheless, the spread of EHNV by regurgitation of ingested material within a few hours of feeding is possible (Whittington et al., 1994).~~

~~### 2.2.87. Known or suspected wild aquatic animal carriers~~

~~None known.~~

Annex 24 (contd)

2.3. Disease pattern

2.3.1. Transmission mechanisms

Rainbow trout: EHNV has spread between rainbow trout farms by transfer of infected fingerlings and probably transport water (Langdon *et al.,* 1988; Whittington *et al.*, 1994; 1999). ~~It is assumed that consignments of fish contain a low proportion of individuals with progressive subclinical or clinical infection, rather than carrier fish.~~ The low prevalence of infection in rainbow trout means that active infection can easily go unrecognised in a population and be spread by trading fish. There are no data on possible vertical transmission of EHNV on or within ova, and disinfection protocols for ova have not been evaluated. EHNV has not yet been isolated from ovarian tissues or from broodstock. Annual recurrence in farmed rainbow trout may be due to reinfection of successive batches of fish from wild ~~redfin~~ European perch present in the same catchment.

~~Redfin~~ European *perch:* The occurrence of infection with EHNV in ~~redfin~~ European perch in widely separated river systems and impoundments, and its upstream progression, indicates that EHNV is spread by means other than water; mechanisms include translocation of live fish or bait by recreational fishers. ~~Redfin~~ European perch migrations in Australia are uncertain (see also Section 2.2.6 Vectors).

2.3.2. Prevalence

Rainbow trout: ~~the~~ clinical disease is generally difficult to ~~identify~~ detect with very low mortality rates and infection with EHNV may be present on a farm without causing suspicion. During outbreaks, EHNV has been detected by virus isolation in 60–80% of moribund or dead fish, but in only 0–4% of in-contact, clinically healthy fish. The 99% confidence limits for the prevalence of subclinical infection are 0–8% (Whittington *et al.*, 1994; 1999). The virus could not be found at all in surviving cohorts after an outbreak. Anti-EHNV antibodies were detected in ~~grower~~ growout fish at low prevalence (0.7%, 95% confidence limits 0.02% to 3.7%).

~~Redfin~~ European *perch:* the disease is recognised by epizootic mortality in fish of any age affecting a very large proportion of the population with dramatic population decline (Langdon *et al.,* 1986; 1987; Whittington *et al.*, 1996). Typically, fingerling and juvenile fish are affected in endemic areas, but in newly infected areas adults are also affected. When the disease is first recognised in an area there is a dramatic population decline (Langdon *et al.,* 1986; 1987; Whittington *et al.*, 1996).

The studies above were conducted prior to the availability of real-time PCR assays, which may have greater diagnostic sensitivity and reveal higher prevalence in subclinically infected populations.

2.3.3. Geographical distribution

Rainbow trout: infection with EHNV is known only from fish farms located in the Murrumbidgee and Shoalhaven river catchments in New South Wales, Australia (Whittington *et al*., 2010). ~~However, some farms within this region have remained free of the disease (Whittington *et al*., 1999).~~

~~Redfin~~ European *perch:* infection with EHNV is endemic in south-eastern Australia, but there is a discontinuous distribution (Whittington *et al*., 2010). The ~~disease~~ infection occurs in many small and large impoundments in Victoria and since 1986 has spread progressively upstream in the Murrumbidgee river catchment through New South Wales and the Australian Capital Territory. Similar spread has been observed in the Murray River in South Australia (Whittington *et al.*, 1996).

2.3.4. Mortality and morbidity

Rainbow trout: It appears that under natural conditions EHNV is poorly infective but has a high case fatality rate. Infection with EHNV may be present on a farm without causing suspicion because the mortality rate may not rise above the usual background rate. Infection with EHNV has most often been reported in young fingerlings <125 mm fork length with daily mortality of less than 0.2% and total mortality of up to 4%. However, rainbow trout of all ages may be susceptible, although infection has not yet been seen in broodstock (Whittington *et al.*, 1994; 1999). There is a low direct economic impact because of the low mortality rate. ~~In keeping with the natural pattern of disease, rainbow trout were resistant to bath exposure in $10^{2.2}$ $TCID_{50}$ (50% tissue culture infective dose) ml^{-1} (Whittington & Reddacliff, 1995), while only 1 of 7 became infected after bath inoculation for 1 hour in 10^{3} $TCID_{50}$ ml^{-1} (Langdon *et al*., 1988).~~ Differences in susceptibility between European and Australian stocks of rainbow trout may exist (Ariel & Bang Jensen, 2009).

~~Redfin~~ European *perch:* There is a very high rate of infection and mortality in natural outbreaks that, over time, leads to loss in wild fish populations (Langdon *et al.,* 1986; 1987; Whittington *et al.*, 1996). Experimental bath inoculation with as few as 0.08 $TCID_{50}$ ml^{-1} was lethal, and doses too low to be detected by virus isolation in BF-2 cells were fatal by intraperitoneal inoculation (Whittington & Reddacliff, 1995). Differences in susceptibility between European and Australian stocks of ~~redfin~~ European perch may exist (Ariel & Bang Jensen, 2009).

2.3.5. Environmental factors

Rainbow trout: ~~Natural~~ Outbreaks appear to be related to poor husbandry, particularly overcrowding, inadequate water flow and fouling of tanks with feed. ~~Water quality parameters are suboptimal, and intercurrent diseases, including skin diseases caused by protozoa and fungi, and systemic bacterial infection are common.~~ Damage to skin may provide a route of entry for EHNV. Outbreaks have been seen on farms at water temperatures ranging from 11 to 20°C (Whittington *et al.*, 1994; 1999). The incubation period after intraperitoneal inoculation was 3–10 days at 19–21°C compared with 14–32 days at 8–10°C (Whittington & Reddacliff, 1995).

~~Redfin~~ European *perch:* Natural epizootics of infection with EHNV affecting juvenile and adult ~~redfin~~ European perch occur mostly in summer (Langdon *et al.,* 1986; 1987; Whittington *et al.*, 1994). It has been assumed that the disease in juvenile fish is related to the annual appearance of large numbers of non-immune young fish and their subsequent exposure to the virus while schooling in shallow waters; adults are uncommonly involved in these outbreaks. It is possible that environmental temperature is the trigger for outbreaks as juvenile fish feed in warm shallow waters on planktonic fauna, whereas adults feed on benthic invertebrates and larger prey in deeper cooler water (Whittington & Reddacliff, 1995). Experimentally the incubation period ranged from 10 to 28 days at 12–18°C compared with 10–11 days at 19–21°C, and adult perch were refractory to infection at temperatures below 12°C (Whittington & Reddacliff, 1995). European stocks of ~~redfin~~ European perch also displayed temperature-dependent susceptibility (Ariel & Bang Jensen, 2009).

2.4. Control and prevention

2.4.1. Vaccination

None available.

2.4.2. Chemotherapy

None available.

2.4.3. Immunostimulation

Not tested.

2.4.4. Resistance breeding

There has been no formal breeding programme for resistant strains of susceptible species. However, experimental trials using a bath exposure have shown that European perch from water bodies in New South Wales, Australia with previous EHNV infections showed lower mortality compared with European perch from neighbouring and distant water bodies in Australia that have no previous history of EHNV (Becker *et al.,* 2016). ~~Not tested.~~

2.4.5. Restocking with resistant species

Not tested.

2.4.6. Blocking agents

Not tested.

2.4.7. Disinfection of eggs and larvae

Not tested.

2.4.8. General husbandry practices

Disease control in rainbow trout at the farm level relies on reducing the impact of infection by maintaining low stocking rates and adequate water quality. ~~The mechanism of protection may be through maintenance of healthy integument.~~

Investigations on one rainbow trout farm indicated that ponds with high stocking rates and low water flow, and thus poorer water quality, may result in higher levels of clinical disease compared with ponds on the same farm with lower stocking rates and higher water flow (Whittington *et al.,* 1994). ~~Disease control in rainbow trout at the farm level relies on reducing the impact of infection by maintaining low stocking rates and adequate water quality.~~ The mechanism of protection may be through maintenance of healthy integument (Whittington *et al.,* 1994).

3. Sampling

3.1. Selection of individual specimens

A simple method for preparation of fish tissues for cell culture and enzyme-linked immunosorbent assay (ELISA) has been validated (Whittington & Steiner, 1993).

Bath large fish for 30 seconds in 70% ethanol; bath fingerlings for 5 seconds in 70% ethanol then rinse in sterile water. Dissect fish aseptically in a Class II biosafety cabinet.

Large fish (*>60 mm fork length*): remove 0.1 g liver, kidney, spleen (± other organs in specific situations) and place in sterile 1.5 ml tubes. Tubes suitable for use with pestles for grinding tissues (see below) are available, but standard 1.5 ml tubes may be suitable. In some situations liver, kidney and spleen may be pooled in a single tube (see Section 3.3).

Medium fish (*30–60 mm fork length*): scrape all viscera into the tube.

Small fish (*<30 mm fork length*): remove head and tail, place rest of fish into the tube.

3.2. Preservation of samples for submission

For cell culture and ELISA, freeze tubes containing tissues at –20°C to –80°C until needed.

For light microscopic examination, fix tissues in 10% neutral buffered formalin.

3.3. Pooling of samples

The effect of pooling tissues from multiple fish on the sensitivity of diagnostic tests has not been evaluated. ~~However, tissues for virus isolation are commonly pooled in lots of 5 or 10 individual fish per test.~~

3.4. Best organs or tissues

Liver, anterior kidney, spleen.

3.5. Samples/tissues that are not suitable

Inappropriate tissues include gonads, gonadal fluids, milt and ova, since there is no evidence of reproductive tract infection.

4. Diagnostic methods

4.1. Field diagnostic methods

4.1.1. Clinical signs

There are no specific clinical signs. Fish are found dead. There may be clinical evidence of poor husbandry practices, such as overcrowding and suboptimal water quality manifesting as skin, fin and gill lesions (Reddacliff & Whittington, 1996).

4.1.2. Behavioural changes

Moribund fish may have loss of equilibrium, flared opercula and may be dark in colour (Reddacliff & Whittington, 1996).

4.2. Clinical methods

4.2.1. Gross pathology

There may be no gross lesions or nonspecific lesions on the skin, fins and gill. A small proportion of fish may have enlargement of kidney, liver or spleen. There may be focal white to yellow lesions in the liver corresponding to areas of necrosis (Reddacliff & Whittington, 1996).

4.2.2. Clinical chemistry

Not applicable.

4.2.3. Microscopic pathology

Acute focal, multifocal or locally extensive coagulative or liquefactive necrosis of liver, haematopoietic kidney and spleen are commonly seen in routine haematoxylin and eosin (H&E)-stained sections of formalin-fixed material. A small number of basophilic intracytoplasmic inclusion bodies may be seen, particularly in areas immediately surrounding necrotic areas in the liver and kidney. Necrotic lesions may also be seen in heart, pancreas, gastrointestinal tract, gill and pseudobranch (Reddacliff & Whittington, 1996).

4.2.4. Wet mounts

Not applicable.

4.2.5. Smears

Not tested.

4.2.6. Electron microscopy/cytopathology

Affected tissues (e.g. kidney liver and spleen) contain cells exhibiting necrosis. Cells contain conspicuous cytoplasmic inclusions that are rarefied areas of the cytoplasm in which the viruses are assembled. Within the cytoplasm, aggregates (paracrystalline arrays) of large (175 nm ± 6 nm) nonenveloped icosahedral viruses are apparent; single viruses are also present. Complete viruses (containing electron-dense cores) bud/egress from the infected cells through the plasma membrane. The nuclei of infected cells are frequently located peripherally and are distorted in shape.

4.3. Agent detection and identification methods

4.3.1. Direct detection methods

4.3.1.1. Microscopic methods

Light microscopy: routine methods can be used for tissue fixation, such as in 10% buffered neutral formalin, paraffin embedding, preparation of 10 µm sections and staining with H&E to demonstrate tissue necrosis and basophilic intracytoplasmic inclusion bodies. These inclusion bodies are indicative but not confirmatory for infection with EHNV. Formalin-fixed paraffin-embedded sections can also be stained using an immunoperoxidase method (see below) to identify EHNV antigen associated with necrotic lesions.

Electron microscopy: Ultrathin routine sectioning methods can be used for preparation of tissues and cell cultures (Eaton *et al.,* 1991) to demonstrate tissue necrosis, presence of viruses and virus inclusion bodies. Tissues and cells fixed with an alternative fixation and embedding regime can be used for antigen detection (Hyatt, 1991).

Negative contrast electron microscopy: supernatants from dounce homogenised tissues (10% [w/v]) and cell cultures can be used to detect viruses. Ranaviruses have a definitive appearance. They vary in diameter (150–180 nm) and have a limiting cell-derived (plasma membrane) envelope that surrounds a capsid of skewed symmetry. Underlying the capsid is a *de novo* membrane that itself surrounds a core containing the double-stranded (ds) DNA and minor proteins. These preparations can also be used to confirm ranavirus antigenicity (Eaton *et al.,* 1991).

4.3.1.1.1. Wet mounts

Not applicable.

4.3.1.1.2. Smears

Not applicable.

4.3.1.1.3. Fixed sections

See Section 4.3.1.1 on microscopic methods.

4.3.1.2. Agent isolation and identification

4.3.1.2.1. Cell culture/artificial media

Preparation of fish tissues for virus isolation and ELISA

A simple method for preparation of fish tissues for cell culture and ELISA has been validated (Whittington & Steiner, 1993) (see sampling Section 3.1).

i) Freeze tubes containing tissues at –80°C until needed.

ii) Add 0.5 ml of homogenising medium (minimal essential medium Eagle, with Earle's salts with glutamine] [MEM] with 200 International Units [IU] ml^{-1} penicillin, 200 µg ml^{-1} streptomycin and 4 µg ml^{-1} amphotericin B) to each tube. Grind tissue to a fine mulch with a sterile fitted pestle.

iii) Add another 0.5 ml of homogenising medium to each tube and mix with a pestle.

iv) Add three sterile glass beads to each tube (3 mm diameter) and close the lid of the tube.

v) Vortex the suspension vigorously for 20–30 seconds and place at 4°C for 2 hours.

vi) Vortex the suspension again as above and centrifuge for 10 minutes at 2500 ***g*** in a benchtop microcentrifuge.

vii) Transfer the supernatant, now called clarified tissue homogenate, to a fresh sterile tube. Homogenates may be frozen at –80°C until required for virus isolation and ELISA.

Cell culture/artificial media

~~Cell culture is the gold standard test but is costly and time consuming.~~ EHNV grows well in many fish cell lines including BF-2 (bluegill fry ATCC CCL 91), FHM (fathead minnow; ATCC CCL 42), EPC (epithelioma papulosum cyprini [Cinkova *et al.,* 2010]), and CHSE-214 (Chinook salmon embryo cell line; ATCC CRL 1681) at temperatures ranging from 15 to 22°C (Crane *et al.,* 2005). Incubation temperatures of 20°C or 24°C result in higher titres than 15°C; 22°C and BF-2 EPC or CHSE-214 cells are recommended to maximise titres, which might be important for the detection of low numbers of viruses in fish tissues (Ariel *et al.,* 2009). BF-2 cells are preferred by the OIE Reference Laboratory with an incubation temperature of 22°C ~~both before and after inoculation with virus has been recommended for many years~~. The procedure for BF-2 cells is provided below. A procedure for CHSE-214 cells is provided under immunoperoxidase staining below (Section 4.3.1.2.2). The identity of viruses in cell culture is determined by immunostaining, ELISA, immuno-electron microscopy, PCR or other methods.

Samples: tissue homogenates.

Cell culture technical procedure: cells are cultured (in flasks, tubes or multi-well plates) with growth medium (MEM + 10% fetal calf serum [FCS] with 100 IU ml^{-1} penicillin, 100 µg ml^{-1} streptomycin and 2 µg ml^{-1} amphotericin B). The cells are incubated until almost confluent at 22°C, which can take up to 4 days depending on the seeding rate. Medium is changed to a maintenance medium (MEM with 2% FCS and 100 IU ml^{-1} penicillin, 100 µg ml^{-1} streptomycin and 2 µg ml^{-1} amphotericin B) on the day of inoculation. A 1/10 dilution using homogenising medium is made of single or pooled homogenates. Each culture is inoculated with 100 µl of sample per ml of culture medium. This represents a final 1/100 dilution of a 0.1 mg ml^{-1} tissue homogenate. A further 1/10 dilution is made representing a final 1/1000 dilution, and two cultures are inoculated. No adsorption step is used. As an alternative, two to three cultures can be inoculated directly with 10 µl undiluted homogenate per ml of culture medium. Note that a high rate of cell toxicity or contamination often accompanies the use of a large undiluted inoculum. The cultures are incubated at 22°C in an incubator for 6 days. Cultures are read at day 3 and day 6. Cultures are passed at least once to detect samples with low levels of virus. On day 6, the primary cultures (P1) are frozen overnight at –20°C, thawed, gently mixed and then the culture supernatant is inoculated onto fresh cells as before (P2), i.e. 100 µl P1 supernatant per ml culture medium. Remaining P1 supernatants are transferred to sterile 5 ml tubes and placed at 4°C for testing by ELISA or PCR or another means to confirm the cause of cytopathic effect (CPE) as EHNV. P2 is incubated as above, and a third pass is conducted if necessary.

Interpretation of results

CPE is well developed and consists of focal lysis surrounded by rounded granular cells. This change extends rapidly to involve the entire monolayer, which detaches and disintegrates.

4.3.1.2.2. Antibody-based antigen detection methods

It should be noted that polyclonal antibodies used in all related methods (immunoperoxidase, antigen-capture ELISA and immunoelectron microscopy) cross-react with all known ranaviruses except Santee Cooper ranaviruses (Ahne *et al.,* 1998; Cinkova *et al.,* 2010; Hedrick *et al*., 1992; Hyatt *et al*., 2000).

4.3.1.2.2.1. Detection of EHNV using immunocyctochemistry ~~immunoperoxidase staining~~ of infected cell cultures

Principle of the test: EHNV replicates within cultured cells. The addition of a mild detergent permeabilises the cells allowing an affinity purified rabbit antibody to bind to intracellular viral proteins. EHNV is detected by a biotinylated anti-species antibody and a streptavidin–peroxidase conjugate. The addition of a substrate results in 'brick-red' staining in areas labelled with antibodies.

Samples: tissue homogenates.

Operating characteristics: when performed as described in this protocol, the staining is conspicuous and specific. However, the test has not been validated with respect to sensitivity or reproducibility.

Preparation of cells: the procedure described below is for CHSE-214 cells. Other recommended cell lines can also be used.

i) CHSE-214, 24-well plates are seeded the day before use with 250,000 cells/well (or 4 million cells in 40 ml of growth medium per plate) in 1.5 ml of growth medium (Earle's MEM with non-essential amino acids [EMEM], 10% FCS, 10 mM N-2-hydroxyethyl-piperazine-N-2-ethanesulfonic acid [HEPES], 2 mM glutamine, 100 IU penicillin and 100 µg streptomycin) and incubated in 5% CO_2 at 22°C overnight. (NOTE: cultures must be nearly confluent and have healthy dividing cells prior to use.)

ii) Discard the medium, inoculate each well with 150 µl of a 10% suspension of ground tissue (e.g. liver, kidney or spleen), incubate for 1 hour (22°C) then add 1.5 ml of fresh maintenance medium (as for growth medium except 2% FCS) and return to the incubator (22°C).

iii) Observe cultures for CPE. If no CPE occurs by day 10, pass the cultures on to fresh CHSE cells by collecting the cells and medium and adding 150 µl to the cells of the fresh plate; note that cells are not freeze–thawed. There is no need to discard the existing medium, just return the new plate to the incubator (22°C). Again, observe daily for CPE.

iv) Fix cells (add 50 µl for 96-well plate cultures with 200 µl culture medium/well or 400 µl (for 24-well plate cultures with 1.6 ml culture medium/well) of a 20% formalin solution to each well), without discarding the culture medium when CPE is first observed. After incubation (22°C) for 1 hour at room temperature (RT), the medium/formalin mixture is discarded and the wells are rinsed twice with PBS-A (phosphate buffered saline, Ca^{++} and Mg^{++} free) to remove the formalin. More PBS-A is added if the plates are to be stored at 4°C.

Protocol

i) Dilute primary anti-EHNV antibody and normal serum to working strength as described below (fixation protocol for immunocytochemistry) for the relevant agent in 1% skim milk (SM) solution (PBS-A [SM]) to the volume required for the test.

ii) Remove PBS-A from wells (with fixed cell cultures) and wash wells twice with 0.05% (v/v) PBS/Tween 20 (PBST). Add 50 µl of primary antibody solutions to each well in a 96-well plate well or 200 µl in a 24-well plate well. Incubate on a plate shaker at 100–200 rpm at RT (22–24°C) for 15–30 minutes or without shaking at 37°C for 1 hour.

iii) Dilute biotinylated anti-species serum (secondary antibody) in 0.1% SM solution as described in the fixation protocol (below) for the relevant agent to the volume required for the test.

iv) Remove primary antibody solution and wash wells three times with PBST. Add secondary antibody to all wells. Incubate on a plate shaker at 100–200 rpm at RT for 15–30 minutes or without shaking at 37°C for 1 hour.

v) Dilute streptavidin–peroxidase conjugate in 0.1% SM solution for the relevant agent to the volume required for the test.

vi) Remove secondary antibody from wells and wash wells three times with PBST. Add conjugate to each well. Incubate on a plate shaker at 100–200 rpm at RT for 15–30 minutes or without shaking at 37°C for 1 hour.

vii) Prepare stock substrate of 3-amino-9-ethylcarbazole (AEC) solution: dissolve one AEC tablet (20 mg) in 2.5 ml of dimethyl formamide.

viii) Remove conjugate from wells. Wash (three times) with PBST.

ix) Dilute dissolved AEC stock in 47.5 ml of acetate buffer (4.1 ml anhydrous sodium acetate in 1 litre of deionised water; the pH is adjusted to 5.0 with glacial acetic acid). Just before use, add 25 µl 30% hydrogen peroxide to AEC solution then add to each well. Incubate at RT for 20 minutes.

x) Remove substrate solution and wash wells twice with deionised water to stop reaction.

xi) To visulise all cells counterstain with Mayer's haematoxylin (50 µl/well or 200 µl/well) for 1 minute and rinse with deionised water.

xii) Add 50 µl Scott's tap water and rinse with deionised water and air dry.

Interpretation of the results

Positive reaction: granular-like, focal, brick-red staining of cells indicates presence of virus identified by the diagnostic antibody.

Negative reaction: no red staining apparent – all cells should be stained pale blue due to counterstain.

Background staining: nongranular, nonfocal, more generalised, pale, pinkish staining may occur throughout the culture. This background staining could be caused by any number of reasons, e.g. nonspecific antibody reaction with nonviral components, inefficient washing, and expiration of other reagents.

Reagents for immunocytochemistry tests

20% Formaldehyde (*PBS-A*) *saline*

Formalin (36–38% formaldehyde)	54 ml
Distilled water	36 ml
10 × PBS-A	10 ml

10 × PBS-A

To make up 1 litre of 10 × PBS-A use:

NaCl	80.0 g
Na_2HPO_4	11.5 g
KCl	2.0 g
KH_2PO_4	2.0 g
Distilled water	1.0 litre

NOTE: some salts are supplied with extra water groups. If using these reagents adjust the masses to ensure the appropriate mass of salt is added, e.g. for $Na_2HPO_4.2H_2O$ add 15 g instead of 11.5 g (156 mw/120 mw × 11.5 g = 14.95 g) to remove the effect of the water molecules.

4.3.1.2.2.2 Detection of EHNV using antigen-capture ELISA

Antigen-capture ELISA has been validated to detect EHNV in cell cultures and directly in fish tissue homogenates. The analytical sensitivity is 10^3 to 10^4 $TCID_{50}$ ml^{-1}. Specificity approaches 100% and sensitivity for direct detection in fish tissues is 60% relative to ~~the gold standard of~~ virus isolation in BF-2 cells (Hyatt *et al.*, 1991; Whittington & Steiner, 1993)~~ and unpublished data)~~. ~~ELISA is useful for both diagnosis and certification.~~ Neutralisation tests cannot be used to identify EHNV because neutralising antibodies are not produced following immunisation of mammals or fish. Mouse monoclonal antibodies produced against EHNV are directed against major capsid protein (MCP) epitopes~~ and are non neutralising (unpublished data)~~. Rabbit-anti-EHNV antibodies have been developed for use in antigen-capture ELISA, immunoperoxidase staining and immunoelectron microscopy (Hengstberger *et al.,* 1993; Hyatt *et al.*, 1991; Reddacliff & Whittington, 1996). Reagents and protocols are available from the reference laboratory.

Samples: tissue homogenate samples prepared using ~~a validated~~ the protocol (see below), and cell cultures.

Principle of the test: EHNV particles are captured from the sample by an affinity purified rabbit antibody that is coated to the plate. EHNV is detected by a second antibody and a peroxidase-labelled conjugate using the chromogen ABTS (2,2'-azino-di-[3-ethyl-benzthiazoline]-6-sulphonic acid). The enzyme is inactivated after 20 minutes and the resulting optical density (OD) is compared with standards.

Operating characteristics: the protocol is based on published procedures (Hyatt *et al.*, 1991; Steiner *et al*., 1991; Whittington, 1992; Whittington & Steiner, 1993). When performed as described in this protocol, the operating characteristics of the test are as given in Table 4.1. The precision of the assay is <10% coefficient of variation, measured as variation in the OD of the controls between plates and over time, when the recommended normalisation procedure is followed.

Annex 24 (contd)

Table 4.1. *EHNV ELISA operating characteristics compared with the ~~gold standard of cell culture~~ virus isolation in BF-2 cells*

Sample	Positive-negative cut-off**	Sensitivity %	Specificity %
Tissues of fish*	OD 0.5	60	>99
Tissue culture supernatants with cytopathic effect (BF2 cells)	OD 0.3	>99	>99

*~~Redfin~~ European perch and rainbow trout only. Higher background OD occurs with golden perch. There are no data for other species.

** these cut-offs are determined by the OIE Reference Laboratory for EHNV and will vary with the batch of control antigen. Values above are for batch 86/8774-4-5-01.

Test components and preparation of reagents

i) Flat bottom microtitre plates are required.

ii) Affinity purified rabbit anti-EHNV immunoglobulin and sheep anti-EHNV antiserum reagents are supplied in freeze-dried form. Reconstitute using 1 ml of purified water and allow the vial to stand at RT for 2 minutes. Mix the vial very gently. These reagents are stable when stored at –20°C for at least 4 years. For routine use in ELISA, it is recommended that working stocks of both antibodies be prepared as a 1/10 dilution in Tris saline glycerol merthiolate (TSGM; formula at end of this section). These are stable at –20°C for at least 5 years and do not solidify at this temperature.

iii) The peroxidise labelled anti-sheep immunoglobulin conjugate (commercial reagent, KPL #14-23-06; 0.5 mg) is supplied as a freeze-dried powder. This reagent has displayed remarkable consistency in activity between different lots over a period of 15 years. The product should be reconstituted in sterile 50% glycerol water, dispensed in 150 µl aliquots and stored at –20°C as undiluted stock. A working stock is prepared by adding 900 µl of TSGM to 100 µl of undiluted stock. The working stock is also stored at –20°C and is stable for at least 1 year. New batches of this conjugate should be titrated against an older batch using standard protocols.

iv) EHNV control antigen, heat-inactivated, is supplied as freeze-dried powder. Reconstitute in 1 ml sterile water and store in small aliquots at –20°C. Prepare dilutions using PBSTG (PBS + Tween + gelatin) on the same day the test is performed. Control EHNV antigen dilutions (A, B, D and F) cover the range of the signal response of the assay and enable a normalisation procedure to be undertaken.

Equipment

An automatic plate washer is recommended although plates can be washed by hand. The assay is sensitive to plate washing conditions. If the OD of the controls is unexpectedly low, and the conjugate and other reagents are within date, the plate washer should be adjusted so that washing pressure during filling of wells and aspiration of wells is minimised.

An automatic plate reader is recommended although plates can be read by eye.

Precision calibrated pipettes (e.g. Gilson) should be used to prepare dilutions of all reagents and to load reagents into microtitre plate wells.

Protocol

i) Coat a 96-well ELISA plate (100 µl/well) with affinity purified rabbit-anti-EHNV diluted 1/12,800 in borate coating buffer. Incubate overnight at 4°C.

ii) Wash plate five times with wash buffer (Milli-Q [MQ] purified water plus 0.05% Tween 20). Note that distilled and deionised water can also be used in this and all other steps.

iii) Prepare a blocking solution: warm the solutions in a microwave oven or water bath to dissolve the gelatin, then cool to RT.

iv) Block remaining binding sites using blocking solution (100 µl/well) (1% [w/v] gelatin in PBSTG diluent [PBS, 0.05% [v/v] Tween 20, 0.1% [w/v] gelatin]). Incubate at RT for 30 minutes.

v) Wash plate five times as above.

vi) Work in a Class II biological safety cabinet. Dilute the control antigen (see below) in PBSTG and add to the lower right-hand corner of the plate. Add tissue homogenate samples or culture supernatant samples and control antigens at 100 µl/well. All samples and controls are added to duplicate wells. Incubate for 90 minutes at RT.

The control antigens are dilutions of a heat killed cell culture supernatant of EHNV 86/8774. The controls are expected to give the following OD, although there will be some variation from laboratory to laboratory and ±10% variation should therefore be allowed:

Control	Dilution in PBS*	OD (405 nm)*
A	1/5	>2.0
B	1/40	1.90
D	1/200	0.68
F	1/3000	0.16

*These dilutions and OD values are determined by the OIE Reference Laboratory for infection with EHNV and will vary with the batch of control antigen. The values above are for batch 86/8774-4-5-01. The positive-negative cut-off for clarified tissue homogenate samples from ~~redfin~~ European perch and rainbow trout in this ELISA is approximated by the OD value of control D on each plate.

vii) Wash the plate by hand to avoid contamination of the plate washer. Work in a Class II cabinet. Aspirate wells using a multichannel pipette. Rinse the plate twice.

viii) Wash the plate five times on the plate washer, as above.

ix) Add the second antibody sheep-anti-EHNV diluted 1/32,000 in PBSTG (100 µl/well). Incubate for 90 minutes at RT.

x) Wash the plate five times on the plate washer.

xi) Add the conjugate diluted 1/1500 in PBSTG (100 µl/well). Incubate for 90 minutes at RT.

xii) Wash the plate five times on the plate washer.

xiii) Add ABTS substrate (22 ml ABTS + 10 µl H_2O_2) (100 µl/well) and place the plate on a plate shaker. Time this step from the moment substrate is added to the first wells of plate 1. Incubate for 20 minutes.

xiv) Immediately add ABTS stop solution (50 µl/well), shake the plate briefly and read OD at 405 nm. Calculate mean ELISA OD of duplicate wells. Calculate the coefficient of variation of the duplicates: samples with CV >15% should be retested if the mean OD lies near the positive-negative cut-off.

Normalisation of data and decision limit quality control

If it is desired to normalise data from plate to plate and over time, or to undertake decision limit quality control, the following procedure can be followed. Run control antigens in ELISA on at least five occasions over a period of 3 weeks (a total of 20 separate ELISA plates). Calculate the mean OD for each control antigen. Then, for each plate subsequently used, calculate a plate correction factor (PCF) as follows:

PCF = (mean OD control A/actual OD + mean OD control B/actual OD + mean OD control D/actual OD + mean OD control F/actual OD)/4. Multiply the actual mean OD of each sample by the PCF for that plate and report these values.

PCF is allowed to vary between 0.8 and 1.2, which approximates to a coefficient of variation of 10%. Values outside this range suggest that a plate needs to be retested. Plots of PCF over time provide a ready means for monitoring the stability of reagents, procedural variations and operator errors. This QC method has been validated for antigen capture ELISA.

Annex 24 (contd)

Buffers and other reagents

Borate coating buffer

Boric acid	6.18 g
Disodium tetraborate ($Na_2B_4O_7.10H_2O$)	9.54 g
NaCl	4.38 g
MQ water to	1 litre
Autoclave	

10 × phosphate buffered saline

NaCl	80.00 g
KCl	2.00 g
Na_2HPO_4	11.50 g
KH_2PO_4	2.00 g
MQ water to	900 ml
Adjust pH to 7.2 with HCl or NaOH; make up to 1 litre	
Autoclave	

For working strength dilute 1/10 and recheck pH.
For storage of powder in jars, make up twice the above quantity of powder; store; to make up add 1.8 litres MQW, pH, make up to 2 litres.

ABTS

Citrate phosphate buffer	
Citric acid	21.00 g
Na_2HPO_4	14.00 g
MQ water to 800 ml; adjust pH to 4.2; make up to 1 litre	
ABTS	0.55 g
Citrate phosphate buffer to	1 litre
Dispense in 22-ml aliquots and freeze.	
Immediately prior to use add 10 µl H_2O_2 per 22-ml aliquot.	

ABTS stop solution (0.01% NaN_3 in 0.1 M citric acid)

Citric acid	10.5 g
MQW to	500 ml
Add 50 mg sodium azide or 1 ml of 5% solution.	

KPL Conjugate #14-23-06[2]

TSGM cryoprotectant

10 × Tris/saline, pH 7.4	50 ml
Glycerol	250 ml
Sterile purified water to	500 ml
Autoclave	
Add 10% Merthiolate	1 ml
Store in dark bottle at 4°C.	

10 × Tris/saline (250 mM Tris, 1.5 M NaCl)

Tris	15.14 g
NaCl	43.83 g
Sterile purified water	500 ml
pH adjust to	7.4

2 Reagent Supplier: Bio-Mediq DPC Australia, P.O. Box 106, Doncaster, Victoria 3108, Australia; Tel.: (+61-3) 9840 2767; Fax: (+61-3) 9840 2767. Visit: www.kpl.com for links to worldwide network distributors. 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*.

4.3.1.2.2.3. Immunoelectron microscopy

Gold-labelling of sections containing tissues or cell cultures

Principle of the test: cell cultures, tissues and/or tissue homogenates can be used for examination by electron microscopy. Conventional electron microscopy (examination of ultra-thin sections) will generate data on virus structure and morphogenesis. Negative contrast electron microscopy will produce images that can be used to examine the particulate structure of the virus. The use of ranavirus-specific antibodies and conjugated gold with these preparations permits both ultrastructure and antigenicity to be examined (Hyatt, 1991). These collective data enable classification to the genus Ranavirus.

Cell cultures and tissues

i) Fix tissues or cell cultures as described in Drury *et al.,* 2002. Briefly, 2.5% (v/v) buffered glutaraldehyde (cacodylate or phosphate) is used to fix cells for 40 minutes. Following primary fixation the cells are rinsed in the same buffer (3 × 20 minutes), post-fixed in 1% (w/v) buffered osmium tetroxide (1 hour), washed (3 × 5 minutes) in double-distilled/reverse osmosis (RO) water, dehydrated through graded alcohol (70–100%) and infiltrated and embedded in an epoxy resin (e.g. Spurrs or epon). For gold labelling of ultra-thin resin sections, attention must be given to fixation and embedding regimes. For example, cells should be fixed in 0.25% (v/v) glutaraldehyde with 2–4% paraformaldehyde. No secondary fixation is used and the cells are infiltrated and embedded in an acrylic resin such as LR White.

ii) Following fixation and embedding, cut and transfer ultrathin sections onto filmed nickel grids.

iii) Cut sections from the appropriate blocks.

iv) Block in 2% (w/v) skim milk powder in PBS-A (10 minutes).

v) Block free aldehydes with 0.1 M glycine in PBS-A (20 minutes).

vi) Wash in PBS-A (3 × 1 minutes). This is an optional step used only if there is an excess of free aldehydes (a high background may be indicative of this).

vii) If protein A-gold is not being used then block in normal species serum – this serum should be homologous to that complexed to gold. Recommended dilution is approximately 1/40 (10 minutes).

viii) Incubate in primary antibody. If incubation details are unknown then perform initial reactions with 1/100 to 1/2700 dilutions (with three-fold dilutions). Dilute antibodies in 1% (v/v) cold water fish gelatin in PBS-A, (60 minutes, RT).

ix) Rinse in 1% (v/v) coldwater fish gelatin in PBS-A, (6 × 3 minutes).

x) Incubate in gold-labelled secondary antibody or protein A-gold or protein G-gold. Suggested dilution 1/40 in a PBS-A containing 1% (w/v) bovine serum albumin (BSA), 0.1% (v/v) Tween 20 and 0.1% (v/v) Triton X, 60 minutes, RT.

xi) Rinse in PBS-A (6 × 3minutes, RT).

xii) Post-fix in 2.5% (v/v) glutaraldehyde in PBS-A (5 minutes, RT).

xiii) Rinse in water (RO) (3 × 3 minutes, RT).

xiv) Dry on filter paper (type not critical).

xv) Stain in uranyl acetate and lead acetate.

Interpretation of results

Viruses within the cytoplasm of infected cells will be specifically gold-labelled. Viruses will be located singularly, within assembly bodies (inclusion bodies) and within paracrystalline arrays.

Gold-labelling of virus particles (viruses adsorbed to grids)

i) Dounce homogenise 10% (w/v) liver, kidney or spleen and clarify (5 minutes, 2500 ***g***).

ii) Adsorb the supernatant (from homogenate or cell cultures) to grid substrate.

iii) Use carbon-coated 200 mesh gold grids.

iv) Fix the sample with 0.1% (v/v) glutaraldehyde and 1% (v/v) Nonidet P40 (NP40) in PBS (2 minutes).

v) Wash in PBS (3 × 3 minutes).

vi) Block with 5% (v/v) cold water fish gelatin (Sigma) in PBS (10 minutes) followed with incubation buffer (PBS/0.1% cold water fish gelatin).

vii) Incubate with antibody (affinity purified rabbit anti-EHNV, Lot No. M708; supplied by the OIE Reference Laboratory; suggested dilution 1/500) for 1 hour, at RT.

viii) Wash grids (6 × 3 minutes) in incubation buffer.

ix) Incubate with 10 nm protein A-gold (for dilution, refer to suppliers recommendation) for 1 hour, at RT.

x) Wash (6 × 3 minutes).

xi) Fix with 2.5% glutaraldehyde (5 minutes).

xii) Wash with distilled water (3 × 3 minutes) and stain with 2% phosphotungstic acid (pH 6.8) for 1 minute.

Interpretation of results

The inclusion of NP40 will permit antibodies and protein A-gold to penetrate the outer membrane and react with the underlying capsid. Labelling should be specific for the virus. Non-EHNV affinity purified rabbit serum (1/500) should be included as a negative control.

4.3.1.2.2.4. Immunohistochemistry (immunoperoxidase stain)

Samples: formalin-fixed paraffin-embedded tissue sections.

Technical procedure

The following protocol is intended for the qualitative demonstration of EHNV antigens in formalin-fixed paraffin-embedded tissue sections (Reddacliff & Whittington, 1996). It assumes that antigens may have become cross linked and therefore includes a protease digestion step that may be omitted if unfixed samples are examined. A commercial kit (DAKO® LSAB K0679) with peroxidase-labelled streptavidin and a mixture of biotinylated anti-rabbit/anti-mouse/anti-goat immunoglobulins as link antibodies is used for staining. Other commercially supplied reagents are also used. For convenience these are also supplied by DAKO[3]. The primary affinity purified rabbit anti-EHNV antibody (Lot No. M708) is supplied freeze-dried by the OIE Reference Laboratory.

i) Cut 5 µm sections and mount on SuperFrost® Plus G/Edge slides (Menzel-Glaser, HD Scientific Cat. No. HD 041300 72P3). Mark around the section with a diamond pencil to limit the spread of reagents.

ii) Deparaffinise the section:

Preheat slides in a 60°C incubator for 30 minutes.

Place slides in a xylene bath and incubate for 5 minutes. Repeat once. Note that xylene replacements can be used without deleterious effects.

Tap off excess liquid and place slides in absolute ethanol for 3 minutes. Repeat once.

Tap off excess liquid and place slides in 95% ethanol for 3 minutes. Repeat once.

Tap off excess liquid and place slides in distilled or deionised water for 30 seconds.

iii) Expose antigens using a protease treatment. Flood slide with proteinase K (5–7 µg ml^{-1}) and incubate for 20 minutes (ready-to-use solution, DakoCytomation Cat. No. S3020). Rinse slide by immersing three times in water. Place in a PBST bath for 5 minutes (PBS pH 7.2, 0.05% [v/v] Tween 20). Tap off the excess wash solution and carefully wipe around the section.

3 Dako Cytomation California Inc., 6392 via Real, Carpinteria, CA 93013, USA, Tel.: (+1-805) 566 6655, Fax: (+1-805) 566 6688; Dako Cytomation Pty Ltd, Unit 4, 13 Lord Street, Botany, NSW 2019, Australia, Fax: (+61-2) 9316 4773; Visit www.dakocytomation.com for links to other countries.

iv) Perform the immunostaining reaction using the Universal DAKO LSAB®+ Kit, Peroxidase (DakoCytomation Cat No. K0679). Ensuring the tissue section is completely covered, add the following reagents to the slide. Avoid drying out.

v) 3% hydrogen peroxide: cover the section and incubate for 5 minutes. Rinse gently with PBST and place in a fresh wash bath.

vi) Primary antibody (affinity purified rabbit anti-EHNV 1:/1500 Lot No. M708) and negative control reagent (non-immune rabbit serum at a dilution of 1/1500) on a second slide. Cover the section and incubate for 15 minutes. Rinse slides.

vii) Link: cover the section and incubate for 15 minutes. Rinse slides.

viii) Streptavidin peroxidase: cover the section and incubate for 15 minutes. Rinse slides.

ix) Substrate–chromogen solution: cover the section and incubate for 5 minutes. Rinse slides gently with distilled water.

x) Counterstain by placing slides in a bath of DAKO® Mayer's Haematoxylin for 1 minute (Lillie's Modification, Cat. No. S3309). Rinse gently with distilled water. Immerse 10 times into a water bath. Place in distilled or deionised water for 2 minutes.

xi) Mount and cover-slip samples with an aqueous-based mounting medium (DAKO® Faramount Aqueous Mounting Medium Cat. No. S3025).

Interpretation of results

EHNV antigen appears as a brown stain in the areas surrounding degenerate and necrotic areas in parenchymal areas. There should be no staining with negative control rabbit serum on the same section.

Availability of test and reagents: antibody reagents and test protocols are available from the OIE Reference Laboratory.

4.3.1.2.3. Molecular techniques

Although several conventional PCR or quantitative real-time PCR methods have been described, none has been validated according to OIE guidelines for primary detection of EHNV or other ranaviruses in fish tissues. However, identification of ranavirus at genus and species level is possible using several published PCR strategies. In the first method described here, two PCR assays using MCP primers are used with restriction analysis to detect and rapidly differentiate EHNV from the European (ECV), North American (FV3) and other Australian ranaviruses (BIV) (Marsh *et al*., 2002). This can be completed in less than 24 hours at relatively low cost. In the second method described here, a single MCP PCR assay is used to generate a 580 bp product, which is then sequenced to identify the type of ranavirus. Alternatively, PCR of the DNA polymerase gene and neurofilament triplet H1-like protein genes can be used (Holopainen *et al*., 2011) (this method is not described in this chapter).

Samples: virus from cell culture or direct analysis of tissue homogenate.

4.3.1.2.3.1. PCR and restriction endonuclease analysis (REA): technical procedure

Amplified product from PCR assay MCP-1 digested with PflM I enables differentiation of Australian iridoviruses (EHNV and BIV) from non-Australian iridoviruses (FV3, Americas; and ECV, Europe). Amplified product from PCR assay MCP-2 digested with Hinc II, Acc I and Fnu4H I (individually) enables differentiation of EHNV and BIV (Australia) from each other and from FV3 (Americas) and ECV (Europe).

Preparation of reagents

EHNV-purified DNA and BIV-purified DNA PCR control reagents are supplied by the reference laboratory in freeze-dried form. Reconstitute using 0.5 ml of Tris-EDTA (TE) buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0) and allow the vial to stand at RT for 2 minutes. Mix the vial very gently. For routine use, as a PCR control, it is recommended that working stocks be prepared as a 1/10 dilution in TE buffer (pH 8.0). Aliquots of 250 µl should be stored at –20°C. Each aliquot is sufficient for at least 50 reactions (1 to 5 µl added to cocktail) and has a minimum shelf life of 6 months from date of diluting.

Primers M151 and M152 (MCP-1, 321 bp), M153 and M154 (MCP-2, 625 bp) are supplied in working strength (100 ng μl^{-1}) and should be stored at –20°C. Primers can also be ordered from commercial suppliers. For primer sequences, refer to Table 4.2.

Table 4.2. *MCP-1 and MCP-2 primer sequences*

PCR assay	Primer	Sequence	Product size	Gene location
MCP-1	M151	AAC-CCG-GCT-TTC-GGG-CAG-CA	321 bp	266–586
	M152	CGG-GGC-GGG-GTT-GAT-GAG-AT		
MCP-2	M153	ATG-ACC-GTC-GCC-CTC-ATC-AC	625 bp	842–1466
	M154	CCA-TCG-AGC-CGT-TCA-TGA-TG		

PCR cocktail

Amplification reactions in a final volume of 50 µl (including 5 µl DNA sample) contain 2.5 µl (250 ng) of each working primer, 200 µM of each of the nucleotides dATP, dTTP, dGTP and dCTP, 5 µl of 10 × PCR buffer (66.6 mM Tris/HCl, 16.6 mM $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, 1.65 mg ml^{-1} BSA, 10 mM beta-mercaptoethanol) and 2 U Taq polymerase. Instructions on preparation of 10 × PCR buffer are included in Table 4.3.

Table 4.3. *10* **×** *PCR buffer preparation*

Ingredients	Amount	Final concentration in 50 µl PCR mix
Tris	4.050 g	66.6 mM
Ammonium sulphate	1.100 g	16.6 mM
BSA (albumin bovine fraction V fatty acid free)	0.825 g	1.65 mg ml^{-1}
Magnesium chloride	1.25 ml	2.5 mM
TE buffer (sterile)	50 ml	

NOTE: alternative commercial buffers may also be used.

Two negative controls are included, one comprising PCR cocktail only and the second containing 5 µl TE buffer.

The MCP-1 and MCP-2 reactions have the following profile: 1 cycle of denaturation at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds and extension at 72°C for 1 minute; a final extension of 72°C for 5 minutes, and cooling to 4°C.

NOTE: the annealing temperature may be increased to 60 or 62°C to reduce nonspecific amplification when the assay is used to test fish tissues.

PCR results are assessed by electrophoresis in 2% agarose gels stained with ethidium bromide. EHNV PCR control DNA (1/10 working stock) should give a result similar in intensity to the 10–3 band in both cases.

~~*Restriction endonuclease analysis (REA)*~~

~~PCR amplicons are subjected to REA with the enzymes described in Table 4.4. All endonucleases should be used according to the manufacturers' instructions. REA reactions are prepared by adding 1–4 µl of PCR product, 2 U of the appropriate restriction endonuclease, 1.6 µl of buffer (supplied with each restriction endonuclease), 1.6 µl of 100 µg ml^{-1} BSA (for PflM I and Hinc II) and made up to a final volume of 16 µl with sterile purified water. Restriction digests are incubated for 2–4 hours at the recommended temperatures and assessed by agarose gel electrophoresis in 3% gels. The predicted band sizes after restriction are given in Table 4.4.~~

Table 4.4. *Restriction endonuclease analysis of ranavirus MCP amplicons*

PCR Assay	Restriction enzyme	Predicted band sizes after restriction (bp)	Pattern applies to
MCP-1 (321bp)	*Pfl*M I	321	EHNV, BIV
		131, 190	FV3, WV
MCP 2 (625bp)	*Hinc* II	100, 138, 387	EHNV
		100, 525	BIV, FV3
		100, 240, 285	WV
	Acc I	238, 387	EHNV
		625	BIV, ESV, ECV, WV
		164, 461	FV3, GV
	*Fnu*4H I	33, 38, 44, 239, 271	EHNV
		3, 33, 38, 44, 108, 399	BIV
		3, 38, 44, 108, 432	FV3, GV
		3, 9, 38, 44, 108, 151, 272	ESV, ECV
		3, 44, 71, 108, 399	WV

Aliquot into 500 µl volumes and store at –20°C. For a working solution, add 3.5 µl of beta-mercaptoethanol per 500 µl 10 × buffer. Any remaining buffer should be discarded after preparing the PCR cocktail.

The sensitivity of PCR in diagnostic applications directly on fish tissues is being evaluated.

Detailed protocols to enable completion of the test, worksheets and purified control EHNV DNA are available from the OIE Reference Laboratory.

4.3.1.2.3.2. Alternative PCR and sequencing for viral identification

In this assay two primers, a reverse primer (5'-AAA-GAC-CCG-TTT-TGC-AGC-AAA-C-3') and a forward primer (5'-CGC-AGT-CAA-GGC-CTT-GAT-GT-3'), are used for amplification of the target MCP sequence (580 base pairs [bp]) of EHNV DNA by PCR. This PCR procedure can be used for the specific detection of ranaviruses from ~~redfin~~ European perch, rainbow trout, sheatfish, catfish, guppy fish (*Poecilia reticulata*), doctor fish (*Labroides dimidatus*) and a range of amphibian ranaviruses (Hyatt *et al.*, 2000). Nucleic acid (1 µl) is added to Taq polymerase buffer containing 0.1 µM of each primer, 2.5 U Taq polymerase (Promega) and 2.5 mM $MgCl_2$. The mixture is incubated in an automatic thermal cycler programmed for 35 cycles at 95°C for 60 seconds, 55°C for 60 seconds, and 72°C for 60 seconds, and finally held at 72°C for 15 minutes. Amplified DNA (580 bp) is analysed by agarose gel electrophoresis, excised and sequenced using a range of standard technologies). Each viral species is identified by its unique DNA sequence available from GenBank. Samples can be submitted to the OIE reference laboratory for specific identification.

4.3.1.2.4. Agent purification

Purification of EHNV has been described (Hyatt *et al.*, 1991; Steiner *et al*., 1991) and a protocol is available from the reference laboratory.

4.3.2. Serological methods

Neutralising antibodies have not been detected in fish or mammals exposed to EHNV. Indirect ELISA for detection of antibodies induced following exposure to EHNV has been described for rainbow trout and ~~redfin~~ European perch (Whittington *et al.*, 1994; 1999; Whittington & Reddacliff, 1995). The sensitivity and specificity of these assays in relation to a ~~gold~~ standard test are not known and interpretation of results is currently difficult. Protocols and specific anti-immunoglobulin reagents required to conduct these tests are available from the reference laboratory.

5. Rating of tests against purpose of use

The methods currently available for surveillance, detection, and diagnosis of infection with EHNV 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; d = the method is presently not recommended for this purpose; and NA = not applicable. 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 (see Chapter 1.1.2 *Principles and methods of validation of diagnostic assays for infectious diseases*), 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
	Ova/ milt	Fry/ fingerlings	Juveniles	Adults		
Gross signs	n/a	d	d	d	d	d
Histopathology	n/a	d	d	d	b	d
~~Immunoperoxidase stain~~ Immunohistochemistry	n/a	c	c	c	c	c
Transmission EM	n/a	d	d	d	c	b
Immuno-EM	n/a	d	d	d	c	b
Cell culture	n/a	a	a	a	a	b
Antigen-capture ELISA	n/a	a	a	a	a	a
Antibody-capture ELISA	n/a	d	d	c	c	d
~~PCR-REA~~	~~n/a~~	~~d~~	~~a~~	~~d~~	~~c~~	~~a~~
PCR sequence analysis	n/a	d	d	d	c	a

EM = electron microscopy; ELISA = enzyme-linked immunosorbent assay; PCR = polymerase chain reaction; REA = restriction endonuclease analysis; n/a = not applicable.

6. Test(s) recommended for targeted surveillance to declare freedom from epizootic haematopoietic necrosis

The test recommended for targeted surveillance is cell culture, and antigen-capture ELISA. Serology (antibody-capture ELISA) might also play a useful role in surveys to identify infected trout populations.

~~Statistically valid sampling practices need to be used and the correct organs/samples need to be collected;~~

~~Standardised tests of specified sensitivity and specificity should be used. This restricts certification testing to cell culture, the gold standard test, and antigen capture ELISA.~~

~~The chances of detecting EHNV infection in apparently healthy rainbow trout is extremely low, even where disease is active in the same population, because the prevalence of infection is low and there is a high case fatality rate. For practical purposes, EHNV can only be detected in fish that are clinically affected or that have died with the infection. From a random sample of live rainbow trout it would be possible to misclassify a farm as being free of EHNV even during an outbreak of the disease because the prevalence of infection is generally very low. Consequently, the examination of 'routine' mortalities is recommended (Whittington *et al.*, 1999).~~

~~During a low grade outbreak of disease in rainbow trout, the prevalence of EHNV among mortalities may be 60-80% and the contribution of EHNV to 'background' mortality is high enough to enable detection of the virus in the absence of overt disease in the population. For EHNV detection and certification purposes the population of interest is 'the population of mortalities' and sampling rates can be selected to detect at least one EHNV infected individual at a given level of confidence given a certain prevalence of infection and test sensitivity (Cannon & Roe, 1982; Simon & Schill, 1984). During an outbreak of EHNV the virus was detected in at least 2% of dead fish (Whittington *et al.*, 1999). For this reason, assume a prevalence of 2% for sampling of EHNV for certification purposes. The antigen-capture ELISA used to screen tissue homogenates for EHNV has a sensitivity of at least 60% compared with cell culture (Whittington & Steiner, 1993). The sample size required from a very large population of 'routine' mortalities (Whittington *et al.*, 1999) to provide 95% confidence in detecting at least one infected individual using a test of 60% sensitivity is approximately 250. In practice, 'routine' mortalities should be collected daily and stored in plastic bags in groups of 20 at −20°C until a sample of 250 has been gathered. Where possible, young age classes should be selected to simplify dissections and tissue processing. Individual clarified homogenates that are positive in antigen capture ELISA are then subjected to cell culture to confirm the presence of EHNV. This is an economical approach as it greatly reduces the number of cell cultures required. Alternatively, cell culture could be used and samples from five fish pooled to reduce costs.~~

~~Serology might also play a useful role in surveys to identify infected trout populations. Assuming 1% prevalence of seropositive grower fish on an endemically infected farm, a sample of 300 fish would be required to be 95% certain of detecting at least one infected individual (Cannon & Roe, 1982). Further research is required to confirm the validity of this approach.~~

7. Corroborative diagnostic criteria

7.1. Definition of suspect case

~~Finfish, apparently healthy, moribund or dead in which parenchymal tissues contain histological evidence of focal, multifocal or locally extensive liquefactive or coagulative necrosis with or without intracytoplasmic basophilic inclusion bodies.~~

The presence of EHNV shall be suspected if at least one of the following criteria is met:

i) Histopathology consistent with EHNV, with or without clinical signs of disease;

ii) CPE typical of EHNV in cell cultures;

iii) Positive conventional PCR result;

iv) Positive antigen capture ELISA.

7.2. Definition of confirmed case

The presence of EHNV is considered to be confirmed if, in addition to the criteria in Section 7.1, one or more of the following criteria are met:

i) EHNV isolation is carried out in cell culture followed by virus identification by either an antibody-based test (immunoperoxidase stain, ELISA, ~~neutralisation test,~~ immunohistochemistry) and/or conventional PCR followed by sequencing of the amplicon;

ii) EHNV is detected in histological sections by immunoassay using specific anti-EHNV antibodies;

iii) Detection of EHNV in tissue preparations by conventional PCR followed by sequencing of the amplicon.

~~Finfish, apparently healthy, moribund or dead in which parenchymal tissues contain histological evidence of focal, multifocal or locally extensive liquefactive or coagulative necrosis with or without intracytoplasmic basophilic inclusion bodies and/or in which EHNV is demonstrated by the following means:~~

~~1. Characteristic CPE in cell culture and cell culture is positive for EHNV in immunoperoxidase test or antigen capture ELISA or PCR,~~

~~or~~

~~2. Tissues positive in antigen capture ELISA or immunoperoxidase stain or immunoelectron microscopy or PCR~~

~~And for both 1 and 2,~~

~~3. Sequence consistent with EHNV is demonstrated by PCR REA or PCR sequencing.~~

8. References

AHNE W., BEARZOTTI M., BREMONT M. & ESSBAUER S. (1998). Comparison of European systemic piscine and amphibian iridoviruses with epizootic haematopoietic necrosis virus and frog virus 3. *J. Vet. Med.* [*B*], **45**, 373–383.

AHNE W., OGAWA M. & SCHLOTFELDT H.J. (1990). Fish viruses: transmission and pathogenicity of an icosahedral cytoplasmic deoxyribovirus isolated from sheatfish *Silurus glanis*. *J. Vet. Med*. [*B*], **37**, 187–190.

AHNE W., SCHLOTFELDT H.J. & THOMSEN I. (1989). Fish viruses: isolation of an icosahedral cytoplasmic deoxyribovirus from sheatfish (*Silurus glanis*). *J. Vet. Med.* [*B*], **36**, 333–336.

ARIEL E. & BANG JENSEN B. (2009). Challenge studies of European stocks of redfin perch, *Perca fluviatilis* L., and rainbow trout, *Oncorhynchus mykiss* (Walbaum), with epizootic haematopoietic necrosis virus. *J. Fish Dis.,* **32**, 1017–1025.

ARIEL E., NICOLAJSEN N., CHRISTOPHERSEN M.-B., HOLOPAINEN R., TAPIOVAARA H. & BANG JENSEN B. (2009). Propagation and isolation of ranaviruses in cell culture. *Aquaculture,* **294**, 159–164.

ARIEL E., TAPIOVAARA H. & OLESEN N.J. (1999). Comparison of Pike-perch (*Stizostedion lucioperca*), Cod (*Gadus morhua*) and turbot (*Scophthalmus maximus*) iridovirus isolates with reference to other piscine and amphibian iridovirus isolates. European Association of Fish Pathologists, VIII. International Conference on Diseases of Fish and Shellfish, Rhodes, Greece, 20–24 September.

BANG JENSEN B., ERSBOLL A.K. & ARIEL E. (2009). Susceptibility of pike *Esox lucius* to a panel of ranavirus isolates. *Dis. Aquat. Org.*, **83**, 169–179.

BECKER J.A., TWEEDIE A., GILLIGAN D., ASMUS M. & WHITTINGTON R. J. (2016). Susceptibility of Australian Redfin Perch *Perca fluviatilis* Experimentally Challenged with Epizootic Hematopoietic Necrosis Virus (EHNV). *J. Aquat. Anim. Health,* **28**, 122–130.

BLOCH B. & LARSEN J.L. (1993). An iridovirus-like agent associated with systemic infection in cultured turbot *Scophthalmus maximus* fry in Denmark. *Dis. Aquat. Org.*, **15**, 235–240.

BRYAN L.K., BALDWIN C.A., GRAY M.J. & MILLER D.L. (2009). Efficacy of select disinfectants at inactivating Ranavirus. *Dis. Aquat. Org.,* **84**, 89–94.

CANNON R.M. & ROE R.T. (1982). Livestock Disease Surveys: A Field Manual for Veterinarians. Australian Government Publishing Service, Canberra, 35 p.

CHINCHAR G., ESSBAUER S., HE J.G., HYATT A., MIYAZAKI T., SELIGY V. & WILLIAMS T. (2005). Family Iridoviridae. *In:* Virus Taxonomy. Classification and Nomeclature of Viruses. Eight Report of the International Committee on the Taxonomy of Viruses, Fauquet C.M., Mayo M.A., Maniloff J., Desselberger U. & Ball L.A., eds. Academic Press, San Diego, California, USA, 145–161.

CHINCHAR V.G. (2002). Ranaviruses (family Iridoviridae): emerging cold-blooded killers – brief review. *Arch. Virol.*, **147**, 447–470.

CINKOVA K., RESCHOVA S., KULICH P. & VESELY T. (2010). Evaluation of a polyclonal antibody for the detection and identification of ranaviruses from freshwater fish and amphibians. *Dis. Aquat. Org.,* **89**, 191–198.

CRANE M.S.J., YOUNG J. & WILLIAMS L. (2005). Epizootic haematopoietic necrosis virus (EHNV): growth in fish cell lines at different temperatures. *Bull. Eur. Assoc. Fish Pathol.*, **25**, 228–231.

DRURY S.E.N., GOUGH R.E. & CALVERT I. (2002). Detection and isolation of an iridovirus from chameleons (*Chamaeleo quadricornis* and *Chamaeleo hoehnelli*) in the United Kingdom. *Vet. Rec.,* **150**, 451–452.

EATON B.T., HYATT A.D. & HENGSTBERGER S. (1991). Epizootic haematopoietic necrosis virus: purification and classification. *J. Fish Dis.,* **14**, 157–169.

FIJAN N., MATASIN Z., PETRINEC Z., VALPOTIC I. & ZWILLENBERG L.O. (1991). Isolation of an iridovirus-like agent from the green frog (*Rana esculenta* L.). *Veterinarski Arhiv,* **61**, 151–158.

GOBBO F., CAPPELLOZZA E., PASTORE M.R. & BOVO G. (2010). Susceptibility of black bullhead *Ameiurus melas* to a panel of ranavirus isolates. *Dis. Aquat. Org.,* **90**, 167–174.

HEDRICK R.P., MCDOWELL T.S., AHNE W., TORHY C. & DE KINKELIN P. (1992). Properties of three iridovirus-like agents associated with systemic infections of fish. *Dis. Aquat. Org.,* **13**, 203–209.

HENGSTBERGER S.G., HYATT A.D., SPEARE R. & COUPAR B.E.H. (1993). Comparison of epizootic haematopoietic necrosis and Bohle iridoviruses, recently isolated Australian iridoviruses. *Dis. Aquat. Org.*, **15**, 93–107.

HOLOPAINEN R., HONKANEN J., JENSEN B.B., ARIEL E. & TAPIOVAARA H. (2011). Quantitation of ranaviruses in cell culture and tissue samples. *J. Virol. Methods,* **171**, 225–233.

HOLOPAINEN R., OHLEMEYER S., SCHÜTZE H., BERGMANN S.M. & TAPIOVAARA H. (2009). Ranavirus phylogeny and differentiation based on major capsid protein, DNA polymerase and neurofilament triplet H1-like protein genes. *Dis. Aquat. Org.,* **85**, 81–91.

HYATT A.D. (1991). Immunogold labelling techniques, *In:* Electron Microscopy in Biology: a Practical Approach, Harris R., ed. IRL Press, Oxford, UK, 59–81.

HYATT A.D., EATON B.T., HENGSTBERGER S. & RUSSEL G. (1991). Epizootic hematopoietic necrosis virus: detection by ELISA, immuno-histochemistry and electron microscopy. *J. Fish Dis.*, **14**, 605–617.

HYATT A.D., GOULD A.R., ZUPANOVIC Z., CUNNINGHAM A.A., HENGSTBERGER S., WHITTINGTON R.J., KATTENBELT J. & COUPAR B.E.H. (2000). Comparative studies of piscine and amphibian iridoviruses. *Arch. Virol.,* **145**, 301–331.

HYATT A.D., WILLIAMSON M., COUPAR B.E.H., MIDDLETON D., HENGSTBERGER S.G., GOULD A.R., SELLECK P., WISE T.G., KATTENBELT J., CUNNINGHAM A.A.& LEE J. (2002). First identification of a ranavirus from green pythons (*Chondropython viridis*). *J. Wildl. Dis.,* **38**, 239–252.

LANGDON J.S. (1989). Experimental transmission and pathogenicity of epizootic haematopoietic necrosis virus (EHNV) in redfin perch, *Perca fluviatilis* L., and 11 other teleosts. *J. Fish Dis.*, **12**, 295–310.

LANGDON J.S. & HUMPHREY J.D. (1987). Epizootic Hematopoietic Necrosis a New Viral Disease in Redfin Perch *Perca fluviatilis* L. in Australia. *J. Fish Dis.*, **10**, 289–298.

LANGDON J.S., HUMPHREY J.D. & WILLIAMS L.M. (1988). Outbreaks of an EHNV-like iridovirus in cultured rainbow trout, *Salmo gairdneri* Richardson, in Australia. *J. Fish Dis.*, **11**, 93–96.

LANGDON J.S., HUMPHREY J.D., WILLIAMS L.M., HYATT A.D. & WESTBURY H.A. (1986). First virus isolation from Australian fish: an iridovirus-like pathogen from redfin perch, *Perca fluviatilis* L. *J. Fish Dis.*, **9**, 263–268.

Annex 24 (contd)

MAO J., THAM T.N., GENTRY G.A., AUBERTIN A. & CHINCHAR V.G. (1996). Cloning, sequence analysis, and expression of the major capsid protein of the iridovirus frog virus 3. *Virology*, **216**, 431–436.

MAO J.H., HEDRICK R.P. & CHINCHAR V.G. (1997). Molecular characterisation, sequence analysis and taxonomic position of newly isolated fish iridoviruses. *Virology*, **229**, 212–220.

MARSH I.B., WHITTINGTON R.J., O'ROURKE B., HYATT A.D. & CHISHOLM O. (2002). Rapid differentiation of Australian, European and American ranaviruses based on variation in major capsid protein gene sequence. *Molec. Cell. Probes*, **16**, 137–151.

POZET F., MORAND M., MOUSSA A., TORHY C. & DE KINKELIN P. (1992). Isolation and preliminary characterization of a pathogenic icosahedral deoxyribovirus from the catfish (*Ictalurus melas*). *Dis. Aquat. Org.,* **14**, 35–42.

REDDACLIFF L.A. & WHITTINGTON R.J. (1996). Pathology of epizootic haematopoeitic necrosis virus (EHNV) infection in rainbow trout (*Oncorhynchus mykiss* Walbaum) and redfin perch (*Perca fluviatilis* L.). *J. Comp. Pathol.,* **115**, 103–115.

SIMON R.C. & SCHILL W.B. (1984). Tables of sample size requirements for detection of fish infected by pathogens: three confidence levels for different infection prevalence and various population sizes. *J. Fish Dis*., **7**, 515–520.

SPEARE R. & SMITH J.R. (1992). An iridovirus-like agent isolated from the ornate burrowing frog *Limnodynastes ornatus* in northern Australia. *Dis. Aquat. Org.*, **14**, 51–57.

STEINER K.A., WHITTINGTON R.J., PETERSEN R.K., HORNITZKY C. & GARNETT H. (1991). Purification of epizootic haematopoietic necrosis virus and its detection using ELISA. *J. Virol. Methods,* **33**, 199–210.

WHITTINGTON R.J. (1992). Evaluation of a simple method for improving the precision of an ELISA detecting antibody in serum. *J. Immunol. Methods*, **148**, 57–64.

WHITTINGTON R.J., BECKER J.A. & DENNIS M.M. (2010). Iridovirus infections in finfish – critical review with emphasis on ranaviruses. *J. Fish Dis.,* **33**, 95–122.

WHITTINGTON R.J., KEARNS C., HYATT A.D., HENGSTBERGER S. & RUTZOU T. (1996). Spread of epizootic haematopoietic necrosis virus (EHNV) in redfin perch (*Perca fluviatilis*) in southern Australia. *Aust. Vet. J.*, **73**, 112–114.

WHITTINGTON R.J., PHILBEY A., REDDACLIFF G.L. & MACGOWN A.R. (1994). Epidemiology of epizootic haematopoietic necrosis virus (EHNV) infection in farmed rainbow trout, *Oncorhynchus mykiss* (Walbaum): findings based on virus isolation, antigen capture ELISA and serology. *J. Fish Dis.*, **17**, 205–218.

WHITTINGTON R.J. & REDDACLIFF G.L. (1995). Influence of environmental temperature on experimental infection of redfin perch (*Perca fluviatilis*) and rainbow trout (*Oncorhynchus mykiss*) with epizootic haematopoietic necrosis virus, an Australian iridovirus. *Aust. Vet. J.,* **72**, 421–424.

WHITTINGTON R.J., REDDACLIFF L.A., MARSH I., KEARNS C., ZUPANOVIC Z. & CALLINAN R.B. (1999). Further observations on the epidemiology and spread of epizootic haematopoietic necrosis virus (EHNV) in farmed rainbow trout *Oncorhynchus mykiss* in southeastern Australia and a recommended sampling strategy for surveillance. *Dis. Aquat. Org.,* **35**, 125–130.

WHITTINGTON R. J. & STEINER K. A. (1993). Epizootic haematopoietic necrosis virus (EHNV): improved ELISA for detection in fish tissues and cell cultures and an efficient method for release of antigen from tissues. *J. Virol. Methods,* **43**, 205–220.

WOLF K., BULLOCK G.L., DUNBAR C.E. & QUIMBY M.C. (1968). Tadpole edema virus: a viscerotrophic pathogen for anuran amphibians. *J. Infect. Dis.,* **118**, 253–262.

ZUPANOVIC Z., MUSSO C., LOPEZ G., LOURIERO C.L., HYATT A.D., HENGSTBERGER S. & ROBINSON A.J. (1998). Isolation and characterisation of iridoviruses from the giant toad *Bufo marinus* in Venezuela. *Dis. Aquat. Org.,* **33**, 1–9.

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* *

Annex 24 (contd)

NB: There is an OIE Reference Laboratory for infection with Epizootic haematopoietic necrosis virus (EHNV) (see Table at the end of this *Aquatic Manual* or consult the OIE web site for the most up-to-date list: http://www.oie.int/en/our-scientific-expertise/reference-laboratories/list-of-laboratories/).
Please contact the OIE Reference Laboratories for any further information on infection with EHNV.
The OIE Reference Laboratory can supply purified EHNV DNA, heat killed EHNV antigen and polyclonal antibodies against EHNV together with technical methods.
A fee is charged for the reagents to cover the costs of operating the laboratory.

NB: FIRST ADOPTED IN 1995 AS EPIZOOTIC HAEMATOPOIETIC NECROSIS; MOST RECENT UPDATES ADOPTED IN 2012.

Annex 25

CHAPTER 2.3.3

INFECTION WITH *GYRODACTYLUS SALARIS*

EU position

The EU thanks the OIE and in general supports the adoption of this modified chapter.

We note with thanks that parts of our previous comments raised for Section 4.3.1.2.3. have been referred to the OIE Reference Laboratory expert for consideration and look forward to hearing the outcome in due course.

Comments are inserted in the text below.

1. Scope

Infection with *Gyrodactylus salaris* means infection with the pathogenic agent *Gyrodactylus salaris* (*G. salaris*) of the Genus *Gyrodactylus* and Family Gyrodactylidae, ~~(Platyhelminthes; Monogenea) is a viviparous freshwater parasite that may cause infection in Atlantic salmon (*Salmo salar*)~~.

EU comment

The Scope states that "infection with *G. salaris*" means "infection with the pathogenic agent *G. salaris*". It should be noted that there is evidence of mixed pathogenicity of *G. salaris* with reports of some strains which are pathogenic to rainbow trout and not Atlantic salmon (in Denmark) and vice versa (widespread throughout Europe). We request that the Scope, or subsequent disease information, makes clear what is regarded as the "pathogenic agent" due to the mixed pathogenicity issue for *G. salaris*.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent, agent strains

Several strains or clades of *G. salaris* have been identified on the basis of genotyping with the mitochondrial cytochrome oxidase 1 (CO1) marker (Hansen *et al*., 2003; 2007b; Meinilä *et al.,* 2002; 2004). Although there does not seem to be any correspondence between strains as identified by CO1 and pathogenicity (Hansen *et al*., 2007a), all strains recovered from Atlantic salmon that have been studied in laboratory experiments, so far, are highly pathogenic to strains of Atlantic salmon. ~~Recently,~~ Strains non-pathogenic to salmon ~~were~~ have been recovered from non-anadromous Arctic charr (*Salvelinus alpinus*) in Norway (Olstad *et al*., 2007a; Robertsen *et al*., 2007) and from rainbow trout (*Oncorhynchus mykiss*) in Denmark (Jørgensen *et a*l., 2007; Lindenstrøm *et al*., 2003).

EU comment

The EU notes that in the paragraph above, the term "strains" is used for both *G. salaris* and Atlantic salmon. To avoid any possible confusion, the EU suggests that "clades" be used instead of "strains" in relation to different variants of *G. salaris*, whereas "strains" be used when referring to different variants of Atlantic salmon.

2.1.2. Survival outside the host

Survival of detached parasites is temperature ~~dependant~~ dependent, e.g. about 24 hours at 19°C, 54 hours at 13°C, 96 hours at 7°C and 132 hours at 3°C (Olstad *et al*., 2006). Likewise, survival attached

to a dead host is temperature ~~dependant~~ dependent: maximum survival time for *G. salaris* on dead Atlantic salmon are ~~for~~ 72, 142 and 365 hours at 18, 12 and 3°C, respectively (Olstad *et al*., 2006).

2.1.3. Stability of the agent (effective inactivation methods)

Gyrodactylus salaris is known to survive~~s~~ between ~~all~~ temperatures of ~~between~~ 0°C to ~~and~~ 25°C. Tolerance to temperatures above 25°C is unknown. It is not resistant to freezing. *Gyrodactylus salaris* is sensitive to desiccation ~~not drought resistant and must be surrounded by water for survival~~. *Gyrodactylus salaris* dies after a few days at pH≤5. It is more sensitive to low pH (5.1<pH<6.4) in association with aluminium and zinc than the host Atlantic salmon (Poléo *et al*., 2004; Soleng *et al*., 2000) (see also Section 2.4.2).

2.1.4. Life cycle

Gyrodactylus salaris is an obligate parasite with a direct life cycle. Parasites give birth to live offspring, and there are no other life stages ~~eggs, resting stages, specialised transmission stages or intermediate hosts~~.

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing a species as susceptible to infection with *G. salaris* according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) include: Arctic char (*Salvelinus alpinus*), Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*), grayling (*Thymallus thymallus*), North American brook trout (*Salvelinus fontinalis*) and rainbow trout (*Oncorhynchus mykiss*).

~~*Gyrodactylus salaris* is an ectoparasite mainly on Atlantic salmon (*Salmo salar*), but can survive and reproduce on several salmonids, such as rainbow trout (*Oncorhynchus mykiss*), Arctic charr (*Salvelinus alpinus*), North American brook trout (*Salvelinus fontinalis*), grayling (*Thymallus thymallus*), North American lake trout (*Salvelinus namaycush*) and brown trout (*Salmo trutta*) (in declining order of susceptibility).~~

~~Strains of Atlantic salmon have shown variable susceptibility to *G. salaris* (Bakke *et al*., 2002). The Baltic strains have been considered resistant. However, this has only been shown for salmon from the Russian River Neva, the Swedish River Torneälven and the Finnish landlocked Lake Saima population. Salmon from the Baltic Swedish River Indalsälven are almost as susceptible as the Norwegian salmon and salmon from the Scottish River Conon (Bakke *et al*., 2004). Salmon from other Baltic rivers have shown intermediate susceptibility.~~

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: none known ~~nil~~.

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have identified *G. salaris* on the following organisms, but a long-term active infection has not been demonstrated: [Under study].

EU comment

For *G. salaris*, transient infections in non-susceptible species such as European eel (*Anguilla anguilla*) have been reported, contributing to spread of the disease. The inclusion of information on such species would be beneficial in this chapter to raise awareness of the risk, either as an addition to the section above or covered within section 2.2.7 Vectors.

References:

T. A. Bakke, P. A. Jansen, and , L. P. Hansen (1991). Experimental transmission of Gyrodactylus salaris Malmberg, 1957 (Platyhelminthes, Monogenea) from the Atlantic salmon (*Salmo salar*) to the European eel (*Anguilla anguilla*). Canadian Journal of Zoology, 69(3): 733-737

Arnulf Soleng1, Peder A. Jansen and Tor A. Bakke1 (1999). Transmission of the monogenean *Gyrodactylus salaris*. FOLIA PARASITOLOGICA 46: 179-184

2.2.32. Susceptible stages of the host

All stages of the host are susceptible but mortality has only been observed in fry and parr stages.

2.2.43. Species or subpopulation predilection (probability of detection)

Not applicable.

2.2.54. Target organs and infected tissue

Gyrodactylus salaris usually occurs on the fins of ~~most~~ infected Atlantic salmon, but the parasite distribution on the host may vary depending ~~site preference is dependent~~ on intensity of infection (Jensen & Johnsen, 1992; Mo, 1992). Parasites are also commonly found on the body and less commonly on the gills. On other hosts, the distribution may be different, but in general ~~on some host species~~ the parasite is relatively less abundant on the fins and relatively more common on the body compared with Atlantic salmon.

2.2.65. Persistent infection ~~with lifelong carriers~~

Not applicable.

2.2.76. Vectors

Not applicable.

2.3. Disease pattern

2.3.1. Transmission mechanisms

Gyrodactylus salaris has spread between rivers and farms mainly by the ~~transport/restocking~~ translocation of live fish. ~~Migrating~~ Fish migrating ~~swimming~~ through brackish water can also spread ~~cause~~ the parasite ~~to be spread~~ between rivers (see also Section 2.3.5). ~~Rivers with susceptible Atlantic salmon~~ Populations located near infected rivers ~~with infected populations~~ are at great risk of infection if they ~~these rivers~~ are located within the same brackish water system. If *G. salaris* is introduced into a farm~~/tank~~ with ~~susceptible~~ Atlantic salmon, there is a high probability that all fish in the farm will become infected, depending on the layout of the farm~~. Rivers with susceptible Atlantic salmon located near infected rivers are at great risk of infection if these rivers are located within the same brackish water system.~~

2.3.2. Prevalence

Prevalence in susceptible strains of Atlantic salmon reaches close to 100% in parr in rivers (Appleby & Mo, 1997; Johnsen & Jensen, 1991); ~~and farms reaches~~ similarly prevalence in farmed Atlantic salmon (in freshwater) rises to close to 100% within a short time after introduction of the parasite. Prevalence in resistant strains in rivers and farms is unknown. Prevalence in other susceptible species is usually much lower and can be below 10% (e.g. in farmed rainbow trout; Buchmann & Bresciani, 1997).

2.3.3. Geographical distribution

Gyrodactylus salaris is restricted in its distribution to Europe. It has been recovered from farmed Atlantic salmon or farmed rainbow trout in several (mainly northern) European countries. In the wild, the parasite has been found on wild salmonids, mainly Atlantic salmon parr, in rivers in Russia, Sweden and Norway. Infection with *G. salaris* is more common in farmed rainbow trout than previously thought, and is likely to be present in more countries than those currently known. In 2006, infection with *G. salaris* was reported from fish farms in Italy (Paladini *et al.,* 2009) and, in 2007, from fish farms in Poland (Rokicka *et al*., 2007) and Macedonia (Ziętara *et al.,* 2007). In 2009, *G. salaris* was identified by the OIE Reference Laboratory, from fish farms in Romania. ~~Great Britain~~ The United Kingdom and Ireland have been demonstrated to be free of the parasite.

2.3.4. Mortality and morbidity

Mortality in farmed ~~susceptibe~~ Atlantic salmon fry and parr can be 100% ~~in susceptible farmed Atlantic salmon~~ if not treated. Mortality in wild Atlantic salmon fry and parr in Norwegian rivers can be as high as 98%, with an average of about 85%. Mortality in other susceptible host species is usually low or not observed.

2.3.5. Environmental factors

Although *G. salaris* mainly lives in fresh water, it reproduces normally at salinities up to 5–6 ppt. Survival at higher salinities is temperature dependent. For example at 1.4°C, *G. salaris* may survive for 240 hours, 78 hours and 42 hours at 10 ppt, 15 ppt and 20 ppt salinity, respectively, while at 12°C it may survive for 72 hours, 24 hours and 12 hours at the same three salinities, respectively (Soleng & Bakke, 1997).

2.4. Control and prevention

2.4.1. Vaccination

Vaccines are not available.

2.4.2. Chemotherapy

Gyrodactylus salaris is sensitive to changes in the chemical composition of the water. It is sensitive to the most commonly used chemicals for bath treatment of farmed salmon parr and salmon eggs (e.g. high salinity salt water, formaldehyde and compounds containing chlorine and iodine). Furthermore, *G. salaris* is sensitive to acidic solutions (pH 5.0–6.0) of aluminium sulphate ($[Al_2(SO_4)_3]$~~; AlS~~) (Soleng *et al.,* 1999). As ~~AlS~~ aluminium sulphate is less toxic to fish than to *G. salaris* in moderately acidified waters, this chemical has been used in attempts to eradicate the parasite from river systems in Norway.

2.4.3. Immunostimulation

Immunostimulation is not available.

2.4.4. Resistance breeding

In laboratory experiments, selected breeding has resulted in increased survival among the offspring (Salte *et al.,* 2010). However, selected breeding has not been applied to wild salmon stocks, mainly because the stock will remain infected and thus the parasite may spread to more rivers.

2.4.5. Restocking with resistant species

Restocking with resistant strains of Atlantic salmon (e.g. Baltic Neva strain) in affected rivers is not compatible with existing strain management of Atlantic salmon.

2.4.6. Blocking agents

Not applicable.

2.4.7. Disinfection of eggs and larvae

Eggs that are transferred from infected farms should be disinfected (iodine-containing compounds have been used).

2.4.8. General husbandry practices

The general recommended husbandry practices for avoiding the spread of infective agents between units in freshwater fish farms apply to *G. salaris*. Equipment (e.g. fish nets) used in one unit should not be used in another without adequate disinfection.

3. Sampling

3.1. Selection of individual specimens

In cases where sampling is performed and infection is not suspected, a random sample with an adequate number of fish should be taken from, for example, a river. In farms, if fish show clinical signs of infection (as described in Section 4.1.1), these fish should be selected.

3.2. Preservation of samples for submission

Fish should be killed immediately and should not be allowed to dry out before preservation. Whole fish should be preserved in ~~96~~ 80–100% EtOH in bottles large enough to provide excess space and preservative. The concentration of EtOH after preservation should not be below 70%. As a rule of thumb this concentration is obtained if the proportion of fish to EtOH does not exceed 1:9. If the concentration is lower, the mucous and epidermis may disintegrate and *Gyrodactylus* specimens, even if they are preserved, may drop off. Bottles should have an opening wide enough to avoid the possibility of scraping off *Gyrodactylus*

specimens when fish are put into the bottle or when taken out for examination. Bottles should be stored in a horizontal position until the tissue is fixed/preserved to prevent the fish curling. This facilitates examination of the fish as they can easily be turned with a pair of forceps under the microscope. When preservation of the fish is complete, the bottles can be stored in a vertical position.

As *G. salaris* is common on fins of Atlantic salmon, fins cut off from the body and stored in EtOH as described above can also be submitted. This is especially suitable for larger fish and under field conditions where, for example, transport is limited.

3.3. Pooling of samples

Samples ~~from a river or a farm~~ can be pooled, although each fish is subsequently examined and analysed separately. Fins of fish from a farm or a river can be pooled and are also examined and analysed separately, but in this instance each fin cannot be related to a certain fish host.

3.4. Best organs or tissues

Fish can be examined as whole specimens either live under anaesthesia (for example, with MS222), freshly killed, or preserved. In addition, fresh or preserved fins can be examined. The same examination method (see Section 4.3.1) is used in all cases. Examination of live, anaesthetised fish is very time-consuming and not recommended.

Instead of examining the whole fish, the fins can be examined (by the method described in Section 4.3.1). When Norwegian salmon parr are infected, almost all fish have at least one *G. salaris* on one of the fins. On some fish, *G. salaris* specimens may occur on the body or head, including the nostrils, the gills and the mouth cavity. The distribution of *G. salaris* on fins and other parts of the fish varies among fish species and seems to vary among salmon strains.

3.5. Samples/tissues that are not suitable

Dead fish, stored on ice, are not acceptable for *Gyrodactylus* examination, even if the fish are kept separately in plastic bags, etc. The parasites quickly ~~soon~~ die if not covered in water, and as these parasites do not have an exoskeleton, dead parasites disintegrate quickly. If such dead fish are rinsed in water, *Gyrodactylus* specimens may be found in the sediment. However, if specimens are not found in the sediment, it cannot be concluded that the fish were uninfected. ~~Examination of formaldehyde-fixed fish is not recommended for reasons of operator safety.~~ Formaldehyde-fixed *Gyrodactylus* specimens are ~~also very~~ difficult to identify morphologically and are unsuitable for DNA analysis.

4. Diagnostic methods

4.1. Field diagnostic methods

4.1.1. Clinical signs

Usually there are no clinical signs in fish with one or up to a few tens of parasite specimens.

In the early disease phase, increased flashing (fish scratch their skin on the substrate) is typical. Later, fish may become greyish because of increased mucous production and the fins may be eroded. Diseased fish are lethargic and are usually found in slower-moving water.

4.1.2. Behavioural changes

Flashing is common among moderate to heavily infected farmed fish as they scratch their skin on the bottom or wall of a tank or pond. Heavily infected fish may have reduced activity and stay in low current areas.

4.2. Clinical methods

4.2.1. Gross pathology

Heavily infected fish may become greyish as a result of increased mucification, and at a later stage the dorsal and pectoral fins may become whitish as a result of increased thickness (mainly hypertrophy) of the epidermis.

Heavily infected fish may have eroded fins, especially dorsal, tail and pectoral fins, because of parasite feeding.

Secondary fungal infections (*Saprolegnia* spp.) are commonly observed in fish with infection with *G. salaris*.

4.2.2. Clinical chemistry

Not applicable.

4.2.3. Microscopic pathology

Not applicable.

4.2.4. Wet mounts

Scrapings (wet mounts) from skin or fins can be used to detect *Gyrodactylus* specimens on infected fish. In these cases, with high intensity infestation, hundreds or thousands of *Gyrodactylus* specimens are present all over the body and fins. Preparations of wet mounts are usually not suitable for identification of *Gyrodactylus* to the species level and other preparations for morphological or DNA analysis must be made (see below). If the number of *Gyrodactylus* specimens is low, the chances of detecting the parasites by scrapings are limited.

4.2.5. Smears

Not applicable.

4.2.6. Fixed sections

Not applicable.

4.2.7. Electron microscopy/cytopathology

Not applicable.

4.3. Agent detection and identification methods

4.3.1. Direct detection methods

Detection of *Gyrodactylus* and identification of *G. salaris* is a two-step process. Firstly, parasite specimens are observed using optical equipment and secondly, parasites are identified, usually on an individual basis using other equipment and methods.

Optical equipment must be used to detect *Gyrodactylus*. In the case of a suspected outbreak of infection with *G. salaris* where only light microscopy is available, wet mounts can be used to detect *Gyrodactylus* specimens. However, it is strongly advised not to use this method in a surveillance programme as the presumed specificity and sensitivity is ~~very~~ low (value not known) and, therefore, the number of fish examined is ~~needs to be unreasonably~~ high.

Fish can be examined as live whole specimens (under anaesthesia), freshly killed or preserved~~/fixed~~. The same examination method (see below) is used in all cases. Examination of live, anaesthetised fish is very time-consuming and not recommended. ~~Examination of formaldehyde-fixed fish is not recommended for reasons of operator safety.~~ *Gyrodactylus* specimens fixed in formaldehyde are also very difficult to identify and are not suitable for DNA analysis. Instead of examining the whole fish, the fins can be examined (by the method described below). When parr of ~~very~~ susceptible Atlantic salmon strains ~~parr~~ are infected ~~infested~~, almost all fish have at least one *G. salaris* on one of the fins. On some fish, *G. salaris* specimens may occur on the body or head, including the nares ~~nostrils~~, the gills and the mouth cavity. The distribution of *G. salaris* on fins and other parts of the fish varies among fish species and the distribution also seems to vary among salmon strains.

Live anaesthetised fish, freshly cut fins or EtOH-preserved fish or fins should be examined under a binocular dissecting microscope with good illumination. The fish should be placed in a box and completely covered in fresh water. Preserved fish can also be examined in EtOH. Living parasites are more easily detected by their movements, thus disturbing light refraction on the skin of the fish should be avoided. Live *Gyrodactylus* are colourless while EtOH-preserved *Gyrodactylus* specimens are usually only slightly opaque. If the dissecting microscope is illuminated from above, the bottom of the microscope stage should be black. This will increase the contrast and the parasites will be detected more easily. The whole surface of the fish, including gills and mouth cavity, must be examined. It is best to use two forceps for this process. The fins of relatively small fish, usually less than 10 cm, can also be studied using illumination through the bottom of the microscope stage. This way, *Gyrodactylus* specimens on the fins can usually be easily observed.

~~If examination is carried out in EtOH, the use of gloves should be considered. For operator protection purposes, the dissecting microscope could be placed on a suction bench with a downwards outlet to avoid inhalation of evaporated preservative.~~

4.3.1.1. Microscopic methods

Identification of *Gyrodactylus* species is based on morphology and morphometry of marginal hooks anchors (hamuli) and bars in the opisthaptor (the attachment organ). Good preparation of specimens is a prerequisite for species identification.

Digestion of the soft tissue, leaving the hard parts only, is recommended when high-resolution morphometrics is required for reliable morphometric diagnosis. The soft tissue can be digested in a solution (approx. 1 µl) of 75 mM Tris, 10 mM EDTA (ethylene diamine tetra-acetic acid), 5% SDS (sodium dodecyl sulphate) and 100 mg ml^{-1} proteinase K, pH 8.0. After adding the digestion solution, the reaction should be inspected in the microscope until completion and then ended by adding a stop solution (1:1 glycerol and 10% neutral buffered formalin). The procedure for digestion is described in detail in (Harris *et al.,* 1999). Identification of *G. salaris* should be in accordance with references: Cunningham *et al.,* 2001; Malmberg *et al.,* 1957;1970; McHugh *et al*., 2000; Olstad *et al*., 2007b; Shinn *et al.,* 2004.

The size of the opisthaptoral hard parts in *Gyrodactylus* varies extensively with, for example, temperature, whereas shape is more stable (Mo, 1991a; 1991b; 1991c). The capability of linear measurements to capture morphology might therefore not always be sufficient for reliable diagnosis (Olstad *et al*., 2007b).

Gyrodactylus salaris is morphologically similar to *G. teuchis* from brown trout, Atlantic salmon, and rainbow trout, and to *G. thymalli* from grayling (Figure 1). The species can be differentiated by trained morphologists on the basis of the shape of the marginal hook sickle. *Gyrodactylus teuchis* has a longer and more constantly curved sickle blade, while *G. thymalli* has a small angle on the shaft of the sickle (Cunningham *et al.,* 2001; McHugh *et al*., 2000; Shinn *et al.,* 2004).

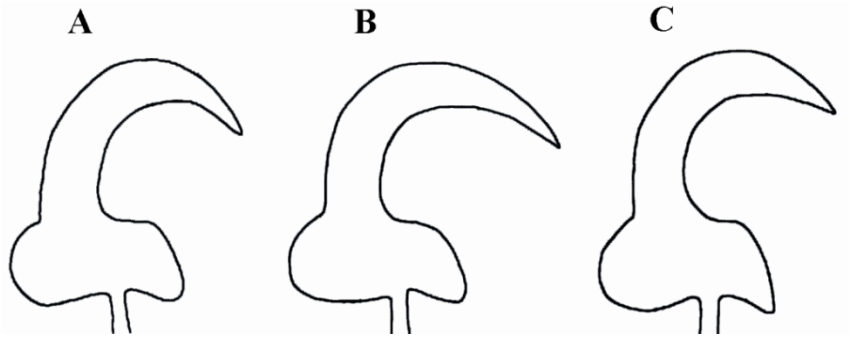

Figure 1. *Marginal hooks of* (*A*) *Gyrodactylus salaris*, (*B*) *G. teuchis and* (*C*) *G. thymalli.*
Drawings are modified after Cunningham et al., *2001.*

4.3.1.1.1. Wet mounts

Not applicable.

4.3.1.1.2. Smears

Not applicable.

4.3.1.1.3. Fixed sections

Not applicable.

4.3.1.2. Agent isolation and identification

4.3.1.2.1. Cell culture/artificial media

Not applicable.

4.3.1.2.2. Antibody-based antigen detection methods

Not applicable.

4.3.1.2.3. Molecular techniques

Preparation of samples

Template DNA should be prepared from live/fresh or EtOH-preserved specimens using a suitable DNA preparation protocol. A DNA extraction kit may be used in accordance with the manufacturer's recommendations.

4.3.1.2.3.1. Analysis of the ribosomal RNA gene internal transcribed spacer region

i) PCR amplification of the internal transcribed spacer (ITS)

For amplification of a 1300 base pair product of the ITS-region, primers, such as 5'-TTT-CCG-TAG-GTG-AAC-CT-3' and 5'-TCC-TCC-GCT-TAG-TGA-TA-3', may be used. The cycling conditions for PCR are as follows, initial denaturation at 95°C for 5 minutes; 30 cycles of 94°C for 1 minute, 50°C for 1 minute, 72°C for 2 minutes; final extension at 72°C for 7 minutes (Cunningham, 1997). If partially degraded material is analysed, the ITS1 and ITS2 spacers can be amplified in two separate reactions using primer sets and PCR conditions described in Matejusova *et al*. (2001).

ii) ITS sequencing and sequence analysis

Amplified ITS fragments prepared as in Section 4.3.1.2.3.1.i above should be sequenced and the sequences subjected to a BLAST search in GenBank/EMBL to establish identity with known sequences. In addition to the PCR primers, at least two internal primers should be used such as; 5'-ATT-TGC-GTT-CGA-GAG-ACC-G and 5'-TGG-TGG-ATC-ACT-CGG-CTC-A (Ziętara & Lumme, 2003). Several sequences of other species infecting salmonids, e.g. *G derjavini*, *G. derjavinoides*, *G. truttae*, *G. teuchis* and *G. thymalli* are available in GenBank/EMBL. *Gyrodactylus salaris* and *G. thymalli* cannot be distinguished by this method, but sequences of ITS distinguishes *G. salaris* and *G. thymalli* from all other known species.

~~Note: Several sequences of *G. salaris* and *G. thymalli* are available in GenBank/EMBL, all differing by only a few point mutations, but with no specific mutations that distinguish *G. salaris* from *G. thymalli*.~~

4.3.1.2.3.2. Analysis of the mitochondrial cytochrome oxidase I gene

i) PCR amplification of the mitochondrial cytochrome oxidase 1 (CO1) gene

For amplification of the CO1-gene, the primers 5'-TAA-TCG-GCG-GGT-TCG-GTA-A-3' and 5'-GAA-CCA-TGT-ATC-GTG-TAG-CA-3') (Meinilä *et al.,* 2002) may be used. The cycling conditions for PCR are as follows, initial denaturation at 95°C for 5 minutes; 35 cycles of 95°C for 1 minute, 50°Cfor1 minute, 72°C for 2 minutes; final extension at 72°C for 7 minutes. Additional primer sets for amplification of CO1 can be found in references: 4 Meinilä *et al.,* 2002; 2004.

ii) CO1 sequencing and sequence analysis

Amplified CO1 fragments prepared as described above should be sequenced and compared with other sequences using a BLAST search in GenBank/EMBL. In addition to the PCR primers, at least two internal primers can be used, such as 5'-CCA-AAG-AAC-CAA-AAT-AAG-TGT-TG-3'), and 5'-TGT-CYC-TAC-CAG-TGC-TAG-CCG-CTG-G-3' (Hansen *et al*., 2003).

If the obtained sequence does not have a 100% match in GenBank/EMBL, a phylogenetic analysis should be performed to establish the relationship to other available sequences. Different clades of *G. salaris* and *G. thymalli* can be distinguished with this method.

NOTE: CO1 sequences cannot unambiguously differentiate between *G. salaris* and *G. thymalli* but can be used to assign specimens to a clade. Clades of *G. salaris* and *G. thymalli* generally correspond well to host preferences and/or the geographical distribution of the parasites, with a few exceptions. ~~CO1 cannot be applied as a pathogenicity marker.~~

Note that some researchers have chosen to submit all their sequences from both Atlantic salmon and grayling as *G. salaris*, causing confusion when comparing sequences (both ITS and CO1) with those in GenBank/EMBL in a BLAST search. Host identity of sequences in GenBank/EMBL should thus always be checked.

Where the sequence is not assigned to one of the recognised clades of *G. salaris* or *G.thymalli,* advice should be sought from the OIE Reference Laboratory.

4.3.1.2.4. Agent purification

Not applicable.

4.3.2. Serological methods

Not applicable.

5. Rating of tests against purpose of use

Not applicable.

6. Test(s) recommended for targeted surveillance to declare freedom from infection with *G. salaris*

Diagnostic/detection methods to declare freedom are the same as those mentioned in for Section 4.3.

7. Corroborative diagnostic criteria

7.1. Definition of suspect case

Observation of *Gyrodactylus* specimen(s) on Atlantic salmon or rainbow trout (or other susceptible hosts) either in skin scrapings examined in a light microscope or on fins or skin examined under a stereo-microscope.

7.2. Definition of confirmed case

~~A molecular identification of *Gyrodactylus* specimen(s) to *G. salaris* (or *G. thymalli*) by sequencing of ITS followed by sequencing and phylogenetic analysis of CO1 to assign the sequence to the nearest known relative is preferred. Trained morphologists can perform morphological identification of *Gyrodactylus* specimen(s) to *G. salaris* based on structures of the attachment organ. However, a morphological diagnosis should be confirmed by molecular tools. A combination of both morphological and molecular methods as described in this chapter is recommended.~~

Infection with *G. salaris* shall be confirmed if the following criteria are met:

i) Morphology consistent with *G. salaris*;

Or

ii) Molecular identification of *Gyrodactylus* specimen(s) to *G. salaris* (or *G. thymalli*) by sequencing of ITS followed by sequencing and phylogenetic analysis of CO1 to assign the sequence to the nearest known relative is preferred.

8. References

Appleby C. & Mo T.A. (1997). Population Dynamics of *Gyrodactylus salaris* (Monogenea) Infecting Atlantic Salmon, *Salmo salar*, Parr in the River Batnfjordselva, Norway. *J. Parasitol.*, **83**, 23–30. https://doi.org/10.2307/3284312.

Bakke T.A., Harris P.D. & Cable J. (2002). Host specificity dynamics: observations on gyrodactylid monogeneans. *Int. J. Parasitol.,* **32**, 281–308.

Bakke T.A., Harris P.D., Hansen H., Cable J. & Hansen L. P. (2004). Susceptibility of Baltic and East Atlantic salmon *Salmo salar* stocks to *Gyrodactylus salaris* (Monogenea). *Dis. Aquat. Org.,* **58**, 171–177.

Buchmann K. & Bresciani J. (1997). Parasitic infections in pond-reared rainbow trout Oncorhynchus mykiss in Denmark. *Dis. Aquat. Org.*, **28**, 125–138. https://doi.org/10.3354/dao028125

Cunningham C.O. (1997). Species variation within the internal transcribed spacer (ITS) region of *Gyrodactylus* (Monogenea: Gyrodactylidae) ribosomal RNA genes. *J. Parasitol*., **83**, 215–219.

Cunningham C.O., Mo T.A., Collins C.M., Buchmann K., Thiery R., Blanc G. & Lautraite A. (2001). Redescription of *Gyrodactylus teuchis* Lautraite, Blanc, Thiery, Daniel & Vigneulle, 1999 (Monogenea: Gyrodactylidae), a species identified by ribosomal RNA sequence. *Syst. Parasitol*., **48**, 141–150.

HANSEN H., BACHMANN L. & BAKKE T.A. (2003). Mitochondrial DNA variation of *Gyrodactylus* spp. (Monogenea, Gyrodactylidae) populations infecting Atlantic salmon, grayling, and rainbow trout in Norway and Sweden. *Int. J. Parasitol.,* **33**, 1471–1478.

HANSEN H., BAKKE T.A. & BACHMANN L. (2007a). DNA taxonomy and barcoding of monogenean parasites: lessons from *Gyrodactylus*. *Trends Parasitol.*, **23** (8), 363–367.

HANSEN H., BAKKE T.A. & BACHMANN L. (2007b). Mitochondrial haplotype diversity of *Gyrodactylus thymalli* (Platyhelminthes; Monogenea): extended geographic sampling in United Kingdom, Poland, and Norway reveals further lineages. *Parasitol. Res.,* **100**, 1389–1394.

HARRIS P.D., CABLE J., TINSLEY R.C. & LAZARUS C.M. (1999). Combined ribosomal DNA and morphological analysis of individual gyrodactylid monogeneans. *J. Parasitol.,* **85**, 188–191.

JENSEN A.J. & JOHNSEN B.O. (1992). Site Specificity of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea) on Atlantic Salmon (*Salmo salar* L.) in the River Lakselva, northern Norway. *Can. J. Zool.*, **41**, 264–267.

JOHNSEN B.O. & JENSEN A.J. (1991). The Gyrodactylus story in Norway. *Aquaculture*, **98**, 289–302. https://doi.org/10.1016/0044-8486(91)90393-L

JØRGENSEN T.R., LARSEN T.B., JØRGENSEN L.G., BRESCIANI J., KANIA P. & BUCHMANN K. (2007). Characterisation of a low pathogenic strain of *Gyrodactylus salaris* from rainbow trout. *Dis. Aquat. Org.,* **73**, 235–244.

LINDENSTRØM T., COLLINS C.M., BRESCIANI J., CUNNINGHAM C.O. & BUCHMANN K. (2003). Characterization of a *Gyrodactylus salaris* variant: infection biology, morphology and molecular genetics. *Parasitology,* **127**, 165–177.

MALMBERG G. (1957). Om förekomsten av *Gyrodactylus* på svenska fiskar. *Skr. söd. Sver. Fisk För.,* (*Årsskr.*) 1956, 19–76. (In Swedish, species descriptions and summary in English).

MALMBERG G. (1970). The excretory systems and the marginal hooks as a basis for the systematics of *Gyrodactylus* (Trematoda, Monogenea). *Ark. Zool.* [*Ser. 2*], **23**, 1–235.

MATEJUSOVÁ I., GELNAR M., MCBEATH A.J.A., COLLINS C.M. & CUNNINGHAM C.O. (2001). Molecular markers for gyrodactylids (Gyrodactylidae: Monogenea) from five fish families (Teleostei). *Int. J. Parasitol.,* **31**, 738–745.

MCHUGH E.S., SHINN A.P. & KAY J.W. (2000). Discrimination of the notifiable pathogen *Gyrodactylus salaris* from *G. thymalli* (Monogenea) using statistical classifiers applied to morphometric data. *Parasitology,* **121**, 315–323.

MEINILÄ M., KUUSELA J., ZIĘTARA M. & LUMME J. (2002) Primers for amplifying approximately 820 bp of highly polymorphic mitochondrial COI gene of *Gyrodactylus salaris. Hereditas,* **137**, 72–74.

MEINILÄ M., KUUSELA J., ZIĘTARA M.S. & LUMME J. (2004). Initial steps of speciation by geographic isolation and host switch in salmonid pathogen *Gyrodactylus salaris* (Monogenea: Gyrodactylidae). *Int. J. Parasitol.,* **34**, 515–526.

MO T.A. (1991a). Seasonal variations of opisthaptoral hard parts of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea: Gyrodactylidae) on parr of Atlantic salmon *Salmo salar* L. in the River Batnfjordselva, Norway. *Syst. Parasitol.*, **19**, 231–240.

MO T.A. (1991b). Variations of opisthaptoral hard parts of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea: Gyrodactylidae) on rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) in a fish farm, with comments on the spreading of the parasite in south-eastern Norway. *Syst. Parasitol.*, **20**, 1–9.

MO T.A. (1991c). Variations of opisthaptoral hard parts of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea: Gyrodactylidae) on parr of Atlantic salmon *Salmo salar* L. in laboratory experiments. *Syst. Parasitol.*, **20**, 11–19.

MO T.A. (1992). Seasonal variations in the prevalence and infestation intensity of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea: Gyrodactylidae) on Atlantic salmon parr, *Salmo salar* L., in the River Batnfjordselva, Norway. *J. Fish Biol.*, **41**, 697–707.

OLSTAD K., CABLE J., ROBERTSEN G. & BAKKE T. A. (2006). Unpredicted transmission strategy of *Gyrodactylus salaris* (Monogenea: Gyrodactylidae): survival and infectivity of parasites on dead hosts. *Parasitology,* **133**, 33–41.

OLSTAD K., ROBERTSEN G., BACHMANN L. & BAKKE T.A. (2007A). Variation in host preference within *Gyrodactylus salaris* (Monogenea): an experimental approach. *Parasitology,* **134**, 589–597.

OLSTAD K., SHINN A.P., BACHMANN L. & BAKKE T.A. (2007B). Host-based identification is not supported by morphometrics in natural populations of *Gyrodactylus salaris* and G. *thymalli* (Platyhelminthes, Monogenea). *Parasitology,* **134**, 2041–2052.

PALADINI G., GUSTINELLI A., FIORAVANTI M.L., HANSEN H. & SHINN A.P. (2009). The first report of *Gyrodactylus salaris* Malmberg 1957 (Platyhelminthes, Monogenea) on Italian cultured stocks of rainbow trout (*Oncorhynchus mykiss* Walbaum). *Vet. Parasitol*., **165** (3–4), 290–297.

POLÉO A.B.S., SCHJOLDEN J., HANSEN H., BAKKE T.A., MO T.A., ROSSELAND B.O. & LYDERSEN E. (2004). The effect of various metals on *Gyrodactylus salaris* (Platyhelminthes, Monogenea) infections in Atlantic salmon (*Salmo salar*). *Parasitology,* **128**, 169–177.

ROBERTSEN G., HANSEN H., BACHMANN L. & BAKKE T. A. (2007). Arctic charr (*Salvelinus alpinus*) is a suitable host for *Gyrodactylus salaris* (Monogenea, Gyrodactylidae) in Norway. *Parasitology,* **134**, 257–267.

ROKICKA M., LUMME J. & ZIĘTARA M. (2007). Identification of *Gyrodactylus* ectoparasites in Polish salmonid farms by PCR-RFLP of the nuclear ITS segment of ribosomal DNA (Monogenea, Gyrodactylidae). *Acta Parasitologica,* **52**, 185–195.

SALTE R., BENTSEN H.B., MOEN T., TRIPATHY S., BAKKE T.A., ØDEGÅRD J., OMHOLT S. HANSEN L.P. (2010). Prospects for a genetic mangement strategy to control *Gyrodactylus salaris* infection in wild Atlantic salmon (*Salmo salar*) stocks. *Can. J. Fish. Aquat. Sci.*, **67**, 121–129.

SHINN A.P., HANSEN H., OLSTAD K., BACHMANN L. & BAKKE T.A. (2004). The use of morphometric characters to discriminate specimens of laboratory-reared and wild populations of *Gyrodactylus salaris* and *G. thymalli* (Monogenea). *Folia Parasitol.,* **51**, 239–252.

SOLENG A. & BAKKE T.A. (1997). Salinity tolerance of *Gyrodactylus salaris* (Platyhelminthes, Monogenea): laboratory studies. *Can. J. Fish. Aquat. Sci.*, **55**, 1837–1845.

SOLENG A., POLEO A.B.S., ALSTAD N.E.W. & BAKKE T. A. (1999). Aqueous aluminium eliminates *Gyrodactylus salaris* (Platyhelminthes, Monogenea) infections in Atlantic salmon. *Parasitology,* **119**, 19–25.

ZIĘTARA M.S. & LUMME J. (2003). The crossroads of molecular, typological and biological species concepts: two new species of *Gyrodactylus* Nordmann, 1832 (Monogenea: Gyrodactylidae). *Syst. Parasitol.*, **55**, 39–52.

ZIĘTARA M.S., ROKICKA, M., STOJANOVSKI S., SKORKOWSKI E.F. & LUMME J. (2007). Alien mitochondrial DNA in variant clones of *Gyrodactylus salaris* indicates a complex hybrid history in salmonid farms. 7th International Symposium on Fish Parasites, Viterbo, Italy. *Parassitologia*, **49**, 119.

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* *

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

NB: FIRST ADOPTED IN 1997 AS GYRODACTYLOSIS OF ATLANTIC SALMON (*GYRODACTYLUS F*);
MOST RECENT UPDATES ADOPTED IN 2012.

Annex 26

CHAPTER 2.3.5.

INFECTION WITH HPR-DELETED OR HPR0 INFECTIOUS SALMON ANAEMIA VIRUS

EU position

The EU thanks the OIE and in general supports the adoption of this modified chapter.

Comments are inserted in the text below.

1. Scope

~~For the purpose of this chapter,~~ Infection with infectious salmon anaemia virus (ISAV) means infection with the pathogenic agent highly polymorphic region (HPR)-deleted ISAV ~~or HPR0 ISAV (with a non-deleted HPR),~~ or the non-pathogenic HPR0 (non-deleted HPR) ISAV of the Genus *Isavirus* of the Family *Orthomyxoviridae*.

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

Detection of HPR0 ISAV has never been associated with clinical signs of ~~infection with ISAV~~ disease ~~ISA~~ in Atlantic salmon (Christiansen *et al.,* 2011). This virus genotype replicates transiently and has mainly been localised to the gills. A link between non-pathogenic HPR0 ISAV and pathogenic HPR-deleted ISAV, with some outbreaks potentially occurring as a result of the emergence of HPR-deleted ISAV from HPR0 ISAV has been suggested (Cardenas *et al.,* 2014; Christiansen *et al.,* 2017; Cunningham *et al*., 2002; Gagné & Leblanc, 2017; Mjaaland, *et al*., 2002).

EU comment

The EU notes that in point 1 above, whereas it is stated that HPR-deleted ISAV may cause disease, the text then refers to "clinical signs of disease" never having been associated with HPR0 ISAV. For reasons of consistency, and bearing in mind the Glossary definition of disease in the Aquatic Manual (which encompasses both clinical and subclinical infection), the EU would suggest using the term "clinical disease" in both cases.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent, agent strains

ISAV is an enveloped virus, 100–130 nm in diameter, with a genome consisting of eight single-stranded RNA segments with negative polarity (Dannevig *et al.,* 1995). The virus has haemagglutinating, receptor-destroying and fusion activity (Falk *et al*., 1997; Mjaaland *et al*., 1997; Rimstad *et al*., 2011).

The morphological, physiochemical and genetic properties of ISAV are consistent with those of the *Orthomyxoviridae,* and ISAV has been classified as the type species of the genus *Isavirus* (Kawaoka *et al*., 2005) within this virus family. The nucleotide sequences of all eight genome segments, encoding at least ten proteins, have been described (Clouthier *et al.,* 2002; Rimstad *et al*., 2011), including the 3' and 5' non-coding sequences (Kulshreshtha *et al.,* 2010). Four major structural proteins have been identified, including a 68 kDa nucleoprotein, a 22 kDa matrix protein, a 42 kDa haemagglutinin-esterase (HE) protein responsible for receptor-binding and receptor-destroying activity, and a 50 kDa

surface glycoprotein with putative fusion (F) activity, encoded by genome segments 3, 8, 6 and 5, respectively. Segment 1, 2, and 4 encode the viral polymerases PB2, PB1 and PA. The two smallest genomic segments, segments 7 and 8, each contain two open reading frames (ORF). The ORF1 of segment 7 encodes a protein with type I interferon antagonistic properties, while ORF2 has been suggested to encode for a nuclear export protein (NEP). Whether the ORF1 gene product is nonstructural or a structural component of the virion remains to be determined. The smaller ORF1 of segment 8 encodes the matrix protein, while the larger ORF2 encodes an RNA-binding structural protein also with type I interferon antagonistic properties.

Sequence analysis of various gene segments has revealed differences between isolates both within and between defined geographical areas. ~~According to sequence differences in the 5' region of the HE gene, ISAV isolates have been divided into two major groups, one European and one North American group~~. According to sequence differences in a partial sequence of segment 6 ~~all eight genomic segments~~, two groups have been ~~are clearly~~ defined: one designated as a European clade and one designated as a North American clade (Gagné & LeBlanc, 2017). In the HE gene, a small HPR near the transmembrane domain has been identified. This region is characterised by the presence of gaps rather than single-nucleotide substitutions (Cunningham *et al*., 2002; Mjaaland *et al*., 2002). A full-length gene (HPR0) has been suggested to represent a precursor from which all ISAV HPR-deleted (pathogenic) variants of ISAV originate. The presence of non-pathogenic HPR0 ISAV genome has been reported in both apparently healthy wild and farmed Atlantic salmon, but has not been detected in ~~diseased~~ fish with clinical disease and pathological signs consistent with infection with HPR-deleted ISAV ~~ISA~~ (Christiansen *et al*., 2011; Cunningham *et al*., 2002; Lyngstad *et al*., 2012; Markussen *et al*., 2008; McBeath *et al*., 2009; Nylund *et al*., 2007). A mixed infection with ~~of~~ HPR-deleted and HPR0 ISAV variants has been reported (Cardenas *et al.,* 2014; Kibenge *et al.,* 2009). Recent studies show that HPR0 ISAV variants occur frequently in sea-reared Atlantic salmon. ~~The~~ HPR0 ISAV ~~strain seems to be more~~ is seasonal and transient in nature and displays a tissue tropism with high prevalence in gills (Christiansen *et al*., 2011; Lyngstad *et al*., 2011). To date there has been no direct evidence linking the presence of HPR0 ISAV to a ~~subsequent~~ clinical disease ~~infection with ISAV ISA~~ outbreak. The risk of emergence of pathogenic HPR-deleted ISAV variants from a reservoir of HPR0 ISAV is considered to be low but not negligible (Cardenas *et al.,* 2014; Christiansen *et al.,* 2011; 2017; EFSA, 2012; Lyngstad *et al.,* 2012).

In addition to the variations seen in the HPR of the HE gene, other gene segments may also be of importance for development of clinical disease ~~infection with ISAV ISA~~. A putative virulence marker has been identified in the fusion (F) protein. Here, a single amino acid substitution, or a sequence insertion, near the protein's putative cleavage site has been found to be a prerequisite for virulence (Kibenge *et al.,* 2007; Markussen *et al*., 2008). Aside from insertion/recombination, ISAV also uses gene segment reassortment in its evolution, with potential links to virulence (Cardenas *et al.,* 2014; Devold *et al*., 2006; Gagné & Leblanc, 2017; Markussen *et al*., 2008; Mjaaland *et al*., 2005).

2.1.2. Survival outside the host

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

2.1.3. Stability of the agent (effective inactivation methods)

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

2.1.4. Life cycle

The main route of infection ~~route~~ is most likely through the gills for both HPR0 and HPR-deleted ISAV, but infection via the intestine or skin cannot be excluded. HPR-deleted ISAV has been used in the studies referred to below. Endothelial cells lining blood vessels seem to be the primary target cells for ISAV as demonstrated by electron microscopy immunohistochemistry and *in-situ* hybridisation. Virus

replication has also been demonstrated in leukocytes, and sinusoidal macrophages in kidney tissue stain positive for ISAV using immunohistochemistry (IHC). As endothelial cells are the target cells (see Section 2.2.4), virus replication may occur in any organ (Aamelfot *et al.,* 2012; Rimstad *et al*., 2011).

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

~~The route of~~ Shedding of ISAV from infected fish may occur ~~be~~ through natural excretions/secretions.

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

2.2. Host factors

2.2.1. Susceptible host species

~~Natural outbreaks of ISA have only been recorded in farmed Atlantic salmon, and in Coho salmon (*Oncorhynchus kisutch*) in Chile (Kibenge *et al*, 2001). Subclinically infected feral Atlantic salmon, brown trout and sea trout (*S. trutta*) have been identified by RT-PCR (Kibenge *et al*., 2004; Plarre *et al*., 2005). In marine fish, detection of ISAV by RT-PCR has been reported in tissues of pollock (*Pollachius virens*) and cod (*Gadus morhua*), but only in fish collected from cages with Atlantic salmon exhibiting ISA (MacLean SA *et al*., 2003). Following experimental infection by bath immersion, ISAV has been detected by RT-PCR in rainbow trout (*Oncorhynchus mykiss*) (Biacchesi *et al*., 2007) and herring (*Clupea harengus*), the latter in a subsequent transmission to Atlantic salmon. Attempts have been made to induce infection or disease in pollock, *Pollachius virens*, but with negative results.~~

Species that fulfil the criteria for listing as susceptible to infection with ISAV according to Chapter 1.5. of the Aquatic Animal Health Code (Aquatic Code) include: ~~amago trout (*Oncorhynchus masou*),~~ Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*).

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence for susceptibility according to Chapter 1.5. of the *Aquatic Code* include: Atlantic herring (*Clupea harengus*) and amago trout (*Oncorhynchus masou*).

In addition, pathogen-specific positive PCR results have been reported in the following organisms, but an active infection has not been demonstrated: coho salmon (*Oncorhynchus kisutch*).

2.2.3~~2~~. Susceptible stages of the host

In Atlantic salmon, life stages from yolk sac fry to adults are known to be susceptible. Disease outbreaks are mainly reported in seawater cages, and only a few cases have been reported in the freshwater stage, including one case in yolk sac fry (Rimstad *et al*., 2011). Infection with HPR-deleted ISAV ~~ISA~~ ISA has been experimentally induced in both Atlantic salmon fry and parr kept in freshwater. ~~Genetics may also play an important role in the susceptibility of Atlantic salmon to infection with ISAV ISA, as differences in susceptibility among different family groups have been observed~~.

2.2.4~~3~~. Species or subpopulation predilection (probability of detection)

HPR-deleted ~~forms of infection with~~ ISAV ~~ISA is primarily a~~ is only known to cause clinical disease ~~of~~ in Atlantic salmon.

2.2.5~~4~~. Target organs and infected tissue

For fish that have developed infection with HPR-deleted ISAV ~~ISA:~~ endothelial cells in all organs become infected (gills, heart, liver, kidney, spleen and others) (Aamelfot *et al.,* 2012). HPR0 ISAV

~~variants~~ seem primarily to target epithelial cells of the gills (Aamelflot *et al.*, 2016), but ~~this variant~~ has also been detected in kidney and heart (Christiansen *et al.,* 2011; Lyngstad *et al.,* 2011).

2.2.6~~5~~. Persistent infection ~~with lifelong carriers~~

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

2.2.7~~6~~. Vectors

~~Passive transfer~~ Transmission of ISAV by salmon lice (*Lepeophtheirus salmonis and Caligus rogercresseyi* (Oelkers *et al.,* 2014)) has been demonstrated under experimental conditions. Although natural vectors have not been identified, several different vector groups could be possible vectors under certain defined conditions (~~reviewed in~~ Rimstad *et al.,* 2011).

2.2.8~~7~~. Known or suspected wild aquatic animal carriers

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

2.3. Disease pattern

2.3.1. Transmission mechanisms

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

Many ~~ISA~~ outbreaks of clinical disease caused by ~~infection with~~ HPR-deleted ISAV in Norway appear to be isolated in space and time from other outbreaks with unknown sources of infection (Aldrin *et al*., 2011). A suggested hypothesis for disease emergence is occasional transition of HPR0 ISAV into HPR-deleted ISAV ~~variants~~ causing solitary outbreaks or local epidemics through local transmission (Lyngstad *et al.,* 2011; 2012). The risk of emergence of HPR-deleted ISAV ~~variants~~ from ~~a reservoir of~~ HPR0 ISAV is considered to be low but not negligible (EFSA, 2012). A direct link between HPR0 ~~variants~~ and HPR-deleted ISAV remains to be demonstrated (Cardenas *et al.,* 2014; Gagné & Leblanc, 2017).

As infection with ISAV ~~ISA~~ has also been reported from smolt-producing sites with Atlantic salmon, transmission of ISAV from parent to progeny cannot be excluded. Even though there is no evidence of true vertical transmission, eggs and embryos could be a risk of transmission if ~~ISAV~~ biosecurity measures are not adequate (Mardones *et al.,* 2014; Marshall *et al.,* 2014; Rimstad *et al*., 2011).

2.3.2. Prevalence

In ~~a~~ net pens containing diseased fish, the prevalence of HPR-deleted ISAV may vary widely, while in adjacent net pens (without diseased fish) ISAV may be difficult to detect, even by the most sensitive methods. Therefore, for diagnostic investigations it is important to sample from net pens containing diseased fish.

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

2.3.3. Geographical distribution

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

EU comment

For consideration – it may be beneficial to include, in section 2.3.3. above, updated information for the UK covering the 2008/2009 outbreak in the Scottish Shetland Islands, summary report with more details is available via the following reference:

https://data.marine.gov.scot/dataset/report-epidemiology-and-control-outbreak-infectious-salmon-anaemia-shetland-islands-scotland.

2.3.4. Mortality and morbidity

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

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

2.3.5. Environmental factors

Generally, outbreaks of infection with HPR-deleted ISAV ~~ISA~~ tend to be seasonal with most outbreaks in late spring and late autumn; however outbreaks can occur at any time of the year. Handling of fish (e.g. sorting or treatment, splitting or moving of cages) may initiate disease outbreaks on infected farms, especially if long-term undiagnosed problems have been experienced in advance (Lyngstad *et al*., 2008).

2.4. Control and prevention

2.4.1. Vaccination

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

2.4.2. Chemotherapy

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

2.4.3. Immunostimulation

Not applicable.

2.4.4. Resistance breeding

Differences in susceptibility among different family groups of Atlantic salmon in fresh water have been observed in challenge experiments and in field tests, indicating the potential for resistance breeding (Gjøen *et al*., 1997).

2.4.5. Restocking with resistant species

Not applicable.

2.4.6. Blocking agents

Not applicable.

2.4.7. Disinfection of eggs and larvae

Disinfection of eggs according to standard procedures is suggested as an important control measure (chapter 4.4 of the *Aquatic Code*).

2.4.8. General husbandry practices

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

3. Sampling

3.1. Selection of individual specimens

For detection of HPR-deleted ISAV, fish displaying clinical signs or gross pathology should be sampled.

For detection of HPR0 ISAV, randomly selected individuals should be sampled at different time points throughout the production cycle.

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

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

3.2. Preservation of samples for submission

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

3.3. Pooling of samples

Pooling of samples may be acceptable, however, the impact on sensitivity and design prevalence must be considered.

3.4. Best organs or tissues

3.4.1. Detection of HPR-deleted ISAV

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

Virological examination (cell culture and real-time or conventional RT-PCR): heart (should always be included) and mid-kidney;

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

Immunofluorescence (smears): mid-kidney;

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

3.4.2. Detection of HPR0 ISAV

Gill tissue ~~Gills should be tested by RT-PCR~~.

3.5. Samples/tissues that are not suitable

None known.

4. Diagnostic methods

4.1. Field diagnostic methods

4.1.1. Clinical signs

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

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

Affected fish are generally in good condition, but diseased fish have no feed in the digestive tract.

4.2. Pathological evaluation

4.2.1. Gross pathology

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

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

4.2.2. Clinical chemistry

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

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

4.2.3. Microscopic pathology

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

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

4.2.4. Wet mounts

Not applicable.

4.2.5. Smears

See Section 4.3.1.1.2.

4.2.6 Fixed sections

See Section 4.3.1.1.3.

4.2.7. Electron microscopy/cytopathology

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

~~4.2.8. Differential diagnoses~~

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

4.3. Agent detection and identification methods

4.3.1. Direct detection methods

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

4.3.1.1. Microscopic methods

4.3.1.1.1. Wet mounts

Not applicable.

4.3.1.1.2. Smears

4.3.1.1.2.1 Indirect fluorescent antibody test

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

i) Preparations of tissue smears (imprints)

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

ii) Staining procedure

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

4.3.1.1.3. Fixed sections

4.3.1.1.3.1 Immunohistochemistry (IHC)

Polyclonal antibody against HPR-deleted ISAV nucleoprotein is used on paraffin sections from formalin-fixed tissue. This IHC staining has given positive reactions in both experimentally and naturally infected Atlantic salmon. Preferred organs are mid-kidney and heart (transitional area including all three chambers and valves). Suspected cases due to pathological signs are verified with a positive IHC. Histological sections are prepared according to standard methods.

i) Preparation of tissue sections

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

ii) Staining procedure for IHC

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

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

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

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

iii) Interpretation

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

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

4.3.1.2. Agent isolation and identification

4.3.1.2.1. Cell culture

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

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

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

i) Inoculation of cell monolayers

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

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

ii) Monitoring incubation

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

iii) Subcultivation procedure

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

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

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

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

4.3.1.2.2. Antibody-based antigen detection methods

4.3.1.2.2.1 Virus identification by IFAT

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

i) Prepare monolayers of cells in appropriate tissue culture plates (e.g. 96-well or 24-well plates), in slide flasks or on cover-slips dependent on the type of microscope available (an inverted microscope equipped with UV light is necessary for monolayers grown on tissue

culture plates). SHK-1 cells grow rather poorly on glass cover-slips. The necessary monolayers for negative and positive controls must be included.

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

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

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

v) Incubate with FITC-conjugated goat anti-mouse immunoglobulin for 1 hour (or if antibody raised in rabbits is used as the primary antibody, use FITC-conjugated antibody against rabbit immunoglobulin), according to the instructions of the supplier. To increase the sensitivity, FITC-conjugated goat anti-mouse Ig may be replaced with biotin-labelled anti-mouse Ig and FITC-labelled streptavidin with the described rinsing in between the additional step. Rinse once with PBS/0.05% Tween 20, as described above. The nuclei can be stained with 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 shoul be kept in dark until examination. For long periods of storage (more than 2–3 weeksa solution of 1,4-diazabicyclooctane (DABCO 2.5% in PBS, pH 8.2) or similar reagent may be added as an anti-fade solution.

4.3.1.2.3. Molecular techniques

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

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

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

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

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

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

4.3.1.2.3.2 Real-time RT-PCR

4.3.1.2.3.2.1 Sampling

Target organs are normally the heart, kidney and gills. Under surveillance protocols, pooled organs of three fish are recommended while individual analysis of samples is required for confirmatory purposes as well as for molecular studies. Immediately after organ extraction from fish, 0.5 mm^3 slices are fixed in an appropriate independently imbibed in RNAlater (or ethanol) as preservative for RNA in Eppendorf tubes properly labelled to be sent in isothermal containers with cooling units to the diagnostic laboratories. The cold chain should must be maintained during the delivery process.

4.3.1.2.3.2.2 Processing and analysis of samples via real-time RT-PCR

i) RNA extraction

Samples are removed from the RNA preservative, weighed and the sum of the three target organ slices must be kept in the range from 30 to 40 mg. Samples are then homogenised in lysis buffer (according to the kit used) supplemented with 1.4 mm Zirconium oxide beads in an automated Roche's Magnaliser device kit followed by RNA extraction using the E.Z.N.A.® Total RNA Kit (TRK) I (Catalog Number R683402CH), under the following conditions:

Amount of tissue	Amount of TRK lysis buffer	2-Mercaptoetanol
30–40 mg	700 µl	14 µl

ii) Real-time RT-PCR reactions

Three parallel reactions are normally run for each sample, the first two target viral coding segment 8, and the third is a cellular housekeeping gene acting as quality control: (1) carried out according to Snow *et al.* (2006); (2) under an optimised mix named GIM[4]; (3) measures the reporter gene ELF-1α as a reference for the integrity of the RNA recovered.

Mixes are distributed either in ELISA plates or tube strips and kept at 4°C until use. Reactions are recorded using the SuperScript™ III Platinum™ One-Step qRT-PCR Kit, (Catalogue Number 11732088). Each mix is prepared for a final volume of 20 µl considering a maximum simple volume of 4 µl according to the following tables.

Master Mix	Forward primer 20 µM	Reverse primer 20 µM	Probe 20 µM	ROX	Enzyme	Water	Sample	Final volume
10 µl	1 µl	1 µl	0.3 µl	0.4 µl	0.4 µl	4 µl	4 µl	20 µl

Assay	**Primer/probe**	**Sequence**
Snow et al., 2006	Forward	5' TGC TAC ACA GCA GGA TGC AG 3'
	Reverse	5' CAT CTT CTC TGT CGA GCA GGA 3'
	Probe	6FAM CAT CGT CGC TGC AGT TC MGBNFQ
GIM*	Forward	5' ATC AGT AAA CTT CAG AGG AAC ATC 3'
	Reverse	5' GAA ATG AAG ATG TTG CTC AAC 3'
	Probe	5'-/56-FAM/AGC GAC GAT ZEN GAC TCT CTA CTG TGT GAT G-/3IABkFQ/-3'
ELF-1α Sepulveda *et al.*, 2012	Forward	5' GCC CCT CCA GGA YGT YTA CAA 3'
	Reverse	5' CCA CAC GGC CCA CRG GTA C 3'
	Probe	5' /56-FAM/ATC GGY GGT AT+T+G+G+A+AC /3BHQ

*Developed by the OIE Reference Laboratory in Chile.

iii) Sample processing

[4] GIM: Available from the OIE Reference Laboratory in Chile

ELISA plates or strips with reaction mix are taken from 4°C and loaded with adequate volume of samples. Controls are then loaded: a) a positive amplification control (RNA from an ISAV positive reference tissue); b) a negative extraction control (RNA from an ISAV negative reference tissue, extracted along with the testing samples); c) a negative amplification control (free water). Finally, the plates are sealed with parafilm or the tube strips covered and taken to the thermocycler where they are placed before passing by a spin.

iv) Real-time PCR programme

The three reactions (Snow *et al.*, GIM and ELF 1α) are run in parallel and analysed under a simplex format; temperatures for each were carefully set as follows:

Steps	Temperature	Time	Steps
RT	50°C	15 minutes	1
Initial denaturation	95°C	2 minutes	1
Denaturation, annealing and extension	95°C	10 seconds	45
	60°C	1 minute	

4.3.1.2.3.2.3 Interpretation of the results

Results are read and interpreted using the StepOne software version 2.3, according to the following steps:

i) Thresholds are set manually by assigning 0.1 values to the Snow *et al.* and GIM assay and 0.4 to the ELF 1α assay.

i) Controls are checked. If the results are as expected, the reading is continued. If not, the run is aborted.

iii) Ct values for ELF-1α should be within established ranges (14–25) together with a reasonably shaped curve.

iv) Sample results for Snow *et al.* and GIM should give similar Ct values with delta values ranging from 1 to 2 units and share similar curve shapes.

v) Once this procedure is done, results are recorded in a pre-established form and sent to the OIE Reference Laboratory in Chile no later than 24–48 hours upon sample reception.

v) For positive results, a second analysis is required to determine if the putative virus detected is a HPR deleted variant or a HPRO.

4.3.1.2.4. Agent purification

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

4.3.2. Serological methods

None published or validated.

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

5. Rating of tests against purpose of use

As an example, The methods currently available for targeted surveillance for infection with HPR-deleted ISAV and diagnosis of infection with HPR-deleted ISAV ISA are listed in Table 5.1. For surveillance of infection with for HPR0 ISAV, a real-time RT-PCR followed by conventional RT-PCR and sequencing are the only recommended methods (not included in the table). The designations used in the Table indicate: a = the method is the

recommended method for reasons of availability, utility, and diagnostic specificity and sensitivity; b = the method is a standard method with good diagnostic sensitivity and specificity; c = the method has application in some situations, but cost, accuracy, or other factors severely limits its application; and d = the method is presently not recommended for this purpose. These are somewhat subjective as suitability involves issues of reliability, sensitivity, specificity and utility. Although not all of the tests listed as category a or b have undergone formal standardisation and validation, their routine nature and the fact that they have been used widely without dubious results, makes them acceptable.

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

Method	Targeted surveillance for infection with HPR-deleted ISAV				Presumptive diagnosis	Confirmatory diagnosis
	Fry	Parr	Smolt	Adults		
Gross signs	d	d	d	d	c	b
Histopathology	d	d	d	~~b~~ d	b	b
IFAT on kidney imprints	d	d	d	d	b	a
Immunohistochemistry	d	d	d	d	b	a
Isolation in cell culture with virus identification	~~a~~ b	~~a~~ b	~~a~~ b	~~a~~ b	~~a~~ b	a
RT-PCR ~~or real-time RT-PCR followed by sequencing~~	~~a~~ c	~~a~~ c	~~a~~ c	~~a~~ c	b	~~a~~ c
Real-time RT-PCR	a	a	a	a	a	b
Sequencing	d	d	d	d	d	a

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

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

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

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

7. Corroborative diagnostic criteria

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

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

Infection with HPR0 or HPR-deleted ISAV shall be suspected if the following criterion is met:

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

i) Positive conventional RT-PCR or real-time RT-PCR result

In addition, infection with HPR-deleted ISAV shall be suspected if one of the following criteria is met:

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

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

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

iii) Positive IFAT on tissue imprints

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

The presence of ISAV is considered to be confirmed if, in addition to the criteria in Section 7.1, one or more of the following criteria are met:

i) ISAV isolation is carried out in cell culture followed by virus identification by either an antibody-based test (IFAT) and/or conventional PCR followed by sequencing of the amplicon;

ii) ISAV is detected in histological sections by immunoassay using specific anti-ISAV antibodies;

iii) Detection of ISAV in tissue preparations by conventional PCR followed by sequencing of the amplicon

~~7.2.1. Definition of confirmed ISA~~

~~The following criteria should be met for confirmation of ISA: detection of ISAV in tissue preparations by means of specific antibodies against ISAV (IHC on fixed sections [Section 4.3.1.1.3.1] or IFAT on tissue imprints [Section 4.3.1.1.2] or fixed sections as described in Section 4.3.1.1.3) in addition to either:~~

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

~~or~~

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

~~7.2.2 Definition of confirmed HPR-deleted ISAV infection~~

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

~~i) Isolation and identification of ISAV in cell culture from any fish sample on the farm as described in Section 4.3.1.2.1.~~

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

7.3. Definition of confirmed infection with HPR0 ISAV

7.3.1. Definition of confirmed infection with HPR0 ISAV

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

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

8. References

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

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

BIACCHESI S., LE BERRE M., LE GUILLOU S., BENMANSOUR A., BREMONT M., QUILLET E. & BOUDINOT P. (2007). Fish genotype significantly influences susceptibility of juvenile rainbow trout, *Oncorhynchus mykiss* (Walbaum), to waterborne infection with infectious salmon anaemia virus. *J. Fish Dis*., **30**, 631–636.

CÁRDENAS C. CARMONA M., GALLARDO A., LABRA A. & MARSHALL S.H. (2014). Coexistence in field samples of two variants of the infectious salmon anemia virus: a putative shift to pathogenicity. *PLoS One*, **9,** e87832. doi: 10.1371/journal.pone.0087832.

CHRISTIANSEN D.B., MCBEATH A.J.A., AAMELFOT M., MATEJUSOVA I., FOURRIER M., WHITE P., PETERSEN P.E. & FALK K. (2017). First field evidence of the evolution from a non-virulent HPR0 to a virulent HPR-deleted infectious salmon anaemia virus. *J. Gen. Virol*., **98**, 595–606.

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

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

CLOUTHIER S.C., RECTOR T., BROWN N.E.C. & ANDERSON E.D. (2002). Genomic organization of infectious salmon anaemia virus. *J. Gen. Virol.,* **83**, 421–428.

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

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

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

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

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

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

GAGNÉ N. & LEBLANC F. (2017). Overview of infectious salmon anaemia virus (ISAV) in Atlantic Canada and first report of an ISAV North American-HPR0 subtype. *J. Fish Dis*., DOI: 10.1111/jfd.12670

GJØEN H.M., REFSTIE T., ULLA O. & GJERDE B. (1997). Genetic correlations between survival of Atlantic salmon in challenge and field tests. *Aquaculture*, **158**, 277–288.

GUSTAFSON L.L., ELLIS S.K., BEATTIE M.J., CHANG B.D., DICKEY D.A., ROBINSON T.L., MARENGHI F.P., MOFFETT P.J. & PAGE F.H. (2007). Hydrographics and the timing of infectious salmon anemia outbreaks among Atlantic salmon

(*Salmo salar* L.) farms in the Quoddy region of Maine, USA and New Brunswick, Canada. *Prev. Vet. Med.*, **78**, 35–56.

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

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

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

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

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

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

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

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

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

MACLEAN S.A., BOUCHARD D.A. & ELLIS S.K. (2003). Survey of non-salmonid marine fishes for detection of infectious salmon anemia virus and other salmonid pathogens. *In:* Technical Bulletin 1902. International Response to Infectious Salmon Anemia: Prevention, Control and Eradication, Miller O. & Cipriano R.C., eds. USDA, APHIS; US Dept Interior, US Geological Survey; US Dept Commerce, National Marine Fisheries Service, Washington DC, USA, 135–143.

MARDONES F.O., MARTINEZ-LOPEZ B., VALDES-DONOSO P., CARPENTER T.E. & PEREZ A.M. (2014). The role of fish movements and the spread of infectious salmon anemia virus (ISAV) in Chile, 2007–2009. *Prev. Vet. Med.*, 114, 37–46.

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

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

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

MARSHALL S.H, RAMÍREZ R., LABRA A., CARMONA M. & MUÑOZ C. (2014). Bona Fide Evidence for Natural Vertical Transmission of Infectious Salmon Anemia Virus in Freshwater Brood Stocks of Farmed Atlantic Salmon (Salmo salar) in Southern Chile. *J. Virol.*, **88**, 6012–6018. doi: 10.1128/JVI.03670-13.

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

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

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

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

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

OELCKERS K., VIKE S., DUESUND H., GONZALEZ J., WADSWORTH S. & NYLUND A. (2014). *Caligus rogercresseyi* as a potential vector for transmission of Infectious Salmon Anaemia (ISA) virus in Chile. *Aquaculture*, **420–421**, 126–132.

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

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

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

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

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

*
* *

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

NB: FIRST ADOPTED IN 1995 AS INFECTIOUS SALMON ANAEMIA; MOST RECENT UPDATES ADOPTED IN 2014.

Annex 27

CHAPTER 2.2.3.

INFECTION WITH INFECTIOUS HYPODERMAL AND HAEMATOPOIETIC NECROSIS VIRUS

EU position

The EU supports the adoption of this modified chapter.

[…]

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with IHHNV according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) include: ~~giant river prawn (*Macrobrachium rosenbergii* [under study]),~~ yellowleg shrimp (*Penaeus californiensis*), giant tiger prawn (*Penaeus monodon*), northern white shrimp (*Penaeus setiferus*), blue shrimp (*Penaeus stylirostris*), and white leg shrimp (*Penaeus vannamei*).

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 susceptible to infection with IHHNV according to Chapter 1.5. of the *Aquatic Code* include: northern brown shrimp (*Penaeus aztecus*).

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following organisms, but an active infection has not been demonstrated: giant river prawn (*Macrobrachium rosenbergii),* northern pink shrimp (*Penaeus duorarum*), western white shrimp (*Penaeus occidentalis*), *kuruma prawn* (*Penaeus japonicus*), green tiger prawn (*Penaeus semisulcatus*), *Hemigrapsus penicillatus,* Argentine stiletto shrimp (*Artemesia longinaris*), Cuata swimcrab (*Callinectes arcuatus),* Mazatlan sole (*Achirus mazatlanus*), *yellowfin mojarra* (*Gerres cinereus*), tilapias (*Oreochromis* sp.), Pacific piquitinga (*Lile stolifera*) and blackfin snook (*Centropomus medius*).

[…]

CHAPTER 2.2.1.

ACUTE HEPATOPANCREATIC NECROSIS DISEASE

EU position

The EU supports the adoption of this modified chapter.

[…]

1. Scope

Acute hepatopancreatic necrosis disease (AHPND) means infection with strains of *Vibrio parahaemolyticus* (Vp_{AHPND}) that contain a ~70-kbp plasmid with genes that encode homologues of the *Photorhabdus* insect-related (Pir) toxins, PirA and PirB. Although there are reports of the isolation of other *Vibrio* species from clinical cases of AHPND, only Vp_{AHPND} has been demonstrated to cause AHPND.

[…]

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to AHPND according to Chapter 1.5. of the *Aquatic Code* include: giant tiger prawn (*Penaeus monodon*) and whiteleg shrimp (*Penaeus vannamei*).

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 susceptible AHPND ~~for susceptibility~~ according to Chapter 1.5. of the *Aquatic Code* include: fleshy prawn (*Penaeus chinensis*).

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

[…]

Annex 29

GLOSSARY

EU comment

The EU in general supports the proposed changes to the Glossary.

A comment is inserted in the text below.

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~~:

EU comment

The EU suggests italicising the word "biosecurity", as it is a term defined in the Glossary.

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, ~~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~~.

CHAPTER 1.5.

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

EU comment

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

A comment is inserted in the text below.

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, developing or latent *pathogenic agent* has been demonstrated by the occurrence of natural *cases* or by experimental exposure that mimics natural transmission pathways.

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 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 30B (Track 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 of 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 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~~

~~A~~BA. 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

~~B~~CB. no species within the taxonomic ~~group~~ ranking has been found to be refractory to *infection*.

AND

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

Annex 30B (Track changes) (contd)

~~2)~~ Evidence that 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 *susceptible species*;

OR

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

EU comment

The EU suggests defining what exactly is meant by "controlled challenge".

Annex 31

CHAPTER 10.5.

INFECTION WITH SALMONID ALPHAVIRUS

EU comment

The EU supports the proposed changes to this chapter.

[…]

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.: 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 32

CHAPTER 10.7.

INFECTION WITH KOI HERPESVIRUS ~~DISEASE~~

EU comment

The EU in general supports the proposed changes to this chapter.

Comments are inserted in the text below.

[…]

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.: common carp (*Cyprinus carpio carpio*), 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.~~

[…]

EU comment

We have a request to expand on the application of common carp as a common name to ensure this is clearly understood in light of the removal of the separate reference to Ghost carp, as follows:

"common carp including all varieties (*Cyprinus carpio carpio*)".

Indeed, this would make clear that this includes ghost carp, mirror carp etc., given the change from ghost carp previously being listed separately. We request this because common carp as a common name is not widely used for varieties such as ghost carp, or even mirror carp. For example in the UK, it is understood to apply specifically to the fully scaled common carp, with no ghosting etc.

In addition, the EU notes that there are inconsistencies in the French translation of the common names of the various carp species, and would invite the OIE to review the French version of the paragraph above. (This comment is also valid for the corresponding Aquatic Manual chapter.)

Indeed, the French name of Cyprinus carpio is "Carpe commune" and not to "carpe courante". The name "carpe commune" is also used when referring to hybrids, as are discussed in the paragraph above.

Annex 33

CHAPTER 10.9.

INFECTION WITH SPRING VIRAEMIA OF CARP VIRUS

EU comment

The EU in general supports the proposed changes to this chapter.

Comments are inserted in the text below.

[…]

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.: 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 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)* ~~tench *(Tinca tinca)*. These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally~~.

[…]

EU comment

We have a request to expand on the application of common carp as a common name to ensure this is clearly understood in light of the removal of the separate reference to Ghost carp, as follows:

"common carp including all varieties (*Cyprinus carpio carpio*)".

Indeed, this would make clear that this includes ghost carp, mirror carp etc., given the change from ghost carp previously being listed separately. We request this because common carp as a common name is not widely used for varieties such as ghost carp, or even mirror carp. For example in the UK, it is understood to apply specifically to the fully scaled common carp, with no ghosting etc.

This comment is also reflected in Annex 35 - Manual Chapter 2.3.7. – Infection with Koi Herpesvirus.

Furthermore, the scientific name for zebrafish is incorrect, it should be "zebrafish (~~*Sander vitreus*~~ *Danio rerio*)". Indeed, *Sander vitreus* is Walleye.

Finally, we note that the scientific name for "grass carp" should be "*Ctenopharyngodon idella*", instead of "*Ctenopharyngodon idellus*". In addition, to avoid any confusion with the first parenthesis after "grass carp", we would also suggest inserting the words "also called" before "white amur", as we understand this is an alternative common name used in some countries.

Annex 34

CHAPTER 2.3.6.

INFECTION WITH SALMONID ALPHAVIRUS

EU comment

The EU supports the proposed changes to this chapter.

1. Scope

For the purpose of this chapter, infection with salmonid alphavirus (SAV) means infection with any ~~subtype~~ genotype of the pathogenic agent SAV, ~~of the~~ Genus *Alphavirus*, 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 ~~subtypes~~ genotypes (SAV1–SAV6) based solely on nucleic acid sequence for the proteins E2 and nsP3 (Fringuelli *et al.,* 2008). The level of antigenic variation among ~~subtypes~~ genotypes is considered low as monoclonal antibodies (MAbs) raised against a specific SAV ~~subtype~~ genotype are likely to cross react with other SAV isolates (Graham *et al.,* 2014; Jewhurst *et al.,* 2004). The genotype groups and their geographical distributions are presented in the table below (abbreviations: SW= sea water, FW = fresh water):

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)	France, Germany, Italy, Spain, Switzerland, Poland, UK (England, Scotland)
SAV 2 Marine (PD)	Atlantic salmon (SW)	Norway, UK (Scotland)
SAV 3 (PD)	Rainbow trout (SW) Atlantic salmon (SW)	Norway
SAV 4 (PD)	Atlantic salmon (SW)	Ireland, UK (Northern Ireland, Scotland)

SAV 5 (PD)	Atlantic salmon (SW)	UK (Scotland)
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 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: 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, but an active infection has not been demonstrated: Argentine hake (*Merluccius hubbsi*), 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 show that farmed rainbow trout and Atlantic salmon are susceptible at all stages in sea water, probably reflecting a sea water reservoir of SAV. Experimental infection 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.

Annex 34 (contd)

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 SAV 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

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.

Annex 34 (contd)

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 mortality. Clinically diseased fish may be observed swimming slowly at the water surface. In some cases, extremely weak (“sleeping”) fish can be found at the bottom of tanks or in net-cages. An increased number of faecal casts may also be observed in the water. However, it is important to notice that clinical signs are not pathognomonic and that careful observation and examinations of any dead, weak or abnormally behaving fish is necessary.

Initially, nutritional status is usually normal, but in the months after an outbreak or in the later stages of disease, long slender fish (‘runts’) with low condition are typically observed. The development of long, slender fish can be caused by factors other than SAV.

4.2. Clinical methods

4.2.1. Gross pathology

Yellow mucoid gut contents are a usual post-mortem finding, as is typically seen in fish that are not eating. Occasionally signs of circulatory disturbances, such as petechial haemorrhages, small ascites or reddening of the pancreatic region between the pyloric caeca, may be seen. Some diseased fish may show pale hearts or heart ruptures. It is important to 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 develops before the inflammation response in the heart becomes more pronounced. The pancreatic necrotic debris will seemingly disappear and the typical picture of severe loss of exocrine pancreatic tissue will soon appear simultaneously with the increasing inflammation in the heart. Somewhat later, skeletal muscle degeneration, inflammation and fibrosis 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 (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[5] (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.

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[5] 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*.

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:

	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 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.

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.

RT-PCR: Primer and probe sequences	Named	Genomic segment	Product size	Reference
QnsP1F: 5'-CCG-GCC-CTG-AAC-CAG-TT-3' QnsP1R: 5'-GTA-GCC-AAG-TGG-GAG-AAA-GCT-3' QnsP1probe: 5'FAM-CTG-GCC-ACC-ACT-TCG-A-MGB3'	forward primer reverse primer Taqman®probe	QnsP1	107 nt	Hodneland *et al*., 2006
E2F: 5'-CCG-TTG-CGG-CCA-CAC-TGG-ATG-3' E2R: 5'-CCT-CAT-AGG-TGA-TCG-ACG-GCA-G-3'	forward primer reverse primer	E2	516 nt	Fringuelli *et a*l., 2008
nsP3F: 5'-CGC-AGT-CCA-GCG-TCA-CCT-CAT-C-3' nsP3R: 5'-TCA-CGT-TGC-CCT-CTG-CGC-CG-3'	forward primer reverse primer	nsP3	490 nt	Fringuelli *et al.,* 2008

4.3.2. Serological methods

4.3.2.1 Immunoperoxidase-based 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	a
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.

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 microscopically 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), RT-PCR (Section 4.3.1.1.2) or serology (Section 4.3.2).

8. References

ALDRIN M., STORVIK B., FRIGESSI A., VILJUGREIN H. & JANSEN P.A. (2010). A stochastic model for the assessment of the transmission pathways of heart and skeleton muscle inflammation, pancreas disease and infectious salmon anaemia in marine fish farms in Norway. *Prev. Vet. Med.*, **93**, 51–61.

ANDERSEN L., BRATLAND A., HODNELAND K. & NYLUND A. (2007). Tissue tropism of salmonid alphaviruses (subtypes SAV1 and SAV3) in experimentally challenged Atlantic salmon (*Salmon salar* L.). *Arch. Virol.,* **152**, 1871–1883.

ANDERSEN L., HODNELAND H. & NYLUND A. (2010). No influence of oxygen levels on pathogenesis and virus shedding in Salmonid alphavirus (SAV)-challenged Atlantic salmon (*Salmon salar* L.). *Virol. J.*, **7**, 198.

BANG JENSEN B., KRISTOFFERSEN A.B., MYR C. & BRUN E. (2012). Cohort study of effect of vaccination on pancreas disease in Norwegian salmon aquaculture. *Dis. Aquat. Org.*, **102**, 23–31.

BOUCHER P., RAYNARD R.S., HOUGHTON G. & BAUDIN LAURENCIN F. (1995). Comparative experimental transmission of pancreas disease in Atlantic salmon, rainbow trout and brown trout. *Dis. Aquat. Org.*, **22**, 19–24.

BRATLAND A. & NYLUND A. (2009). Studies on the possibility of vertical transmission of Norwegian salmonid Alphavirus in production of Atlantic salmon in Norway. *J. Aquat. Anim. Health*, **21**, 73–78.

CHRISTIE K.E., FYRAND K., HOLTET L. & ROWLEY H.M. (1998) Isolation of pancreas disease virus from farmed Atlantic salmon, *Salmo salar* L., in Norway. *J. Fish Dis*., **21**, 391–394.

CHRISTIE K.E., GRAHAM D.A., MCLOUGHLIN M. F., VILLOING S., TODD D. & KNAPPSKOG D. (2007). Experimental infection of Atlantic salmon *Salmo salar* pre-smolts by i.p. injection of new Irish and Norwegian salmonid alphavirus (SAV) isolates: a comparative study. *Dis. Aquat. Org.*, **75**, 13–22.

FRINGUELLI E., ROWLEY H.M., WILSON J.C., HUNTER R., RODGER H. & GRAHAM D.A. (2008). Phylogenetic analyses and molecular epidemiology of European salmonid alphaviruses (SAV) based on partial E2 and nsP3 gene nucleotide sequences. *J. Fish Dis*., **31**, 811–823.

GRAHAM D.A., BROWN A., SAVAGE P. & FROST P. (2012). Detection of salmon pancreas disease in the faeces and mucus of Atlantic salmon *Salmon salar* by real-time RT-PCR and cell culture following experimental challenge. *J. Fish Dis*., **35**, 949–951.

GRAHAM D.A., CHERRY K., WILSON C.J. & ROWLEY H.M. (2007a). Susceptibility of salmonid alphavirus to a range of chemical disinfectants. *J. Fish Dis*., **30**, 269–277.

GRAHAM D.A., FROST P., MCLAUGHLIN K., ROWLEY H.M., GABESTAD I., GORDON A. & MCLOUGHLIN M.F. (2011). A comparative study of marine salmonid alphavirus subtypes 1–6 using an experimental cohabitation challenge model. *J. Fish Dis*., **34**, 273–286.

GRAHAM D.A., FRINGUELLI E., WILSON C., ROWLEY H.M., BROWN, A., RODGER H., MCLOUGHLIN M.F., MCMANUS C., CASEY E., MCCARTHY L.J. & RUANE N.M. (2010). Prospective longitudinal studies of salmonid alphavirus infections on two Atlantic salmon farms in Ireland; evidence for virual persistence. *J. Fish Dis*., **33**, 123–135.

GRAHAM D.A., JEWHURST V.A., ROWLEY H.M., MCLOUGHLIN M.F. & TODD D. (2003). A rapid immunoperoxidase-basd neutralization assay for salmonid alphavirus used for a serological survey in Northern Ireland. *J. Fish Dis*., **26**, 407–413.

GRAHAM D.A., ROWLEY H.M., FRINGUELLI E., BOVO G., MANFRIN A., MCLOUGHLIN M.F., ZARZA C., KHALILI M. & TODD D. (2007b).First laboratory confirmation of salmonid alphavirus infection in Italy and Spain. *J. Fish Dis*., **30**, 569–572.

GRAHAM D.A., ROWLEY H.M. & FROST P. (2014). Cross-neutralization studies with salmonid alphavirus subtype 1–6 strains: results with sera from experimental studies and natural infections. *J. Fish Dis*., 37, 683–691.

GRAHAM D.A., STAPLES V., WILSON C.J., JEWHURST H., CHERRY K., GORDON A. & ROWLEY H.M. (2007c). Biophysical properties of salmonid alphaviruses: influences of temperature and pH on virus survival. *J. Fish Dis*., **30**, 533–543.

GRAHAM D.A., WILSON C., JEWHURST H. & ROWLEY H. (2008). Cultural characteristics of salmonid alphaviruses – influences of cell line and temperature. *J. Fish Dis*., **31**, 859–868.

HERATH T., COSTA J., THOMPSON K., ADAMS A. & RICHARDS R. (2009). Alternative cell line for the isolation of salmonid alphavirus-1. *Icelandic Agricultural Sci.,* **22**, 19–27.

HODNELAND K. & ENDRESEN C. (2006). Sensitive and specific detection of salmonid alphavirus using real-time PCR (TaqMan). *J. Virol. Methods,* **131**, 184–192.

JANSEN M.D., BANG JENSEN B. & BRUN E. (2014). Clinical manifestations of pancreas disease (PD) outbreaks in Norwegian marine salmon farming – variations due to salmonid alphavirus (SAV) subtype. (Accepted) *J. Fish Dis*., 24 March, doi: 10.1111/jfd.12238

JANSEN M.D., TAKSDAL T., WASMUTH M.A., GJERSET B., BRUN E., OLSEN A.B., BRECK O. & SANDBERG M. (2010a). Salmonid alphavirus (SAV) and pancreas disease (PD) in Atlantic salmon (*Salmo salar* L.) in freshwater and seawater sites in Norway from 2006 to 2008. *J. Fish Dis*., **33**, 391–402.

JANSEN M.D., WASWUTH M.A., OLSEN A.B., GJERSET B., MODAHL I., BRECK O., HALDORSEN R.N., HJELMELAND R., TAKSDAL T. (2010b). Pancreas disease (PD) in sea-reared Atlantic salmon, *Salmon salar* L., in Norway; a prospective, longitudinal study of disease development and agreement between diagnostic test results. *J. Fish Dis*., **33**, 723–736.

JEWHURST V.A., TODD D., ROWLEY H.M., WALKER I.W., WESTON J.H. MCLOUGHLIN M.F & GRAHAM D.A. (2004). Detection and antigenic characterization of salmonid alphavirus isolates fram sera obtained from farmed Atlantic salmon , *salmo salar* L., and farmed rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J. Fish Dis*., **27**, 143–149.

KERBART BOSCHER S., MCLOUGHLIN M., LE VEN A., CABON J., BAUD M. & CASTRIC J. (2006). Experimental transmission of sleeping disease in one-year-old rainbow trout *Onchorhynchus mykiss* (Walbaum), induced by sleeping disease virus. *J. Fish Dis*., **29**, 263–273.

KONGTORP R.T., STENE A., ANDREASSEN P.A., ASPEHAUG V., GRAHAM D.A., LYNGSTAD T.M., OLSEN A.B., OLSEN R.S., SANDBERG M., SANTI N., WALLACE C. & BRECK O. (2010). Lack of evidence for vertical transmission of SAV 3 using gametes of Atlantic salmon, *salmo salar* L., exposed by natural and experimental routes. *J. Fish Dis*., **33**, 879–888.

KRISTOFFERSEN A.B., VILJUGREIN H., KONGTORP R.T., BRUN E. & JANSEN P.A. (2009). Risk factors for pancreas disease (PD) outbreaks in farmed Atlantic salmon and rainbow trout in Norway during 2003–2007. *Prev. Vet. Med.*, **90**, 127–136.

MCCLEARY S.J., GILTRAP M., HENSHILWOOD K. & RUANE N.M. (2014). Detection of salmonid alphavirus RNA in Celtic and Irish Sea flatfish. Submitted to *Dis. Aquat. Org*. (June 2013).

MCLOUGHLIN M.F. & GRAHAM D.A. (2007). Alphavirus infections in salmonids – a review. *J. Fish Dis*., **30**, 511–531.

MCVICAR A.H. (1990). Infection as a primary cause of pancreas disease in farmed Atlantic salmon. *Bull. Eur. Assoc. Fish Pathol*., **10** (3), 84–87.

PETTERSON E., SANDBERG M. & SANTI N. (2009). Salmonid alphavirus associated with *Lepeoptheirus salmonis* (Copepoda: Caligidae) from Atlantic salmon, *Salmo salar* L. *J. Fish Dis*., **30**, 511–531.

PETTERSON E., STORMOEN, M., EVENSEN O., MIKALSEN A.B. & HAUGLAND O. (2013). Natural infection of Atlantic salmon (*Salmon salar*) with salmonid alphavirus 3 generates numerous viral deletion mutants. *J. Gen. Virol.,* **94**, 1945–1954.

Annex 34 (contd)

RODGER H. & MITCHELL S. (2007). Epidemiological observations of pancreas disease of farmed Atlantic salmon, *Salmo salar* L., in Ireland. *J. Fish Dis.*, **32**, 477–479.

RUANE N., GRAHAM D. & RODGER H. (2008). Pancreas disease in farmed salmon – health management and investigations at Irish farm sites 2005–2008. Marine Environments and Health Series, No. 34, Marine Institute. Available at http://oar.marine.ie/handle/10793/267

SNOW M., BLACK I., MCINTOSH R., BARETTO E., WALLACE I.S. & BRUNO D.W. (2010). Detection of salmonid alphavirus RNA in wild marine fish: implications for the origin of salmon pancreas disease in aquaculture. *Dis. Aquat. Org.*, **91**, 177–188.

STENE A., BANG JENSEN B., KNUTSEN Ø., OLSEN A. & VILJUGREIN H. (2014). Seasonal increase in sea temperature triggers pancreas disease in Norwegian salmon farms. *J. Fish Dis.*, **37**, 739–751.

Stene A., Hellebø A., Viljugrein H., Solevåg S.E., Devold M. & Aspehaug V. (2015). Liquid fat, a potential abiotic vector for horizontal transmission of salmonid alphavirus? *J. Fish Dis.*, doi: 10.1111/jfd.12382. [Epub ahead of print].

STORMOEN M., KRISTOFFERSEN A.B. & JANSEN P.A. (2013). Mortality related to pancreas disease in Norwegian farmed salmonid fish, *Salmo salar* L. and *Oncorhynchus mykiss* (Walbaum). *J. Fish Dis.*, **36**, 639–645.

TAKSDAL T., OLSEN A.B., BJERKAAS I., HJORTAAS M.J.DANNEVIG B.H., GRAHAM D.A. & MCLOUGHLIN M.F. (2007). Pancreas disease in farmed Atlantic salmon, *Salmo salar* L., and rainbow trout, *Oncorhynchus mykiss* (Walbaum), in Norway. *J. Fish Dis.*, **30**, 545–558.

TODD D., JEWHURST V.A., WELSH M.D., BORGHMANS B.J., WESTON J.H., ROWLEY H.M., MACKIE D.P. & MCLOUGHLIN M.F. (2001). Production and characterisation of monoclonal antibodies to salmon pancreas disease virus. *Dis. Aquat. Org.*, **46**, 101–108.

VILJUGREIN H., STAALSTRØM A., MOLVÆR J., URKE H.A. & JANSEN P.A. (2009). Integration of hydrodynamics into a statistical mode on the spread of pancreas disease (PD) in salmon farming. *Dis. Aquat. Org.*, **88**, 35–44.

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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/our-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.

Annex 35

CHAPTER 2.3.7.

INFECTION WITH KOI HERPESVIRUS ~~DISEASE~~

EU comment

The EU in general supports the proposed changes to this chapter.

Comments are inserted in the text below.

1. Scope

Infection with koi herpesvirus ~~disease (KHVD)~~ means infection with the pathogenic agent koi herpesvirus (KHV) of the 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. Disease information

2.1. Agent factors

2.1.1. Aetiological agent, agent strains

The aetiological agent is koi herpesvirus (KHV) in the family *Alloherpesviridae* (Haramoto *et al*., 2007; Waltzek *et al.,* 2009) although prior to taxonomic classification, it was also known as carp interstitial nephritis and gill necrosis virus (CNGV) (Ilouze *et al.,* 2010). Waltzek *et al.* (2005) provided evidence to support the classification of the virus as a herpesvirus, and named it cyprinid herpesvirus 3 (CyHV-3), following the nomenclature of other cyprinid herpesviruses: CyHV-1 (carp pox virus, fish papilloma virus) and CyHV-2 (goldfish haematopoietic necrosis virus). Sequence analysis of part of the genome has shown that KHV is closely related to CyHV-1 and CyHV-2, and distantly related to channel catfish virus (Ictalurid herpesvirus: IcHV-1) and Ranid (frog) herpesvirus (RaHV-1) (Waltzek *et al.,* 2005). Aoki *et al.* (2007) described the complete genome sequence of KHV and identified 156 unique protein-coding genes. They suggested that the finding that 15 KHV genes are homologous with genes in IcHV-1 confirms the proposed place of KHV in the family Herpesviridae. Forty viral proteins and 18 cellular proteins are incorporated into mature virions (Michel *et al.,* 2010). Recently, CyHV-3 was designated the type species of the new *Cyprinivirus* genus within the *Alloherpesviridae* family, that also contains CyHV-1 and CyHV-2. Early estimates of the genome size of KHV varied from at least 150 kbp to 277 kbp but the size is now confirmed as 295 kbp. Virus nucleocapsids have been measured at 100–110 nm in diameter and are surrounded by an envelope (Review: Ilouze *et al.,* 2010).

Comparisons of the genomes of KHV isolates from different geographical areas by restriction enzyme analysis (Haenen *et al.,* 2004) or nucleotide sequence analysis (Sano *et al.,* 2004) have shown them to be practically identical. Likewise, the polypeptides of KHV isolates from different geographical areas were similar, although one isolate from Israel had two additional polypeptides (Gilad *et al.,* 2003). Aoki *et al.* (2007) compared the complete genome sequences of three KHV strains isolated from Japan, Israel and the United States of America (USA). The genomes were found to be highly similar to each other at the sequence level (>99%), with the Israel and USA strains more closely related to each other than either is to the Japan strain. The three isolates were interpreted as having arisen as two lineages (J & U/I) from a wild-type parent. However, further studies in Japan suggest that the lineages were independently brought to those regions and caused infection with KHV epidemics (Review: Ilouze *et al*. 2010). A more recent study in France has identified a third intermediate between the J and U/I lineages and suggested that the three lineages of CyHV-3 have been introduced into Europe since 2001 via imported koi carp (Bigarré *et al.,* 2009). More recently, a further intermediate lineage has been discovered that may have emerged in Indonesia (Sunarto *et al.,* 2011).

Annex 35 (contd)

2.1.2. Survival outside the host

Studies in Israel have shown that KHV remains active in water for at least 4 hours, but ~~not for~~ less than 21 hours, at water temperatures of 23–25°C (Perelberg *et al.,* 2003). Studies in Japan have shown a significant reduction in the infectious titre of KHV within 3 days in environmental water or sediment samples at 15°C. However, ~~the infectivity~~ KHV remained infective for >7 days ~~when KHV was exposed to~~ in similar water samples that had been sterilised by autoclaving or filtration (Shimizu *et al.,* 2006). The study also presented evidence for the presence of bacterial strains in the water with anti-viral activity. More recently, the detection of KHV DNA in river water samples at temperatures of 9–11°C has been reported, 4 months before an outbreak of infection with KHV~~D~~ in a river (Haramoto *et al*., 2007). However, persistence of the virus may have been aided by the presence of animate vectors and detection of DNA ~~may~~ is not ~~always be~~ indicative of the presence of infectious virus.

2.1.3. Stability of the agent

The virus is inactivated by UV radiation and temperatures above 50°C for 1 minute. The following disinfectants are also effective for inactivation: iodophor at 200 mg litre^{-1} for 20 minutes, benzalkonium chloride at 60 mg litre^{-1} for 20 minutes, ethyl alcohol at 30% for 20 minutes and sodium hypochlorite at 200 mg litre^{-1} for 30 seconds, all at 15°C (Kasai *et al.,* 2005).

2.1.4. Life cycle

In early reports investigators suggested that the gills are the major portal of virus entry in carp (Dishon *et al.,* 2005; Gilad *et al.,* 2004; Pikarsky *et al*., 2004). However, a more recent experimental study has demonstrated that the skin covering the fins and body of the carp is the major portal of entry for KHV (Costes *et al.,* 2009). ~~There is then a systemic spread of~~ The virus then spreads systemically from the skin and gills to the internal organs and high levels of KHV DNA have been detected in kidney, spleen, liver and gut tissue (Dishon *et al.,* 2005; Pikarsky *et al*., 2004). The assembly and morphogenesis of KHV in infected cells has been described as the same as other herpesviruses. An ultrastructural examination of experimentally infected carp has provided evidence for immature capsids and mature nucleocapsid assembly in the nucleus and further maturation of the virion in the cytoplasm of infected cells. Hyper-secretion of mucous is very evident in the early stages of infection with KHV ~~infection~~ and KHV DNA has been detected at high levels in mucous sampled from experimentally infected carp (Gilad *et al.,* 2004). This is further evidence for active involvement of the skin in viral pathogenesis and an important site of virus shedding. Excretion of virus via urine and faeces may also be an important mechanism for virus shedding. ~~High levels of KHV DNA have been detected in gut and kidney tissues and~~ Infectious virus has been detected in faeces sampled from infected carp (Dishon *et al.,* 2005~~; Gilad *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: common carp (*Cyprinus carpio carpio*), koi carp (*Cyprinus carpio koi*) and common carp hybrids (e.g. *Cyprinus carpio* × *Carassius auratus*).

EU comment

In line with the EU comment in Annex 32, we have a request to expand on the application of common carp as a common name to ensure this is clearly understood in light of the removal of the separate reference to Ghost carp, as follows:

"common carp including all varieties (*Cyprinus carpio carpio*)".

Indeed, this would make clear that this includes ghost carp, mirror carp etc., given the change from ghost carp previously being listed separately. We request this because common carp as a common name is not widely used for varieties such as ghost carp, or even mirror carp. For example in the UK, it is understood to apply specifically to the fully scaled common carp, with no ghosting etc.

In addition, the EU notes that there are inconsistencies in the French translation of the common names of the various carp species, and would invite the OIE to review the French version of the paragraph above. (This comment is also valid for the corresponding Aquatic Manual chapter.)

Indeed, the French name of Cyprinus carpio is "Carpe commune" and not to "carpe courante". The name "carpe commune" is also used when referring to hybrids, as are discussed in the paragraph above (and throughout the chapter).

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: Gold fish (*Carassius auratus*), grass carp (*Ctenopharyngodon idella*) and Syberian crucian carp (*Carassiuscarassius*).

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

EU comment

The EU notes that the scientific names of Atlantic sturgeon and Russian sturgeon are the wrong way around. Indeed, they should be as follows:

Atlantic sturgeon (*Acipenser oxyrinchus ~~gueldenstaedtii~~*);

Russian sturgeon (*Acipencer gueldenstaedtii ~~oxyrinchus~~*).

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

All age groups of fish, from juveniles upwards, appear to be susceptible to infection with KHV~~D~~ (Bretzinger *et al.,* 1999; Sano *et al.,* 2004) but, under experimental conditions, 2.5–6 g fish were more susceptible than 230 g fish (Perelberg *et al.,* 2003). Carp larvae are resistant to infection with KHV ~~infection~~ but the same carp were susceptible to infection on maturation.

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

Common carp or varieties, such as koi or ghost (koi × common) carp, are most susceptible and should be preferentially selected for virus detection, followed by any common carp hybrids present on the site, such as goldfish × common carp or crucian carp × common carp.

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

Gill, kidney, and spleen are the organs in which KHV is most abundant during the course of overt infection (Gilad *et al.,* 2004).

2.2.5~~6~~.Persistent infection with lifelong carriers

There is evidence to indicate that survivors of infection with KHV~~D~~ are persistently infected with virus and may retain the virus for long periods. The virus has been shown to persist in common carp experimentally infected at a permissive temperature and subsequently maintained at a lower than permissive temperature (St-Hilaire *et al.,* 2005). More recently, evidence for KHV persistence in carp has been presented in a study to determine the distribution of the virus in a wild common carp population. Researchers in Japan conducted a PCR and serological survey of KHV in Lake Biwa in 2006 (Uchii *et al.,* 2009), where episodic outbreaks of infection with KHV~~D~~ had been reported in the 2 years following a major outbreak in 2004. Further analysis of the surviving population showed that 54% of the older carp were seropositive and 31% PCR positive. The maintenance of high levels of antibody to the virus suggests that latent virus may be reactivating periodically and boosting the immune response.

2.2.6~~7~~. Vectors

Water is the major abiotic vector. However, animate vectors (e.g. other fish species, parasitic invertebrates and piscivorous birds and mammals) and fomites may also be involved in transmission.

2.2.7~~8~~.Known or ~~S~~suspected wild aquatic animal carriers

~~There is evidence to indicate that other fish species and some aquatic invertebrates are potential vectors of KHV. The viral DNA has been detected in tissues of healthy goldfish after cohabitation with koi carp experimentally infected with KHV and also in goldfish exposed during natural KHV epizootics in koi (Ilouze *et al.,* 2010). In studies in Germany, KHV has been detected by nested PCR in several different varieties of goldfish (red, lion-head & shubunkin) as well as grass carp (*Ctenopharyngodon idella*), ide (*Leuciscus idus*) and ornamental catfish (*Ancistrus* sp.) (Bergmann *et al.,* 2009). The detection in the goldfish and grass carp was confirmed by *in situ* hybridisation using different primers to those used in the PCR. Also, in a recent study in Poland, KHV was detected by PCR in Russian sturgeon (*Acipenser gueldenstaedtii*) and Atlantic sturgeon (*A. oxyrinchus*) from fish farms in Northern Poland (Kempter *et al.,* 2009). All of the sturgeon samples were taken from farms holding common carp with previous history of KHVD outbreaks. The presence, in sturgeon gill and kidney tissue, of KHV protein and viral genome was confirmed by an indirect fluorescent antibody test and *in situ* hybridisation, respectively.~~

There is ~~also~~ increasing evidence to indicate that aquatic invertebrates may be KHV vectors. Studies in Japan have reported the detection of KHV DNA in plankton samples and in particular Rotifera species (Minamoto *et al.,* 2010). The plankton samples were collected in 2008 from Iba-naiko, a shallow lagoon connected to Lake Biwa, that is a favoured carp spawning area. Statistical analysis revealed a significant positive correlation between KHV in plankton and the numbers of Rotifera and the authors suggested that KHV binds to and/or is concentrated by the filter feeding behaviour of Rotifera species. In an earlier report of a small study in Poland, KHV was detected in swan mussels (*Anodonta cygnea*) and freshwater shrimp (*Gammarus pulex*) (Kielpinski *et al.,* 2010). The invertebrates were collected from ponds in Southern Poland ~~that had experienced KHVD outbreaks~~ where outbreaks had occurred in ~~their~~ common carp populations over 5 or 6 years. More work is needed to determine how long the infectious virus persists in the invertebrates in the absence of the host species and also if the virus remains viable.

~~Recent studies have provided increasing evidence to indicate that goldfish (*Carassius auratus*) are susceptible to KHV infection. The RNA transcript of the viral thymidine kinase gene has been detected in gill, brain and intestinal tissue from goldfish that had been exposed to KHV by co-habitation with infected koi carp. Goldfish from the same population were then shown to transmit KHV to naïve common carp when water temperature fluctuation was used as a stressor (El-Matbouli & Soliman, 2010). Bergmann *et al.* (2010a) also reported the replication of KHV in goldfish after experimental infection by immersion. KHV DNA and antigen was detected in leucocytes separated from goldfish blood samples by PCR (at 45 days post infection) and by indirect fluorescent antibody test (at 60 days post infection).~~

2.3. Disease pattern

2.3.1. Transmission mechanisms

The mode of transmission of KHV is horizontal but 'egg-associated' transmission (usually called 'vertical' transmission) cannot currently be ruled out. Horizontal transmission may be direct (fish to fish) or indirect through water (or vectors) ~~vectorial, water being the major abiotic vector~~. The reservoirs of KHV~~D~~ are clinically infected fish and covert virus carriers among cultured, feral or wild fish. Virulent virus is shed via faeces, urine, gills and skin mucous. Under experimental conditions, infectious virus was continuously shed for a longer period from infected common carp at 16°C than those at 23°C or

28°C (Yuasa *et al.,* 2008). The disease course can be rapid, particularly at optimal temperatures (23–25°C), but less rapid at temperatures below 23°C. The disease may manifest itself in 3 days ~~following the addition of naïve~~ after fish have been introduced to a pond containing diseased fish, but other investigators have reported 8–21 days for the disease to be observed in naïve fish (Bretzinger *et al.,* 1999; Hedrick *et al*., 2000).

2.3.2. Prevalence

There are limited published observations of virus prevalence in wild or farmed populations of carp. There is evidence from experimental trials of virus persistence in common carp infected at a permissive temperature and subsequently maintained at a lower than permissive temperature (St-Hilaire *et al.,* 2005; see Section 2.2.5). Analysis of blood serum samples from the study showed that a proportion of the carp (at least 10–25%) developed high antibody titres and the immunological response was detectable for several months (St-Hilaire *et al.,* 2009). In other studies, viral DNA was detected in carp by PCR assay, in the absence of disease, at 13°C and it is possible that infected fish surviving at low temperatures may act as reservoirs of the virus (Gilad *et al.,* 2004). In wild populations that have survived an ~~KHV~~ outbreak there is evidence for a high prevalence of seropositive carp. In the PCR and antibody survey of KHV in Lake Biwa in 2006, further analysis of the surviving carp population showed that 54% of the older carp were seropositive and 31% PCR positive (Uchii *et al.,* 2009). As part of a KHV distribution survey of England and Wales, four sites experiencing clinical outbreaks ~~of KHV~~ in 2006, and having no introductions of fish since that time, were revisited in 2007 and tested for the presence of KHV antibody by enzyme-linked immunosorbent assays (ELISA) (Talyor *et al.,* 2010). Three of these sites produced positive results and showed 85 to 93% sero-prevalence in the samples of surviving carp population. The fourth site tested negative.

2.3.3. Geographical distribution

Following the first reports of infection with KHV~~D~~ in Israel and Germany in 1998 and detection of KHV DNA in tissue samples taken during a mass mortality of carp in the UK in 1996 (Bretzinger *et al.,* 1999; Perelberg *et al.,* 2003), the geographical range of the disease has become extensive. The disease has been spread to many countries worldwide, predominantly through the trade in koi carp, before the current knowledge of the disease and means to detect it were available. It is now known to occur in, or has been recorded in fish imported into, at least 28 ~~different~~ countries. In Europe KHV has been detected in many countries across the continent (Bergmann *et al.,* 2006; Haenen *et al.,* 2004; Novotny *et al.,* 2010). ~~Most recently~~ Infection with KHV~~D~~ outbreaks have been reported to the OIE from Belgium, Ireland, Italy, Romania, Slovenia, Spain, ~~and~~ Sweden and South Africa. In Asia, KHV has been reported from China (Hong Kong), Chinese Taipei, Indonesia, Japan, Korea (Rep. of), Malaysia, Singapore (in fish imported from Malaysia) and Thailand (Haenen *et al.,* 2004; Ilouze *et al.,* 2010; Pikulkaew *et al.,* 2009; Sano *et al.,* 2004). Elsewhere, South Africa, Canada and the USA (Garver *et al.,* 2010; Haenen *et al.,* 2004; Hedrick *et al*., 2000) have reported occurrence of infection with KHV~~D~~. It is likely that the virus is present in many more countries, but has not yet been identified or reported.

2.3.4. Mortality and morbidity

Morbidity of affected populations can be 100%, and mortality 70–80%, but the latter can be as high as 90 or 100% (Bretzinger *et al.,* 1999; Haenen *et al.,* 2004). Secondary and concomitant bacterial ~~and/~~or parasitic infections are commonly seen in diseased carp and may affect the mortality rate and display of clinical signs of disease (Haenen *et al.,* 2004).

2.3.5. Environmental factors

Disease patterns are influenced by water temperature, virulence of the virus, age and condition of the fish, population density and stress factors (e.g. transportation, spawning, poor water quality). The disease is temperature dependent, occurring between 16 and 25°C (Haenen *et al.,* 2004; Hedrick *et al*., 2000; Perelberg *et al.,* 2003; Sano *et al.,* 2004). Under experimental conditions the disease has caused high mortality at 28°C but not at 29 or 30°C, nor at 13°C (Gilad *et al.,* 2004; Ilouze *et al.,* 2010). However, viral DNA was detected in the fish by PCR at 13°C, and it is possible that infected fish surviving at low temperatures may be reservoirs of the virus (Gilad *et al.,* 2004).

2.4. Control and prevention

Methods to control and prevent infection with KHV~~D~~ should mainly rely on avoiding exposure to the virus coupled with good hygiene and biosecurity practices. This is feasible on small farms supplied by spring or borehole water and a secure system to prevent fish entering the farm via the discharge water.

2.4.1. Vaccination

A safe and effective vaccine is not currently widely available. However, live attenuated virus has been used to vaccinate carp and protect the fish from virus challenge. The vaccine preparation induced antibody against the virus and the duration of the protection was at least 8 months (Ilouze *et al.,* 2010). The vaccine was licensed for emergency use in Israel and has been widely used in carp farms across the country. Results of studies in Japan have shown that oral administration of a liposome-based vaccine containing inactivated KHV was effective in protecting carp against infection with KHV ~~infection~~ (Review: Ilouze *et al.,* 2010).

2.4.2. Chemotherapy

Not applicable.

2.4.3. Immunostimulation

There is currently no published information on the use of immunostimulants to control infection with KHV~~D~~ in carp. However, it is known to be an area of research interest.

2.4.4. Resistance breeding

Differential resistance to infection with KHV~~D~~ has been shown among different carp strains. The progeny of crosses of two strains of domesticated carp and one strain of wild carp were challenged by experimental or natural infection. The lowest survival rate was approximately 8%, but the survival rate of the most resistant strain was 61–64% (Shapira *et al.,* 2005). In a more recent resistance study, 96 families derived from di-allele crossing of four European/Asian strains of common carp were experimentally challenged with KHV. Survival rates of the five most resistant crosses in the final virus challenge trial ranged from 42.9 to 53.4% (Dixon *et al.,* 2009).

2.4.5. Restocking with resistant species

Natural outbreaks of infection with KHV~~D~~ have not been reported in commonly farmed herbivorous carp species, including silver carp (*Hypophthalmichthys molitrix*), grass carp (*Ctenopharyngodon idella*), and bighead carp (*Aristichthys nobilis*). Herbivorous carp species are often raised in polyculture with common carp, but no signs of disease or mortalities have been observed in these species, either under normal polyculture conditions or following experimental cohabitation with infected fish, or direct exposure to the virus (Ilouze *et al.,* 2010). Common carp hybrids also represent a potential control method to prevent serious losses from infection with KHV~~D~~. Studies on a population of hybrid male goldfish × female common carp found them to be resistant to infection with KHV~~D~~ (Hedrick *et al*., 2006). These hybrids display rapid growth and have a morphological appearance most similar to their maternal parent. However, KHV DNA was detected by PCR in surviving hybrids suggesting that they are potential virus carriers (Hedrick *et al*., 2006). In contrast, a Polish study reported mortality of 35–42% in a goldfish × koi carp hybrids and 91–100% in a crucian carp × koi carp hybrids, challenged with KHV by bath immersion (Bergmann *et al.,* 2010b; see Section 2.2.1). There may be a high level of genetic variation among hybrids from different crossings and consequently a variation in resistance to KHV. This will be heavily influenced by the strain of common or koi carp used. Different strains of common carp have been shown to vary in their level of resistance to infection with KHV~~D~~ (Dixon *et al.,* 2009; Shapira *et al.,* 2005).

2.4.6. Blocking agents

Not applicable.

2.4.7. Disinfection of eggs and larvae

Disinfection of eggs can be achieved by iodophor treatment. KHV has been shown to be inactivated by iodophor at 200 mg litre^{-1} for 30 seconds at 15°C (Kasai *et al.,* 2005).

2.4.8. General husbandry practices

Biosecurity measures should include ensuring that new introductions of fish are from disease-free sources and installation of a quarantine system where new fish can be held with sentinel fish at permissive temperatures for infection with KHV~~D~~. The fish are then quarantined for a minimum of 4 weeks to 2 months before transfer to the main site and mixing with naïve fish. Hygiene measures on site should be similar to those recommended for SVC and include disinfection of eggs, regular disinfection of ponds, chemical disinfection of farm equipment, careful handling of fish to avoid stress and safe disposal of dead fish.

3. Sampling

3.1. Selection of individual specimens

All age groups of carp appear to be susceptible to infection with KHV~~D~~, although, generally, younger fish up to 1 year are more susceptible to clinical disease and are recommended for sampling. ~~The suitability of selected fish~~ Specimens selected during a suspected infection with KHV~~D~~ outbreak will depend on the diagnostic test used. Moribund or freshly dead carp displaying typical clinical disease signs are suitable for testing by most of the ~~tests~~ assays described in Section 4. Fish carcasses showing signs of tissue decomposition may only be suitable for testing by PCR-based methods. Likewise, samples taken from apparently healthy fish, in a suspected diseased population, may only be reliably tested by more sensitive PCR-based methods.

3.2. Preservation of samples for submission

Whole fish should be sent to the laboratory alive or killed and packed separately in sealed aseptic containers. However, it is highly preferable and recommended to collect organ samples from the fish immediately after they have been selected at the fish production site. Whole fish or selected organ samples should be sent to the laboratory in refrigerated containers or on ice. The freezing of collected fish or dissected organs should be avoided. However, if frozen fish or organs are received they may only be suitable for testing by PCR-based methods. Small samples of tissue may also be submitted preserved in alcohol (e.g. 80–100% ethanol) for testing by PCR-based methods.

3.3. Pooling of samples

~~When testing clinically affected fish by PCR-based methods, and particularly if virus isolation is to be attempted, pooling of samples should be avoided or restricted to a maximum of two fish per pool. For health surveillance testing, by PCR-based methods, pooling should be restricted to a maximum of five fish per pool.~~

Pooling of samples may be acceptable, however, the impact on sensitivity and design prevalence must be considered.

3.4. Best organs or tissues

When testing clinically affected fish by PCR-based methods, and particularly if virus isolation is to be attempted, it is recommended to sample gill, kidney, and spleen tissues. The virus is most abundant in these tissues during the course of overt infection and high levels of virus have also been detected in encephalon and intestine (gut) tissue (Dishon *et al*., 2005; Gilad *et al.,* 2004). When testing subclinical, apparently healthy, fish by PCR-based methods, it is recommended to also include intestine (gut) and encephalon.

3.5. Samples/tissues that are not suitable

Fish carcasses showing very advanced signs of tissue decomposition may not be suitable for testing by any methods.

4. Diagnostic methods

Diagnosis of infection with KHV~~D~~ in clinically affected fish can be achieved by a number of methods. Cell culture isolation of KHV is not currently considered to be as sensitive as the published PCR-based methods for detecting KHV DNA. The virus is isolated in only a limited number of cell lines and these cells can be difficult to handle. Consequently, virus isolation in cell culture is not a reliable diagnostic method for infection with KHV~~D~~ (Haenen *et al.,* 2004). Immunodiagnostic methods, similar to those used for the diagnosis of spring viraemia of carp (SVC) (e.g. immunofluorescence [IF] tests or ELISAs), may be suitable for rapid identification and diagnosis of KHVD but have not been extensively reported, compared or validated. Until such time as validated tests are available, diagnosis of infection with KHV~~D~~ should not rely on just one test but a combination of two or three tests (Haenen *et al.,* 2004).

4.1. Field diagnostic methods

4.1.1. Clinical signs

During an ~~KHVD~~ outbreak of infection with KHV there will be a noticeable increase in mortality in the population. All age groups of fish appear to be susceptible to infection with KHV~~D~~, although, under experimental infection, younger fish up to 1 year old are more susceptible to the disease. On closer examination of individual fish, typical clinical signs include pale discolouration or reddening of the skin, which may also have a rough (sandpaper-like) texture, focal or total loss of epidermis, over- or under-production of mucous on the skin and gills, and pale discolouration of the gills. Other gross signs

include enophthalmia (sunken eyes) and haemorrhages on the skin and base of the fins, and fin erosion.

4.1.2. Behavioural changes

Fish become lethargic, separate from the shoal and gather at the water inlet or sides of a pond and gasp at the surface of the water. Some fish may experience loss of equilibrium and disorientation but they may also show signs of hyperactivity.

4.2. Clinical methods

4.2.1. Gross pathology

There are no pathognomic gross lesions. Final diagnosis must await direct detection of viral DNA or virus isolation and identification. However, the most consistent gross pathology is seen in the gills and this can vary in extent from pale necrotic patches to extensive discolouration, severe necrosis and inflammation. Another commonly observed gross pathology is pale, irregular patches on the skin associated with excess mucous secretion and also under production of mucous where patches of skin have a sandpaper-like texture. Other commonly reported clinical signs include anorexia, enophthalmia (sunken eyes) and superficial haemorrhaging at the base of the fins. Other internal lesions are variable in occurrence and often absent in cases of sudden mortality. Other gross pathologies that have been reported include adhesions in the abdominal cavity with or without abnormal colouration of internal organs (lighter or darker). The kidney or liver may be enlarged, and they may also exhibit petechial haemorrhages. Presence of gross lesions may also be complicated because diseased fish, particularly common carp, are also infested with ectoparasites, such as *Argulus* sp., *Chilodonella* sp., *Cryptobia* sp., *Dactylogyrus* sp., *Gyrodactylus* sp., *Ichthyobodo* sp., *Ichthyophthirius* sp., *Trichodina* sp. and gill monogeneans, as well as numerous species of bacteria, especially *Flavobacterium columnare* at warmer water temperatures.

4.2.2. Clinical chemistry

No published information available.

4.2.3. Microscopic pathology

Further examination of the gills, by low-power microscopy, can reveal erosion of primary lamellae, fusion of secondary lamellae, and swelling at the tips of the primary and secondary lamella. The histopathology of the disease can be nonspecific and variable, but inflammation and necrosis of gill tissues is a consistent feature. Gills also exhibit hyperplasia and hypertrophy of branchial epithelium, and fusion of secondary lamellae and adhesion of gill filaments can be seen. Gill necrosis, ranging from small areas of necrotic epithelial cells of secondary lamellae to complete loss of the lamellae is observed. Branchial epithelial cells and leucocytes may have prominent nuclear swelling, margination of chromatin to give a ‘signet ring’ appearance, and pale diffuse eosinophilic intranuclear inclusions are commonly observed. Inflammation, necrosis and nuclear inclusions have been observed (individually or together) in other organs, particularly the kidney, but also in the spleen, pancreas, liver, brain, gut and oral epithelium.

4.2.4. Wet mounts

Not applicable.

4.2.5. Smears

KHV has been identified in touch imprints and smears of liver, kidney and brain of infected fish by immunofluorescence (IF). Highest levels of positive IF were seen in the kidney and the virus could be detected by IF on a kidney imprint 1 day post-infection (Pikarsky *et al*., 2004; Shapira *et al.,* 2005).

4.2.6. Electron microscopy/cytopathology

Detection of viral particles by transmission electron microscopy (TEM) examination of tissues from clinically infected carp is not a reliable diagnostic method. Pieces of gill and kidney tissue fixed in glutaraldehyde should be sampled from heavily infected ($>10^6$ virus particles) carp. Best results are obtained from sampling a number of carp in an affected population at different stages of infection. This helps to ensure that some of the tissue samples are from heavily infected individuals.

4.3.Agent detection and identification methods

In this section, not all methods are presented in great detail because there has been no extensive comparison and validation of detection and identification methods for KHV. Where this is the case however, a short description of available published methods is provided. Method recommendations will rely on further testing and validation and further data being obtained, from laboratories that have developed the methods, in order to decide if they are 'fit-for-purpose'.

4.3.1. Direct detection methods

KHV has been identified in touch imprints of liver, kidney and brain of infected fish by immunofluorescence (IF). Highest levels of positive IF were seen in the kidney and the virus could be detected by IF on a kidney imprint 1 day post-infection (Pikarsky *et al*., 2004; Shapira *et al.,* 2005). Virus antigen has also been detected in infected tissues by an immunoperoxidase staining method. The virus antigen was detected at 2 days post-infection in the kidney, and was also observed in the gills and liver (Pikarsky *et al*., 2004). However, the detection of KHV by immunostaining must be interpreted with care, as positive-staining cells could result from cross-reaction with serologically related virus (e.g. CyHV-1) or a non-viral protein (Pikarsky *et al*., 2004). A method for direct detection of KHV from kidney imprints by indirect fluorescent antibody test (IFAT) is detailed below.

Immunofluorescence (IF) and *in-situ* hybridisation (ISH) methods, performed on separated fish leucocytes, have been used in research applications for detection or identification of KHV. Although these methods have not been thoroughly compared with other techniques, they are non-destructive (non-lethal) techniques and some laboratories may find them useful in a diagnostic setting. Details of the methods are not given here but detailed protocols for separation of leucocytes from blood and for IF and ISH can be found in published reports by Bergmann *et al.* (2009; 2010a).

ELISA-based methods for direct detection of KHV antigen in infected tissues are under development in a number of laboratories worldwide but no validated methods have been published. Currently, one published ELISA method is available and it was developed in Israel to detect KHV in fish droppings (faeces) (Dishon *et al.,* 2005). The ELISA methods developed will have low sensitivity that may be suitable for detection of the high levels of KHV found in clinically diseased fish tissue but not suitable for surveillance for infection with KHV in healthy populations.

The most commonly used method for detection of KHV directly in fish tissues is using PCR-based assays specific for KHV.

4.3.1.1. Microscopic methods

4.3.1.1.1. Wet mounts

Not applicable.

4.3.1.1.2. Smears/Imprints

4.3.1.1.2.1. Indirect fluorescent antibody test on kidney 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) Allow the imprint to air-dry for 20 minutes.

iv) Rinse once with 0.01 M phosphate-buffered saline (PBS), pH 7.2, then three times briefly with cold acetone (stored at –20°C) for glass slides or a mixture of 30% acetone/70% ethanol, also at –20°C, for plastic wells.

v) Let the fixative act for 15 minutes. A volume of 0.5 ml/2 cm^2 well is adequate for imprints in cell culture plates.

vi) Allow the fixed imprints to air-dry for at least 30 minutes and process immediately or freeze at –20°C.

vii) Rehydrate the dried imprints by four rinsing steps with 0.01 M PBS solution, pH 7.2, containing 0.05% Tween 20 (PBST), and remove this buffer completely after the last rinsing.

viii) Prepare a solution of purified antibody or serum to KHV in 0.01 M PBS, pH 7.2, containing 0.05% Tween 20 (PBST), at the appropriate dilution (which has been established previously or is given by the reagent supplier).

ix) Block with 5% skim milk or 1% bovine serum albumin, in PBST for 30 minutes at 37°C.

x) Rinse four times with PBST.

xi) Treat the imprints with the antibody solution (prepared at step viii) for 1 hour at 37°C in a humid chamber and do not allow evaporation to occur. A volume of 0.25 ml/2 cm^2 well is adequate for imprints in cell culture plates.

xii) Rinse four times with PBST.

xiii) Treat the imprints for 1 hour at 37°C with a solution of fluorescein isothiocyanate (FITC)-conjugated antibody to the immunoglobulin used in the first layer and prepared according to the instructions of the supplier. These FITC antibodies are most often rabbit or goat antibodies.

xiv) Rinse four times with PBST.

xv) Add PBS at 0.5 ml/2 cm^2 well to the treated imprints in cell culture plates and examine immediately, or mount the glass slides with cover-slips using glycerol saline at pH 8.5 prior to microscopic observation.

xvi) Examine under incident UV light using a microscope with ×10 eye pieces and ×20–40 objective lenses having numerical apertures of >0.65 and >1.3, respectively. Positive and negative controls must be found to give the expected results prior to any other observation.

4.3.1.1.3. Fixed sections

The method detailed in Section 4.3.1.1.2 above is also suitable for detection of KHV antigen in paraffin wax tissue sections fixed in 10% neutral buffered formalin (NBF). However, the deparaffinised sections, rehydrated in PBS, may need to be further treated to reveal antigen that may be masked by over fixation of the tissue. A common treatment is incubation of the sections with 0.1% trypsin in PBS at 37°C for 30 minutes. The sections are then washed in cold PBS before proceeding with steps viii–xvi in Section 4.3.1.1.2 above.

NOTE: For direct detection of viral antigen by IFAT or immunohistochemistry, tissues should be fixed for 24–48 hours in 10% NBF and then the fixative should be replaced with 70% ethanol for prolonged storage.

4.3.1.2. Agent detection, isolation and identification

4.3.1.2.1. Cell culture

~~Diagnosis of~~ Detection of KHV~~D~~ in clinically affected fish can be achieved by virus isolation in cell culture. However, the virus is isolated in only a limited number of cell lines and these cells can be difficult to handle. Also, cell culture isolation is not as sensitive as the published PCR-based methods to detect KHV DNA and is not considered to be a reliable diagnostic method for infection with KHV~~D~~ (Haenen *et al.,* 2004).

Cell line to be used: KF-1 or CCB

Virus extraction

Use the procedure described in Chapter 2.3.0 *General information* (on diseases of fish), Section A.2.2.2.

Inoculation of cell monolayers

i) Prior to inoculation of cells organ pool homogenates can be treated with antibiotics as detailed in Chapter 2.3.0, Sections A.2.2.1 and A.2.2.2.

ii) If cytotoxic effects have been observed after inoculation of antibiotic-treated homogenate, filter at least 1 ml of the 1/10 organ homogenate supernatant through a 0.45 µm disposable cellulose acetate filter unit (or unit fitted with a similar low protein binding filter membrane).

iii) For direct inoculation, transfer an appropriate volume of the antibiotic-treated or filtered homogenate on to 24- to 48-hour-old cell monolayers in tissue culture flasks or multi-well plates. Inoculate at least 5 cm^2 of cell monolayer with 100 µl of the filtered supernatant. Alternatively, make a further tenfold dilution of the filtered supernatant in cell culture medium, buffered at pH 7.6 and supplemented with 2% fetal calf serum (FCS), and allow to adsorb for 0.5–1 hour at 18–22°C. Then, without withdrawing the inoculate, add the appropriate volume of cell culture medium (1–1.5ml/5 cm^2 for cell culture flasks), and incubate at 20°C to 25°C. NOTE: When using multi-well plates, incubation under CO_2 atmosphere or addition of HEPES to the cell culture medium (HEPES = N-2-hydroxyethyl-piperazine-N-2-ethanesulfonic acid) will maintain the correct pH during incubation.

Monitoring incubation

i) Follow the course of infection in positive controls and other inoculated cell cultures by daily microscopic examination at ×40–100 magnification for 14 days. The use of a phase-contrast microscope is recommended.

ii) Maintain the pH of the cell culture medium at between 7.3 and 7.6 during incubation. This can be achieved by the addition to the inoculated cell culture medium of sterile bicarbonate buffer for tightly closed cell culture flasks or HEPES-buffered medium for multiwell plates.

iii) If a cytopathic effect (CPE) appears in those cell cultures inoculated with the dilutions of the tested homogenate supernatants, identification procedures must be undertaken immediately (see Section 4.3.1.2.2 below).

iv) If no CPE develops in the inoculated cultures (despite normal progression of CPE in the virus controls), the inoculated cultures should be subcultured for a further 14 days. Should the virus control fail to develop CPE, the process should be repeated with fresh susceptible cells and new batches of samples.

Subcultivation procedures

i) Transfer aliquots of cell culture medium from all monolayers inoculated with organ homogenate supernatant onto fresh cell cultures.

ii) Inoculate cell monolayers as described above in Section 4.3.1.2.1, Inoculation of cell monolayers, step iii.

iii) Incubate and monitor as described above in Section 4.3.1.2.1.

If no CPE occurs, the test may be declared negative.

Confirmatory identification

The most reliable method for confirmatory identification of a CPE is by PCR, followed by sequence analysis of the PCR product. The PCR methods recommended for identification of KHV are the same methods recommended for direct detection in fish tissues (section 4.3.1.2.3 below). For final confirmation, PCR products of the correct size should be identified as KHV in origin by sequence analysis (see section 4.3.1.2.3 below).

Confirmation by PCR

i) Extract DNA from the virus culture supernatant using a suitable DNA extraction kit or reagent. An example of extraction of DNA using a salt-based extraction method (DNAzol®[6] reagent) is described below in section 4.3.1.2.3.1.

ii) Extracted DNA is then amplified using the PCR protocols described below in section 4.3.1.2.3.1.

Amplified PCR products may then be excised from the gel and sequenced as described in section 4.3.1.2.3.

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6 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*.

Annex 35 (contd)

4.3.1.2.2 Antibody-based antigen detection methods

Enzyme-linked immunosorbent assay (ELISA)-based methods for direct detection of KHV antigen in infected tissues are under development in a number of laboratories and these methods may also be suitable for confirmatory identification of KHV. Currently, one published ELISA method is available and was developed in Israel to detect KHV in fish droppings (faeces) (Dishon *et al.,* 2005).

Virus identification methods that rely on the production of KHV-infected cell cultures (e.g. IFAT, immunoperoxidase and serum neutralisation tests) are not recommended. This is because virus growth is slow and unpredictable in the susceptible cell cultures.

4.3.1.2.3. Molecular techniques

Of the published single-round PCR methods, the protocols detailed below are currently considered to be the most sensitive for detection of KHV DNA in fresh tissue samples from clinically diseased carp. The protocols may also allow detection of subclinical levels of virus. The first uses the TK primer set developed by Bercovier *et al.* at the Hebrew University-Hadassah Medical School in Israel (Bercovier *et al.,* 2005). The second was developed by Yuasa *et al.* at the National Research Institute of Aquaculture (NRIA), Watarai, Mie, Japan (Yuasa *et al.,* 2005) and is an improvement of a published protocol. If the tissue shows evidence of decomposition then primer sets targeting shorter regions of the genome may need to be used.

Alternative PCR assays that are favoured by many diagnostic laboratories over conventional PCR, include quantitative PCR assays such as real-time PCR. The most commonly used quantitative assay for detection of KHV is the Gilad Taqman real-time PCR assay (Gilad *et al.,* 2004). Real-time Taqman PCR is now a common diagnostic procedure that has been shown to detect and quantitatively assess very low copy numbers of target nucleic acid sequences. Taqman PCR avoids much of the contamination risk inherent to nested PCR assays by minimising the handling of samples through automation during sample preparation and thermal cycling procedures.

The sample preparation protocol detailed below uses a salt-based extraction method (DNAzol® reagent) for extraction of KHV DNA. This is an easy to use, short-duration protocol that is also relatively inexpensive compared with some kits. Laboratories that are not familiar with DNAzol® or similar salt-based extraction reagents may find the method less reliable in their hands. However, a number of, salt-based and silica-matrix based, DNA extraction kits are available commercially (popular manufacturers include Roche, Qiagen and Invitrogen) that will produce high quality DNA suitable for use with the PCR protocols detailed.

4.3.1.2.3.1. Direct detection by PCR

Sample preparation and extraction of DNA using the DNAzol® reagent

Virus extraction from organ tissues should be carried out using the procedure described in Chapter 2.3.0, Section A.2.2.2.

i) Add 100 µl of tissue homogenate (1/10 [w/v]) to a 1.5 ml microcentrifuge tube containing 1 ml DNAzol® reagent.

ii) Mix gently by inverting the tube five times and stand at room temperature for 5 minutes, then centrifuge at 10,600 ***g*** (rcf) for 10 minutes using a microcentrifuge.

iii) Remove 1 ml of the supernatant to a new 1.5 ml microcentrifuge tube containing 0.5 ml of ethanol.

iv) Mix gently by inverting the tube five times and stand at room temperature for 5 minutes, then centrifuge at 18,000 ***g*** (rcf) for 30 minutes using a microcentrifuge.

v) Remove the supernatant and rinse the pellet with 250 µl of 70% ethanol in molecular biology grade water.

vi) Spin samples for 5 minutes at 18,000 ***g*** (rcf).

vii) Remove the ethanol using a pipette and air-dry the pellet by leaving the tubes open on the bench for 5 minutes.

viii) Resuspend the pellet in 50 µl molecular biology grade water, prewarmed to 60°C, and incubate at 60° for 5 minutes. Samples can be stored at –20°C until required.

PCR

General notes

PCR is prone to false-positive and false-negative results. False-positive results (negative samples giving a positive reaction), may arise from either product carryover from positive samples or, more commonly, from cross-contamination by PCR products from previous tests. Therefore, each assay and tissue extraction should include a negative control to rule out contamination. To minimise the risk of contamination, aerosol-preventing pipette tips should be used for all sample and PCR preparation steps. Additionally, all PCRs should be prepared in a clean area that is separate from the area where the amplifications and gel electrophoresis are performed. Do not share equipment (e.g. laboratory coats and consumables) between areas and, where possible, restrict access between areas. Contaminating PCR products can be carried on equipment, clothes and paper (e.g. workbooks). Also, ensure all work-tops and air-flow hoods used for the extractions and PCR set up are regularly cleaned and decontaminated using UV light and bleach. Reagents and consumables should also be systematically decontaminated using UV-light irradiation. To ensure sample integrity, always store the samples (e.g. in a freezer or refrigerator) in a location away from the molecular biology laboratory or area.

Protocol 1 (with Bercovier TK primers)

i) For each sample, prepare a master mix containing:

10 µl	Reaction buffer (×5 conc.)
5 µl	$MgCl_2$ (25 mM stock)
0.5 µl	dNTPs (25 mM mix)
0.5 µl	Forward primer (10 pmol μl^{-1} stock)
0.5 µl	Reverse primer (10 pmol μl^{-1} stock)
0.25 µl	DNA polymerase 500 µ (5 µ μl^{-1})
30.75 µl	Molecular biology grade water

Bercovier TK primers:
Forward = 5'-GGG-TTA-CCT-GTA-CGA-G-3'
Reverse = 5'-CAC-CCA-GTA-GAT-TAT-GC-3'
Product size = 409 bp
For each sample, dispense 47.5 µl into a 0.5 ml thin-walled microcentrifuge tube. Overlay with two drops of mineral oil.

ii) Add 2.5 µl of the extracted DNA. Store the remainder of the DNA at –20°C.

iii) Place tubes in a thermal cycler and perform the following programme:

1 cycle of 5 minutes at 94°C;
40 cycles of: 1 minute at 95°C
1 minute at 52°C (see note below)
1 minute at 72°C
A final extension step of 10 minutes at 72°C.

Note on cycling conditions: An annealing temperature of 55°C has been used effectively by many laboratories to amplify KHV with the Bercovier TK primers.

iv) Visualise the 409 bp PCR amplicon by electrophoresis of the product in a 2% ethidium bromide-stained agarose gel and observe using UV transillumination. An appropriate molecular weight ladder should be included on the gel to determine the size of the product.

v) Products of the correct size should be confirmed as KHV in origin by sequence analysis.

Protocol 2 (with Gray Sph primers/Yuasa modification)

i) For each sample, prepare a master mix containing:

2 µl	Reaction buffer (×10 conc.)
1.6 µl	dNTPs (2.5 mM mix)
0.2 µl	Forward primer (50 pmol μl^{-1} stock)
0.2 µl	Reverse primer (50 pmol μl^{-1} stock)
0.1 µl	DNA polymerase
14.9 µl	Molecular biology grade water

(NOTE: the final concentration of $MgCl_2$ in the master mix is 2 mM)

Gray Sph primers:
Forward = 5'-GAC-ACC-ACA-TCT-GCA-AGG-AG-3'
Reverse = 5'-GAC-ACA-TGT-TAC-AAT-GGT-CGC-3'
Product size = 292 bp
For each sample, dispense 19 µl into a 0.2 ml thin walled microcentrifuge tube. Overlay with two drops of mineral oil.

ii) Add 1µl of extracted DNA

iii) Place tubes in a thermal cycler and perform the following programme:

1 cycle of 30 seconds at 94°C
40 cycles of: 30 seconds at 94°C
30 seconds at 63°C
30 seconds at 72°C
A final extension step of 7 minutes at 72°C.

iv) Add 3 µl of ×6 loading buffer into each PCR product and electrophorese 7 µl on a 2% ethidium bromide-stained agarose gel at 100 V for 20 minutes and visualise under UV light. An appropriate molecular weight ladder should be included on the gel to determine the size of the product.

v) Products of the correct size should be confirmed as KHV in origin by sequence analysis.

Nucleotide sequence analysis of PCR products

PCR products are excised from the gel and purified using a commercial kit for gel purification (e.g. Geneclean®, Q-BIOgene,UK). Single, intense (bright) PCR products, after purification, are sequenced directly in both directions with the primers used in the initial amplification. Alternatively, less intense (faint) PCR products are cloned using a TA cloning vector (e.g. pGEM T, Promega) and both DNA strands are sequenced using the M13 universal primer sets. The amplification, cloning and sequencing are performed in duplicate to eliminate potential errors introduced by the Taq polymerase. Sequence reactions are then analysed on a Genetic Analyser and the alignments and consensus sequences generated using appropriate computer software (e.g. Sequencher™ 4.0 software, Gene Codes Corporation, Ann Arbour, MI, USA). Testing laboratories that have no sequencing facilities are recommended to use commercial companies that offer a sequencing service. Testing laboratories should follow the instructions supplied by the chosen sequencing service for submission of samples.

4.3.2. Serological methods

The immune status of the fish is an important factor following exposure to KHV, with both nonspecific (interferon) and specific immunity (serum antibodies, cellular immunity) having important roles in herpesvirus infections. Clinical disease dominates at water temperatures of 18°C and above when the host immune response is at its optimum. Infected carp produce antibodies against the virus, and ELISA-based tests that reliably detect these antibodies at high serum dilution have been published (Adkison *et al.,* 2005; Ilouze *et al.,* 2010; St-Hilaire *et al.,* 2005). Antibody has been detected in the serum at 3 weeks after experimental infection and in survivors after 1 year following a natural infection (Adkison *et al.,* 2005; Ilouze *et al.,* 2010; St-Hilaire *et al.,* 2005; Talyor *et al.,* 2010).

Serum from koi carp containing antibodies to KHV has been shown to cross-react, at a low level, with CyHV-1, a further indication that these viruses are closely related. Evidence of cross-reacting antibodies was demonstrated in reciprocal ELISA and western blot analyses of serum from koi infected with CyHV-1 or KHV (Adkison *et al.,* 2005). Diagnostic virologists should also be aware that fish recently vaccinated against KHV may test positive in antibody detection ELISAs.

Detection of antibodies may prove to be a valuable method of establishing previous exposure to KHV in apparently healthy fish, and until PCR-based methods have been developed that are able to reliably detect persistent virus in exposed fish, antibody assays may be the only surveillance tools available. However, due to insufficient knowledge 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 screening method for assessing the viral status of fish populations. Validation of some serological techniques for certain fish virus infections could arise in the near future, rendering the use of fish serology more widely acceptable for health screening purposes.

5. Rating of tests against purpose of use

The methods currently available for targeted surveillance and diagnosis of infection with KHV 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
	Larvae	PLs	Juveniles	Adults		
Gross signs	d	d	c	c	b	d
Direct LM	d	d	c	c	b	d
Histopathology	d	c	c	c	b	c
Isolation in cell culture	d	d	d	d	b	d
Transmission EM	d	d	d	d	b	c
Antibody-based virus detection assays	d	d	c	c	b	b
In-situ DNA probes	d	d	c	c	b	b
PCR	d	b	b	b	a	a
Sequence	n/a	n/a	n/a	n/a	n/a	a
Antibody detection assays (serology)	d	d	c	b	b	d
Bioassay	n/a	n/a	n/a	n/a	n/a	n/a

PLs = postlarvae; LM = light microscopy; EM = electron microscopy; PCR = polymerase chain reaction; n/a = Not applicable.

6. Test(s) recommended for targeted surveillance to declare freedom from koi herpesvirus disease

Targeted surveillance should rely on regular monitoring of sites holding susceptible species. Sites should be monitored when water temperatures have reached levels that are permissive for the development of the disease (>17°C) and no sooner than 3 weeks after such temperatures have been reached. Any diseased fish, or fish showing abnormal behaviour, that are found on the site should be sampled and tested using the most sensitive tests available (e.g. PCR). There are no validated methods that are currently recommended for testing healthy populations of susceptible fish for declaration of freedom from infection with KHV. However, many laboratories use more sensitive molecular-based methods, such as real-time and nested PCR, to detect low levels of persistent virus DNA reliably. These assays may well prove suitable for surveillance programmes. There are no published reports of extensive validation of the more sensitive assays but the most commonly used assay is the Gilad Taqman real-time PCR assay (Gilad *et al.,* 2004). This assay is widely acknowledged to be the most sensitive published PCR method available for detection of low-levels of KHV. Alternatively, detection of antibodies may prove to be a valuable method of establishing previous exposure to KHV in apparently healthy fish. Validation of enzyme immunoassays for detection of antibody to KHV could arise in the near future, rendering the use of these assays more widely acceptable for health screening purposes.

Annex 35 (contd)

7. Corroborative diagnostic criteria

7.1. Definition of suspect case

KHV shall be suspected, in a susceptible fish species, if at least one of the following criteria is met:

i) The presence of typical clinical signs of infection with KHV~~D~~ in a population of susceptible fish.

ii) Presentation of typical histopathology in tissue sections consistent with infection with KHV~~D~~.

iii) A typical CPE observed in susceptible cell cultures without identification of the causative agent.

iv) A single positive result from one of the diagnostic assays ranked as a or b in Table 5.1.

v) Transfer of live fish from a site where presence of infection with KHV~~D~~ has been confirmed, or is suspected, because of the presence of clinical disease, to sites without suspicion of infection with KHV~~D~~.

vi) Other epidemiological links to infection with KHV confirmed sites have been established.

vii) Antibodies to KHV have been detected.

NOTE: When sites have been designated as suspect under criteria v) and vi), testing for infection with KHV should only be attempted if water temperatures have reached levels that are permissive for the development of the disease (>17°C). If water temperatures are below permissive levels then a live sample of the suspect fish may be held at elevated water temperatures (ideally 20–24°C) and tested 14–21 days later.

7.2. Definition of confirmed case

The following criteria should be met for confirmation of infection with KHV:

i) Mortality, clinical signs and pathological changes consistent with infection with KHV ~~disease~~ (Section 4.2) AND detection of KHV by one or more of the following methods:

   a) Detection of KHV by PCR by the methods described in Section 4.3.1.2.3.

   b) OR detection of KHV in tissue preparations by means of specific antibodies against KHV (e.g. IFAT on tissue imprints as described in Section 4.3.1.1.2);

   c) OR isolation and identification of KHV in cell culture from at least one sample from any fish on the site as described in Section 4.3.1.2.1.

ii) In the absence of mortality or clinical disease by one or more of the following methods:

   a) Detection and confirmation of KHV by PCR by the methods described in Section 4.3.1.2.3;

   b) Positive results from two separate and different diagnostic assays ranked as a or b in Table 5.1.

8. References

ADKISON M.A., GILAD O. & HEDRICK R.P. (2005). An enzyme linked immunosorbent assay (ELISA) for detection of antibodies to the koi herpesvirus (KHV) in the serum of koi *Cyprinus carpio*. *Fish Pathol.*, **40**, 53–62.

AOKI T., HIRONO I., KUROKAWA K., FUKUDA H., NAHARY R., ELDAR A., DAVISON A.J., WALTZEK T.B., BERCOVIER H. & HEDRICK R.P. (2007). Genome sequences of three koi herpesvirus isolates representing the expanding distribution of an emerging disease threatening koi and common carp worldwide. *J. Virol.*, **81** (10), 5058–5065.

BERCOVIER H., FISHMAN Y., NAHARY R., SINAI S., ZLOTKIN A., EYNGOR M., GILAD O., ELDAR A. & HEDRICK R.P. (2005). Cloning of the koi herpesvirus (KHV) gene encoding thymidine kinase and its use for a highly sensitive PCR based diagnosis. *BMC Microbiol.*, **5**, 1–9.

BERGMANN S.M., KEMPTER J., SADOWSKI J. & FICHTNER D. (2006). First detection, confirmation and isolation of koi herpesvirus (KHV) in cultured common carp (*Cyprinus carpio L.*) in Poland. *Bull. Eur. Assoc. Fish Pathol.*, **26**, 97–104.

BERGMANN S.M., LUTZE P., SCHUTZE H., FISCHER U., DAUBER M., FICHTNER D. & KEMPTER J. (2010a). Goldfish (Carassius auratus) is a susceptible species for koi herpesvirus (KHV) but not for KHV disease. *Bull. Eur. Assoc. Fish Pathol.*, **30**, 74–84**.**

BERGMANN S.M., SCHUTZE H., FISCHER U., FICHTNER D., RIECHARDT, M., MEYER, K., SCHRUDDE D. & KEMPTER J. (2009). Detection of koi herpes-virus (KHV) genome in apparently healthy fish. *Bull. Eur. Assoc. Fish Pathol.*, **29**, 145-152**.**

BERGMANN S.M., SADOWKSI J., KIELPINSKI M., BARTLOMIEJCZYK M., FICHTNER D., RIEBE R., LENK M. & KEMPTER J. (2010b). Susceptibility of koi x crucian carp and koi x goldfish hybrids to koi herpesvirus (KHV) and the development of KHV disease (KHVD). *J. Fish Dis* , **33**, 267–272.

BIGARRÉ L., BAUD M., CABON J., ANTYCHOWICZ J., BERGMANN S.M., ENGELSMA M., POZET F., REICHERT M. & CASTRIC J. (2009). Differentiation between Cyprinid herpesvirus type-3 lineages using duplex PCR. *J. Virol. Methods* **158**, 51-57.

BRETZINGER A., FISCHER-SCHERL T., OUMOUNA M., HOFFMANN R. & TRUYEN U. (1999). Mass mortalities in koi carp, *Cyprinus carpio*, associated with gill and skin disease. *Bull. Eur. Assoc. Fish Pathol.*, **19**, 182–185.

COSTES B., STALIN RAJ V., MICHEL B., FOURNIER G., THIRION M., GILLET L., MAST J., LIEFFRIG F., BREMONT M. & VANDERPLASSCHEN A. (2009). The major portal of entry of koi herpesvirus in *Cyprinus carpio* is the skin. *J. Virol.*, **83**, 2819–2830.

DISHON A., PERELBERG A., BISHARA-SHIEBAN J., ILOUZE M., DAVIDOVICH M., WERKER S. & KOTLER M. (2005). Detection of carp interstitial nephritis and gill necrosis virus in fish droppings *Appl. Environ. Microbiol*., **71**, 7285–7291.

DIXON P.F., JOINER C.L., WAY K., REESE R.A., JENEY G. & JENEY Z. (2009). Comparison of the resistance of selected families of common carp, *Cyprinus carpio* (L.), to koi herpesvirus: preliminary study. *J. Fish Dis*., **32** 1035–1039.

EL-MATBOULI M. & SOLIMAN H. (2010). Transmission of cyprinid herpesvirus-3 (CyHV-3) from goldfish to naïve common carp by cohabitation. *Res. Vet. Sci.* [published on-line: doi:10.1016/j.rvsc.2010.07.008.

GARVER K.A., AL-HUSSINEE L., HAWLEY L.M., SCHROEDER T., EDES S., LEPAGE V., CONTADOR E., RUSSELL S., LORD S., STEVENSON R.M.W., SOUTER B., WRIGHT E. & LUMSDEN J.S. (2010). Mass mortality associated with koi herpesvirus in wild common carp in Canada. *J. Wildl. Dis.,* **46**, 1242–1251**.**

GILAD O., YUN, S. ADKISON M.A., WAY K., WILLITS N.H., BERCOVIER H. & HEDRICK R.P. (2003). Molecular comparison of isolates of an emerging fish pathogen, koi herpesvirus, and the effect of water temperature on mortality of experimentally infected koi. *J. Gen. Virol.*, **84**, 2661–2667.

GILAD O., YUN S., ZAGMUTT-VERGARA F.J., LEUTENEGGER C.M., BERCOVIER H. & HEDRICK R.P. (2004). Concentrations of a Koi herpesvirus (KHV) in tissues of experimentally infected *Cyprinus carpio koi* as assessed by real-time TaqMan PCR. *Dis. Aquat. Org.*, **60**, 179–187.

HAENEN O.L.M., WAY K., BERGMANN S.M. & ARIEL E. (2004). The emergence of koi herpesvirus and its significance to European aquaculture. *Bull. Eur. Assoc. Fish Pathol.*, **24**, 293–307.

HARAMOTO E., KITAJIMA M., KATAYAMA H. & OHGAKI S. (2007). Detection of koi herpesvirus DNA in river water in Japan. *J. Fish Dis.*, **30**, 59–61.

HEDRICK R.P., GILAD O., YUN S., SPANGENBERG J.V., MARTY G.D., NORDHAUSEN R.W., KEBUS M.J., BERCOVIER H. & ELDAR A. (2000). A herpesvirus associated with mass mortality of juvenile and adult koi, a strain of common carp. *J. Aquat. Anim. Health*, **12**, 44–57.

HEDRICK R.P., WALTZEK T.B. & MCDOWELL T.S. (2006). Susceptibility of koi carp, common carp, goldfish and goldfish × common carp hybrids to cyprinid herpesvirus-2 and herpesvirus-3. *J. Aquat. Anim. Health*, **18**, 26–34.

Annex 35 (contd)

ILOUZE M., DAVIDOVICH M., DIAMANT A., KOTLER M. & DISHON A. (2011). The outbreak of carp disease caused by CyHV-3 as a model for new emerging viral diseases in aquaculture: a review. *Ecol. Res.,* **26**, 885–892.

ITO T., SANO M., KURITA J., YUASA K. & IIDA T (2007). Carp larvae are not susceptible to koi herpesvirus. *Fish Pathol.*, **42**, 107–109.

KASAI H., MUTO Y. & YOSHIMIZU M. (2005). Virucidal effects of ultraviolet, heat treatment and disinfectants against koi herpesvirus (KHV). *Fish Pathol.*, **40**, 137–138.

KEMPTER J., SADOWSKI J., SCHUTZE H., FISCHER U., DAUBER M., FICHTNER D., PANICZ R. & BERGMANN S.M. (2009). Koi herpesvirus: Do Acipenserid restitution programs pose a threat to carp farms in the disease free zones? *Acta Ichthyologica et Piscatoria,* **39**, 119–126.

KIELPINSKI M., KEMPTER J., PANICZ R., SADOWSKI J., SCHUTZE H., OHLEMEYER S. & BERGMANN S.M. (2010). Detection of KHV in freshwater mussels and crustaceans from ponds with KHV history in common carp (*Cyprinus carpio*). *Israeli J. Aquaculture* (*Bamidgeh*), **62**, 28–37.

MINAMOTO T., HONJO M.N., YAMANAKA H., TANAKA N., ITAYAMA T. & KAWABATA Z. (2010). Detection of cyprinid herpesvirus-3 DNA in lake plankton. *Res. Vet. Sci.,* **90**, 530–532.

MICHEL B., LEROY B., STALIN RAJ V., LIEFFRIG F., MAST J., WATTIEZ R., VANDERPLASSCHEN A.F. & COSTES B. (2010). The genome of cyprinid herpesvirus 3 encodes 40 proteins incorporated in mature virions. *J. Gen. Virol.,* **91**, 452–462.

NOVOTNY L., POKOROVA D., RESCHOVA S., VICENOVA M., AXMANN R., VESELY T. & MIKLER J.R. (2010). First clinically apparent koi herpesvirus infection in the Czech Republic. *Bull. Eur. Assoc. Fish Pathol.*, **30**, 85–91.

PERELBERG A., SMIRNOV M., HUTORAN M., DIAMANT A., BEJERANO Y. & KOTLER M. (2003). Epidemiological description of a new viral disease afflicting cultured *Cyprinus carpio* in Israel. *Israeli J. Aquaculture*, **55**, 5–12.

PIKARSKY E., RONEN A., ABRAMOWITZ J., LEVAVI-SIVAN B., HUTORAN M., SHAPIRA Y., STEINITZ M., PERELBERG A., SOFFER D. & KOTLER M. (2004). Pathogenesis of acute viral disease induced in fish by carp interstitial nephritis and gill necrosis virus. *J. Virol.*, **78**, 9544–9551.

PIKULKAEW S., MEEYAM T. & BANLUNARA W. (2009). The outbreak of Koi herpesvirus (KHV) in Koi (*Cyprinus carpio* koi) from Chiang Mai Province, Thailand. *Thai J. Vet. Med*., **39,** 53–58**.**

SANO M., ITO T., KURITA J., YANAI T., WATANABE N., MIWA S. & IIDA T. (2004). First detection of koi herpesvirus in cultured common carp *Cyprinus carpio* in Japan. *Fish Pathol.*, **39**, 165–167.

SHAPIRA Y., MAGEN Y., ZAK T., KOTLER M., HULATA G. & EVAVI-SIVAN B. (2005). Differential resistance to koi herpes virus (KHV)/carp interstitial nephritis and gill necrosis virus (CNGV) among common carp (*Cyprinus carpio* L.) strains and crossbreds. *Aquaculture*, **245**, 1–11.

SHIMIZU T., YOSHIDA N., KASAI H. & YOSHIMIZU M. (2006). Survival of koi herpesvirus (KHV) in environmental water. *Fish Pathol.*, **41**, 153–157.

ST-HILAIRE S., BEEVERS N., JOINER C., HEDRICK R.P. & WAY K. (2009). Antibody response of two populations of common carp, *Cyprinus carpio* L., exposed to koi herpesvirus. *J. Fish Dis*., **32**, 311–320**.**

ST-HILAIRE S., BEEVERS N., WAY K., LE DEUFF R.M., MARTIN P. & JOINER C. (2005). Reactivation of koi herpesvirus infections in common carp *Cyprinus carpio*. *Dis. Aquat. Org.*, **67**, 15–23.

SUNARTO A., MCCOLL K. A., CRANE M. ST J., SUMIATI T., HYATT A. D., BARNES A. C. & WALKER P. J. (2011). Isolation and characterization of koi herpesvirus (KHV) from Indonesia: identification of a new genetic lineage. *J. Fish Dis.,* **34**, 87–101.

TAYLOR N., **WAY K.,** DIXON P.F., PEELER E.J., JEFFREY K. & DENHAM K.L. (2010). Koi herpesvirus (KHV): distribution and prospects for control in England and Wales. *J. Fish Dis.,* **33**, 221–230.

UCHII K., MATSUI K., IIDA T. & KAWABATA Z. (2009). Distribution of the introduced cyprinid herpesvirus 3 in a wild population of common carp, *Cyprinus carpio* L. *J. Fish Dis*., **32,** 857–864**.**

WALTZEK T.B., KELLEY G.O., STONE D.M., WAY K., HANSON L., FUKUDA H., HIRONO I., AOKI T., DAVISON A.J. & HEDRICK R.P. (2005). Koi herpesvirus represents a third cyprinid herpesvirus (CyHV-3) in the family *Herpesviridae*. *J. Gen. Virol.*, **86**, 1659–1667.

WALTZEK T.B., KELLEY G.O., ALFARO M.E., KUROBE T., DAVISON A.J. & HEDRICK R.P. (2009). Phylogenetic relationships in the family *Alloherpesviridae*. *Dis. Aquat. Org.*, **84**, 179–194.

YUASA K., ITO T. & SANO M. (2008). Effect of water temperature on mortality and virus shedding in carp experimentally infected with koi herpesvirus. *Fish Pathol.*, **43**, 83–85.

YUASA K., SANO M., KURITA J., ITO T. & IIDA T. (2005). Improvement of a PCR method with the Sph 1–5 primer set for the detection of koi herpesvirus (KHV). *Fish Pathol.*, **40**, 37–39.

*
* *

NB: There are OIE Reference Laboratories for koi herpesvirus disease
(see Table at the end of this *Aquatic Manual* or consult the OIE web site for the most up-to-date list:
http://www.oie.int/en/our-scientific-expertise/reference-laboratories/list-of-laboratories/).
Please contact the OIE Reference Laboratories for any further information on infection with
koi herpesvirus ~~disease~~ virus

NB: FIRST ADOPTED IN 2009; MOST RECENT UPDATES ADOPTED IN 2012.

Annex 36

CHAPTER 2.2.9.

INFECTION WITH YELLOW HEAD VIRUS GENOTYPE 1

EU comment

The EU supports the proposed changes to this chapter.

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 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: 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: *Acetes* sp., *Callinectes sapidus, Chelonibia patula, Ergasilus manicatus, Fundulus grandis* and *Octolasmis muelleri.*

[...]

Annex 39

PROPOSED NEW *AQUATIC MANUAL* DISEASE CHAPTER TEMPLATE

CHAPTER X.X.X.

INFECTION WITH PATHOGEN NAME

EU comment

The EU thanks the OIE for providing the Aquatic Manual disease chapter template for member country information.

We in general support the new layout, which is indeed very clear. We nevertheless have some further suggestions for consideration, as follows:

1. Section 2.2 Host Factors, 2.2.1 Susceptible Host Species – we request that this section also specifically directs the Reference Laboratory expert to include information, where relevant, on susceptible species that do not typically present with clinical infection. For example, as is the case with certain species susceptible to *Gyrodactylus salaris*. This information is critical for determining effective surveillance methods.

2. Section 2.2 – the addition of a specific summary section for "Conditions conducive to clinical expression…" to unambiguously make clear the intent of this phrase and the information that should be considered where this is used throughout the Aquatic Code chapters and makes cross reference to the Aquatic Manual. This will ensure a clear link to the correct information in the Manual and avoid differences in interpretation and application. In line with this, please also refer to the EU comments in Annex 14 for sections 10.3.4. and 10.3.5. of Code Chapter 10.3.

3. For the on-line version, the addition of a section in '4. Diagnostic Methods' for photographs showing the presentation of clinical signs and gross pathology.

Photographic guidance as provided by the OIE Reference Laboratory will greatly support and facilitate competent "early detection systems" as defined by the OIE. In many cases, both industry employed personnel and veterinarians / aquatic animal health professionals will not have first hand experience of OIE listed diseases. Supplementing the text through provision of good quality photographs as validated by the Expert, that can be used as training and reference tools and readily accessible on-line would greatly help.

Another option for this would be a separate publication, as was done by the OIE a few years ago for terrestrial diseases and which has since become the "OIE bestseller" ("Atlas of Transboundary Animal Diseases", P. Fernandez, W. White, revised English ed. 2016 http://boutique.oie.int/index.php?page=ficprod&id_produit=900&popup=true).

1. Scope

[Please start this chapter with a simple definition of the infection (see glossary in the *Aquatic Code* for definition of infection).]

Infection with disease name is considered to be infection with the pathogenic agent [pathogen name], including its classification or taxonomy.

[Example: 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*. Other genotypes of yellow head complex may also cause disease under certain circumstances but this chapter only considers genotype 1. These other genotypes are only mentioned where relevant to diagnostic testing.]

2. Disease information

[This section should be populated as completely as possible based on available scientific information. It is acknowledged that it may not be possible to provide and reference scientific data for each subsection 2.2 – 2.4 and authors may wish to draw attention to areas where there is a significant lack of information].

2.1. Agent factors

2.1.1. Aetiological agent

[Include description of agent strains and genotypes, their associated pathogenicity, classification and phylogenetic relationships, morphology and structure, genomic characteristics, structural or virulent genes and proteins of the aetiological agent. Include information on appearance and structure from electron microscopic studies if available.]

2.1.2. Survival and stability inside the host tissues

[Describe the stability of the pathogen in host tissues including during storage, treatment or processing.]

2.1.3. Survival and stability outside the host

[Describe the survival and stability of the pathogen outside the host, i.e. in the natural environment, such as water, sediment, air, and surface of tools, etc. Description may be extended to the detectable components of the pathogen, such as nucleic acid or antigen.]

For inactivation methods, see Section 2.4.6.

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with [pathogen name] according to Chapter 1.5 of *Aquatic Animal Health Code* (*Aquatic Code*) are: [insert species names in accordance with the assessment of the relevant *ad hoc* Group**;** see chapter 2.2.8 Infection with yellow head virus genotype 1 for an example.]

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 [pathogen name] according to Chapter 1.5 of the *Aquatic Code* are: [insert species names in accordance with the assessment of the relevant *ad hoc* Group**;** see Chapter 2.2.8 Infection with yellow head virus genotype 1 for an example.] Evidence is lacking for these species to either confirm that the identity of the pathogenic agent is [pathogen name], transmission mimics natural pathways of infection, or presence of the pathogenic agent constitutes an infection.

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

[Evidence indicating which host species, host life stage or subpopulations have the highest likelihood of infection should be provided. This information is to help guide sampling as part of disease investigations or pathogen surveillance (see Section 3).]

[If appropriate indicate for pathogens with more than one host, in which species you are most likely to detect the pathogenic agent . Please ensure consistency with Table 4.1., specifically, define early and juvenile life stages as simply as possible, if they are relevant to diagnostic testing, in this section and use the same definitions in Table 4.1. Be consistent with terms and size definitions used in the relevant general information sections (2.1.0, 2.2.0, 2.3.0, 2.4.0). The default definition of juvenile is any animal older than an early life stage but not yet adult. Early life stages and juvenile shrimp may best be defined by life-cycle stages whilst mollusc species may need to be defined by size. Fish species may need to be described by a combination of size (e.g. length or weight) and physiological state (e.g. pre or post-smolt). Detection should not be used as a synonym for infection in this Section. Populations or

sub-populations at high likelihood of exposure (and therefore potentially infection) include those on farms which purchase or import live animals, populations in rivers or bays with high density or aquaculture or near processing facilities. Within a farm subpopulations at highest risk are those gathered at water outlets, poor growing, etc..

[*Example:* From IHHN chapter: IHHNV has been demonstrated in all life stages (i.e. eggs, larvae, postlarvae [PL, juveniles and adults) of *P. vannamei*. Eggs produced by IHHNV-infected females with high virus loads were found to generally fail to develop and hatch. Those nauplii produced from infected broodstock that do hatch have a high prevalence of IHHNV infection (Motte *et al.,* 2003).]

2.2.4. Distribution of the pathogen in the host

[Describe which organs/tissues are affected and have lesions, and in which tissues, including faeces, mucous or ovarian fluids, etc. the pathogen is mostly likely to be found. Note that organs or tissues demonstrating pathology may be different from those in Section 3.2.]

2.2.5. Reservoirs of infection

[Describe any non-clinical carrier state in susceptible species, the relevant life-stages and whether they are survivors of clinical disease.]

2.2.6. Vectors

[Vectors are not susceptible species. The OIE definition (see Code glossary) needs to be used:: . They are any other living organism that transports a *pathogenic agent* to a susceptible *aquatic animal* or its food or immediate surroundings. The *pathogenic agent* may or may not pass through a development cycle within the vector, and this should be indicated for each vector. Note if only certain life stages act as vectors.]

2.3. Disease pattern

[Patterns of disease, including mortality, morbidity, and prevalence vary across populations. This reflects differences in strains of the agent, host immunity and species susceptibility, and environmental and disease management factors (section 2.3.4).]

2.3.1. Mortality, morbidity and prevalence

[Estimates and variability in mortality, morbidity and prevalence in both wild and farmed infected populations; indicate where estimates vary with species, age and size. Note if data are from experimental studies.]

2.3.2. Clinical signs, including behavioural changes

[Specify appearance or behavioural changes that could be indicative of the disease. Exclude information that would be included in the 2.3.3 Gross pathology.]

2.3.3 Gross pathology

[Macroscopic or anatomical manifestations of disease in organs, tissues, and body cavities for diagnostically useful findings made during the post mortem examination. Do not include clinical signs in this section; clinical signs are in Section 2.3.2]

2.3.4. Modes of transmission and life cycle

[Is there horizontal or vertical (true or due to contamination of gametes) transmission; note evidence of routes of horizontal transmission: live fish movements, by water currents, fomites, cannabalism or other routes of transmission (e.g., via wild birds, anthropogenic movements), Note if infections can or cannot be reproduced under experimental conditions. Information on parasite life cycles, and intermediate hosts, should be included here.]

2.3.5. Environmental and management factors

[Environmental factors include temperature / season, salinity/rainfall, water quality etc..that are directly related to the occurrence of infection and/or disease. Management factors that influence the occurrence of diseases include husbandry practices such as stocking density, grading, co-culture of different species. Please provide references.]

2.3.6. Geographical distribution

[Describe at the continental level and cross reference to WAHIS (http://www.oie.int/animal-health-in-the-world/the-world-animal-health-information-system/data-after-2004-wahis-interface/) for recent information on distribution at the country level. Indicate, if known, where the pathogen was first identified. Information provided by Member Countries on zones or compartments within their territories that are disease free could be included.]

2.4. Biosecurity and disease control strategies

[The discussion of control strategies should be restricted to those that will affect detection of the pathogen in a population or individual animal. Vaccination, antibiotic treatments, formalin baths for ecto-parasites (e.g. *Gyrodactylus*) may reduce DSe because of lower loads, and chemicals may inhibit molecular tests. Vaccination may mean that serological tests cannot be used for disease monitoring, but may be used to determine vaccine coverage.]

2.4.1. Vaccination

Text

2.4.2. Chemotherapy including blocking agents

Text

2.4.3. Immunostimulation

Text

2.4.4. Breeding resistant strains

[Should include the use and stocking of resistant strains. Authors should refer to where detection of the pathogen may be problematic due to subclinical infection.]

2.4.5. Inactivation methods

Text

2.4.6. Disinfection of eggs and larvae

Text

2.4.7. General Husbandry

[Describe husbandry methods that may influence pathogen detection.]

3. Specimen selection, sample collection, transportation and handling

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

3.1. Selection of populations and individual specimens

[Indicate how information provided in section 2.2.3 can be used in surveys of apparently healthy animals, populations at greatest risk of infection may be identified based on knowledge of transmission pathways and population risk factors (e.g. farms which purchase or import live animals, wild or farmed populations where aquaculture is at high density or close to processing facilities). Guidance on selection of animals to be sampled in apparently healthy populations should be based on known animal level risk factors, e.g. age. If sampling animals in a population with ongoing elevated mortality or morbidity, indicate how heavily infected individuals can be identified, e.g. age, clinical signs, stage of the disease. See Aquatic Code article 1.4.8 for further details on sampling].

3.2. Selection of organs or tissues

[Explain the basis for the selection of best organs for testing, which may include field sampling practicalities. Ideally, the whole animals should be sampled and sent to the laboratory. However, where this is not possible, for example if the specimen is too large, specify parts (e.g. organs, tissues, lesions) that should be

sampled in order of priority. Samples should be larger than the analytical unit size required for initial laboratory testing to provide diagnostic material for retesting if needed.]

3.3. Samples or tissues not suitable for pathogen detection

[Indicate for which sample or tissues would not be suitable for pathogen detection is not possible or where likelihood of detection is reduced, e.g. shrimp eyes or other sample types with polymerase chain reaction (PCR) inhibitors, inappropriate fixatives, sample quality, inappropriate tissues and lack of information provided with the submission.]

3.4. Non-lethal sampling

[Identify whether there are any nonlethal options and discuss the diagnostic sensitivity and diagnostic specificity of tests using these specimen types, and implications for animal welfare. In particular, non-lethal sampling needs to be considered for valuable broodstock, for which ovarian fluild and milt should be considered for testing. Include any limitations if test(s) used for non-lethal samples have not been validated to the same level as other samples described in Section 3.2].

3.5. Preservation of samples for submission

[Several factors can affect specimen quality during collection, handling and storage, such as exposure to light, heat, desiccation, and incomplete preservation. Hence, standard operating protocols or recommended practices should be followed at all steps of the diagnostic process.]

[Refer to chapter 2.2.0 or 2.3.0 or 2.4.0 as appropriate. If additional or different information is required, please include it here. Authors should ensure that the recommendations in this section are only relevant to methods described in this chapter.]

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

3.5.1. Samples for pathogen isolation

The success of pathogen isolation and results of bioassay depend strongly on the quality of samples (time since collection and time in storage). Fresh specimens should be kept on ice and preferably sent to the laboratory within 24 hours of collection. To avoid degradation of samples use alternate storage methods only after consultation with the receiving laboratory.

3.5.2. Fixed samples for molecular detection

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

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

Tissue samples for histopathology should be fixed immediately after collection or removal from the shell for molluscs. The recommended ratio of fixative to tissue is 10:1.

3.5.4. Fixed samples for electron microscopy

[The guidance provided needs to have sufficient detail for preservation for TEM, for example see infection with YHV1]

3.5.5. Samples for other tests

[For example, collection of serum.]

3.6. Pooling of samples

[Pooling of samples from more than one individual animal for a given purpose should only be recommended where supporting data on diagnostic sensitivity and diagnostic specificity are available. However, smaller life

stages (e.g. larvae or postlarvae) can be pooled to provide a minimum amount of material for testing. For example see AHPND.]

4. Diagnostic methods

[All recommended tests included in this *Aquatic Manual* chapter are expected to have diagnostic sensitivity and specificity estimates published in the scientific literature for the different purposes of testing. However, it is acknowledged that histopathology, whilst well established for some diseases, is unlikely to have published estimates of diagnostic sensitivity or specificity (whilst noting that validation of histopathological tests is possible by comparison of results of more than one operator). New tests for inclusion in the *Aquatic Manual* must have published estimates of diagnostic sensitivity and specificity. If an assay is not suitable for all susceptible species listed in 2.2.1, then this information needs to be provided].

[Table 4.1 should be used to select the most appropriate method for animals sampled as part of a surveillance programme (e.g. to demonstrate pathogen freedom or for pathogen detection) or from populations that fall under suspicion due to epidemiological links to an infected animal or population. A positive result would be considered a presumptive suspect positive result and can be confirmed using the most appropriate method for confirmatory diagnosis listed in Table 4.1]

[To make a presumptive diagnosis of infection in animals showing clinical signs of disease specified in this chapter, the most appropriate test listed in Table 4.1 should be used. A presumptive positive result can be confirmed using the most appropriate method for confirmatory diagnosis listed in Table 4.1. Tests are provided for three life stages in Table 4.1. which unless otherwise stated should cover the entire life-cycle. The definitions of life stages provided in section 2 should be used. If there are strong reasons to do so the author could use different life stages terminology, but the terms used must be defined in a footnote and be consistent with those used in Section 2.2.3 and Table 2.1. Shade out cells for the lifestages for which the diagnostic test is not appropriate. Delete rows in the table which are not needed and add rows if more than one PCR is described. Replace ‘other antigen detection method and other serological method with the name of the assay.]

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

Key:

+++ =	Recommended method(s) validated for the purpose shown and usually to stage 3 of the OIE Validation Pathway;
++ =	Suitable method(s) but may need further validation;
+ =	May be used in some situations, but cost, reliability, lack of validation or other factors severely limits its application;
Shaded boxes =	Not appropriate for this purpose.

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

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

Method [amend or delete as relevant]	A. Surveillance of apparently healthy animals				B. Presumptive diagnosis of clinically affected animals				C. Confirmatory diagnosis[1] of a suspect result from surveillance or presumptive diagnosis			
	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV
Wet mounts												
Cytopathology[3]												
Histopathology[3]												
Cell or artificial media culture												
Real-time PCR												
Conventional PCR												
Amplicon sequencing[4]												
***In-situ* hybridisation**												
Bioassay												
LAMP												
Ab ELISA												
Ag ELISA												
Other antigen detection methods[5]												
Other serological method[5]												

LV = level of validation, refers to the stage of validation in the OIE Pathway (chapter 1.1.2); PCR = polymerase chain reaction. [give definitions of abbreviations as appropriate; nPCR = nested PCR, etc. NB "RT-PCR" is reserved for reverse transcriptase-polymerase chain reaction methods. "real-time PCR" should always be stated in full and refers to probe-based and SYBR green assays]
[1]For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). [2]Early and juvenile life stages have been defined in Section 2.2.3.

[3]Cytopathology and histopathology can be validated if the results from different operators has been statistically compared. [4]Sequencing of the PCR product.
[5]Specify the test used. Shading indicates the test is inappropriate or should not be used for this purpose.

[Please provide a description of OIE recommended diagnostic methods listed in Table 4.1 in section 4.1 to 4.10. The diagnostic methods should include all the tests that are used for suspect and confirmed case definitions, i.e. provide descriptions of the clinical and histological details, etc., and not simply the agent detection methods. It is acknowledged that not all methods listed will be applicable to all chapters. Only the ones that are appropriate need be described. State if no recommended assay exists in the relevant sections.

⇒ Technical procedure
  - How to use positive/negative controls

⇒ Interpretation of results

⇒ Availability of test (from Reference Laboratories, commercial sources or easily synthesised)

[This section includes methods that detect, possibly isolate and amplify, and identify the agent. For each method, information on the items in the text box above should be provided. This information is required to allow the reader to follow the technique, but also to provide the necessary data – diagnostic specificity and sensitivity, and level of validation (LV) – that are required for the development of a sampling and surveillance programme. The lifestage in which the validation was undertaken should be be noted.]

[Diagnostic sensitivity and diagnostic specificity should be described for all the assays in Table 4.1. It is acceptable to describe only one assay and reference other assays described in the literature (peer reviewed even if just accepted for publication but not yet published).]

[If a test has only been validated in one life stage, the result can be extrapolated to other life stages if there is evidence that the pathogenesis is the same at other life stages.]

[The order of the tests in Section 4.1 to 4.10 follows the typical work flow in a diagnostic laboratory. Some sample preservation methods are described in Section 3. Sections 4.2.to 4.10 are pathogen detection methods and Section 4.12 is for antibody detection methods.]

4.1. Wet mounts

Text

4.2. Cyto- and histopathology

[Describe histopathological changes in affected organs.]

4.3. Cell or artificial media culture for isolation

Cell lines should be monitored to ensure that susceptibility to targeted pathogens has not changed.

4.4. Nucleic acid amplification

[Wherever possible, if you are using different molecular tests, choose tests with different targets. The author should provide protocols in which the amplification products from different PCRs do not overlap. There are risks with conventional PCRs, such as contamination.]

4.4.1. Real-time PCR (qPCR)

[Include nucleic acid extraction method in the PCR description in this section.]

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

4.4.2. Conventional PCR (PCR)

[Describe nucleic acid extraction method in this section or cross reference to method in 4.4.1.]

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

4.4.3. Other nucleic acid amplification methods

[Any other verified nucleic acid amplification methods if available, e.g. LAMP]

4.5. Amplicon sequencing of the amplicon

[PCR is used to amplify the DNA region of interest (the amplicon) prior to amplicon sequencing. Amplicon sequencing primers specific to the pathogen of interest are used to confirm identification of the pathogen. Specify the PCR, e.g. a conventional PCR or other PCR that are used to genotype the pathogen, that generated the amplicon].

4.6. *In-situ* hybridisation (and histoimmunochemistry)

[Include information on the use of positive and negative controls.]

4.7. Bioassay

[Include information on the use of positive and negative controls, well characterised inoculum and well characterised experimental animals.]

4.8. Antibody-based or antigen detection methods (ELISA, etc.)

[Include information on the use of positive and negative controls.]

4.9. Other serological methods

[Include information on the use of positive and negative controls.]

5. Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations

[Please identify the recommended test(s) based on the information provided and assessed in Sections 1–4, for surveillance to demonstrate freedom from infection in apparently healthy populations as outlined in the *Aquatic Code*. Ensure consistency with Table 4.1.]

6. Corroborative diagnostic criteria

[Diagnostic laboratories provide an interpretation of test results either as positive or negative for the targeted pathogen. The results of multiple tests on one or more sample types may be combined into a single interpretation, which is often provided with individual test results to the Competent Authority.]

[The relevant tests are listed in Table 4.1. Appendix 1 cross-references tests in Table 4.1 against the categories i) to vi) below. Appendix 2 provides a worked example. Appendix 3 illustrates the progression through suspect to confirmed cases starting with an apparently healthy or diseased animal.]

This section only addresses the diagnostic test results for detection of infection in the presence (6.1) or absence of clinical signs (6.2) but does not evaluate whether the infectious agent is the cause of the clinical event.

The case definitions for a suspect and confirmed case have been developed to support decision making related to trade and confirmation of disease status at the country, zone or compartment level. Case definitions for disease confirmation in endemically affected areas may be less stringent. It is recommended that all samples that yield suspect positive test results in an otherwise pathogen-free country or zone or compartment should be referred immediately to the OIE Reference Laboratory for confirmation, whether or not clinical signs are associated with the case. If a laboratory does not have the capacity to undertake the necessary diagnostic tests it should seek advice from the appropriate OIE reference laboratory.

6.1. Apparently healthy animals or animals of unknown health status[7]

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

6.1.1. Definition of suspect case in apparently healthy animals

[The information in 6.1.1 and 6.1.2 is pathogen specific. Any positive test result from assays listed in Table 4.1 with a designation of ++ or +++ would usually be considered a suspect positive test result. It should be noted whether a recommended test can be used for all or only some susceptible species. Authors should advise caution if tests are used on species for which validation studies have not been completed.]

The presence of infection shall be suspected if:a positive result has been obtained on at least one animal from at least one of the following diagnostic tests:

[Authors: delete criteria that are non-relevant to the pathogen in question]

i) Positive result by a recommended molecular or antigen or antibody detection test

ii) Cyto- or histopathological changes consistent with the presence of the pathogen or the disease

iii) Visual observation (direct or by microscopy) of the pathogen

iv) Cytopathic effect in cell culture (viruses)

v) Culture and isolation (e.g. bacterial colonies indicative of the pathogen)

vi) Bioassay

6.1.2. Definition of confirmed case in apparently healthy animals

[In principle, at least two independent tests, one from column Test A and one from column Test B in Table A, are required for a confirmed case. One of the tests may have been undertaken as part of a screening programme and resulted in a suspect case. Ideally both tests are run on the same samples with positive test results for both.]

The presence of infection shall be confirmed if:positive results has been obtained on at least one animal from two test used in the following combination:

[number i) to n) combinations of tests]

Reference Laboratories should be contacted for specimen referral when testing laboratories cannot undertake any of the recommended test methods and testing is being undertaken that will result in notification to the OIE.

6.2 Clinically affected animals

Clinical signs are not pathognomonic for a single disease; however they may narrow the range of possible diagnoses.

Annex 39 (contd)

[7] For example transboundary commodities.

[For many diseases, especially those affecting mollusc, 'clinical signs' are extremely limited and mortality may be the only or most dominant observation.]

6.2.1. Definition of suspect case in clinically affected animals

[Clinically affected animals may show signs or gross pathology that are or are not associated with the disease. Gross pathology or clinical signs associated with the disease is a criterion to define a suspect case but confirmation requires 2 assays to be performed. A clinically affected animal which does not show signs associated with the disease becomes a suspect case if one of the tests listed below is positive.]

The presence of infection shall be suspected if at least one of the following criteria are met:

[Authors: delete criteria ii) to vii) that are non-relevant to the pathogen in question]

i) gross pathology or clinical signs associated with the disease as described in this chapter, with or without elevated mortality

ii) Positive result by a recommended molecular or antigen or antibody detection test on at least one animal

iii) Cyto- or histopathological changes consistent with the presence of the pathogen or the disease on at least one animal

iv) Visual observation (direct or by microscopy) of the pathogen on at least one animal

v) Cytopathic effect in cell culture (viruses)

vi) Culture and isolation of the pathogen (e.g. bacterial colonies indicative of the pathogen)

vii) Positive result of a bioassay

6.2.2. Definition of confirmed case in clinically affected animals

[In principle, at least two independent tests, one from column Test A and one from column Test B in Table A, are required for a confirmed case. One of the tests may have been undertaken as part of a screening programme and resulted in a suspect case. Ideally both tests are run on the same samples with positive test results for both.]

The presence of infection shall be confirmed if:positive results has been obtained on at least one animal from two test used in the following combination:

[number i) to n) combinations of tests]

Reference Laboratories should be contacted for specimen referral when testing laboratories cannot undertake any of the recommended test methods and testing is being undertaken that will result in notification to the OIE.

6.3. Diagnostic sensitivity and specificity for diagnostic tests

[Include table from appendix V]

7. References

[List a maximum of 30 references, cited in the text by Author-Date, that provide useful additional information including diagnostic sensitivity and specificity estimates. References should be to documents that are readily accessible. It is important to emphasise that the *Aquatic Manual* is not intended to provide comprehensive reviews of the literature, but rather to provide key, up-to-date references as an entry point to the literature for those who wish to study further. **Longer lists of references will be reduced by the editors**, and it is better that you as the expert do this rather than rely on the editorial team who may be less familiar with this particular disease.]

Annex 39 (contd)

Appendices – provided as a guide to authors (not intended for publication in the manual chapter)

Appendix 1. Methods from Table 4.1 and tests listed in 6.1.1

	Test listed in section 6.1.1					
Methods from Table 4.1	Molecular or antigen or antibody detection test	Cyto- or histo-pathological changes	Visual observation of the pathogen	Cytopathic effect in cell culture	Culture and Isolation	Bioassay
Wet mounts		X	X			
Cyto-/Histo-pathology		X	X			
Cell or artificial media culture				X	X	
Real-time PCR	X					
Conventional PCR	X					
Amplicon sequencing	X					
In-situ hybridization Immunohistochemistry	X		X			
Bioassay						X
LAMP	X					
Ab ELISA	X					
Ag ELISA	X					
Other antigen detection assays	X					
Other serological method	X					

Annex 39 (contd)

Appendix 2. Combination of tests with positive results required for a confirmed case – in apparently healthy or clinically affected animals (for use by experts – not for publication in the Manual)

	Test A		**Test B**
1	Cytopathology or Histopathology	And	Conventional PCR test and amplicon sequencing
2	Any PCR test, including LAMP	And	Conventional PCR targeting a non-overlapping region of the genome and amplicon sequencing
3	Histopathology	And	*In-situ* hybridisation or Immunohistochemistry
4	Pathogen (virus, bacterium, oomycyte, etc.) isolation or Bioassay	And	Conventional PCR and amplicon sequencing or *In-situ* hybridisation or Immunocytochemistry
5	Antigen detection	And	Conventional PCR test and amplicon sequencing or Pathogen isolation and confirmation (see row 4)
6	Visual observation of the pathogen	And	Conventional PCR and amplicon sequencing

[1]A and B do not indicate order in which tests are conducted except where indicated; [2]In-situ hybridisation or immunocytochemistry should follow histopathological examination.

[If one epidemiological unit, e.g. a fish, meets the definition of confirmed case then all the animals in that population are considered to have the same status]

Appendix 2. Worked example for Abalone Herpesvirus (AbHV)

a.6.1. Apparently healthy animals or animals of unknown health status[8]

6.1.1. Definition of suspect case in apparently healthy animals

The presence of infection shall be suspected if:a positive result has been obtained on at least one animal from at least one of the following diagnostic tests:

i) Positive result by real-time PCR or conventional PCR on at least one abalone sample.

ii) Histopathology (ganglioneuritis) observed in neural tissue sections of a single abalone sample.

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection shall be confirmed if positive results has been obtained on at least one animal from following combinations of tests:

i) Positive result by real-time PCR followed by a positive result from a Conventional PCR targeting a non-overlapping region of the genome and sequencing.

ii) Histopathology (ganglioneuritis) observed in neural tissue sections of a single abalone sample followed by a positive results from *in-situ* hybridisation

Reference Laboratories should be contacted for specimen referral when testing laboratories cannot undertake any of the recommended test methods and testing is being undertaken that will result in notification to the OIE.

Use of table in appendix 2. *Combination of tests with positive results required for a confirmed case – apparently healthy animals*

	Test A		Test B
1	Real-time PCR test	And	Conventional PCR targeting a non-overlapping region of the genome and sequencing
2	Histopathology	And	*In-situ* hybridisation

6.2. Clinically affected animals

6.2.1. Definition of suspect case in clinically affected animals

The presence of AbHV shall be suspected if at least one of the following criteria are met:

i) Presence of high mortality rates (up to 90%) associated with clinical signs of abalone viral ganglioneuritis (AVG) as described in this chapter.

ii) Positive result by Real Time PCR or conventional PCR on at least one abalone sample.

iii) Histopathology (ganglioneuritis) observed in neural tissue sections of a single abalone sample.

6.2.2. Definition of confirmed case in clinically affected animals

In principle, at least two independent tests, one from column A and one from column B in Appendix 2, are required for a confirmed case. One of the tests may have been undertaken as part of a screening programme and resulted in a suspect case.

[8] For example transboundary commodities.

Annex 39 (contd)

Use of table in appendix 2 *Combination of tests with positive results required for a confirmed case – clinically affected animals*

	Test A		**Test B**
1	Real-time PCR test	And	Conventional PCR targeting a non-overlapping region of the genome and sequencing
2	Histopathology	And	*In-situ* hybridisation

6.3. Diagnostic sensitivity and specificity for diagnostic tests

6.3.1. For presumptive diagnosis of clinically affected animals

Test type	**Test purpose**	**Source populations**	**Tissue or sample types**	**Species**	**DSe (*n*)**	**DSp (*n*)**	**Reference test**	**Citation**
Real Time PCR	Diagnosis	Clinically diseased abalone from farms and processing plants	Pleuropedal ganglion or pedal nerve cords	Need to check the species to include here	100 (30)	100 (30)	Histopathology	Need to check the reference to insert here

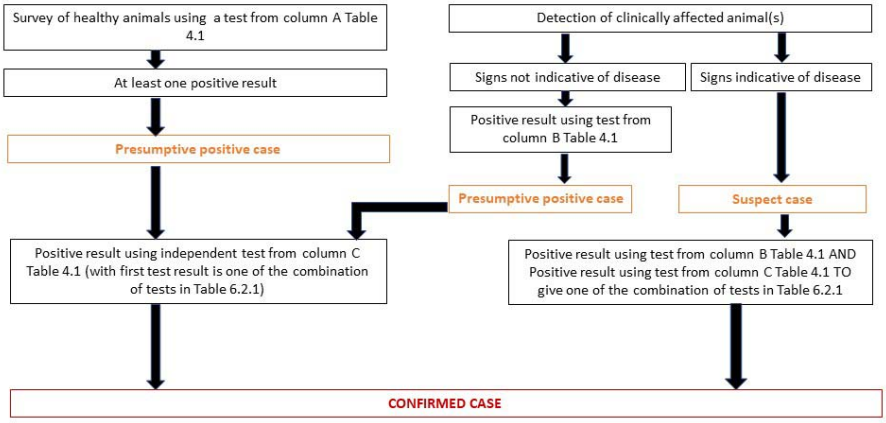
DSe: = diagnostic sensitivity, DSp = diagnostic specificity, qPCR: = real-time polymerase chain reaction.

6.3.2. For surveillance of apparently healthy animals

Test type	**Test purpose**	**Source populations**	**Tissue or sample types**	**Species**	**DSe (*n*)**	**DSp (*n*)**	**Reference test**	**Citation**
Real Time PCR	Surveillance	Naturally AbHV infected and AbHV free	Pleuropedal ganglion or pedal nerve cords	Wild blacklip, and farmed hybrid abalone	96.7 (29)	99.7 (1644)	Histopathology	Corbeil *et al.*, 2010

DSe: = diagnostic sensitivity, DSp = diagnostic specificity, qPCR: = real-time polymerase chain reaction.

Appendix 4. Flow diagram illustrating progression to a presumptive and confirmed case



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Annex 40

AQUATIC ANIMAL HEALTH STANDARDS COMMISSION WORK PLAN 2017–2018

EU comment

The EU thanks the OIE for providing the AAHSC work programme for member country information.

We would suggest including "containment" as a new definition in the Aquatic Code Glossary. Indeed, with the parallel amendments to Code Chapters, we notice that "containment" is being used more consistently throughout the chapters alongside "quarantine"as a specific option for control of animals and products from areas not declared free of disease (for example now used in articles X.X.10 as well as X.X.9). A Glossary definition will ensure that the meaning of "containment" measures is clearly understood, and this is consistent with their already being a Glossary definition for "quarantine".

AQUATIC CODE				
Task	**Sept 2017**	**Feb 2018**	**GS May 2018**	**Sept 2018**
User's Guide	Reviewed and updated in line with changes in the *Terrestrial Code* adopted in 2016 and amended as relevant. Circulated for comment	Reviewed Member comments	Present for adoption	
Glossary	Amended several definitions and circulated for comment	Reviewed Member comments	Present for adoption	
Diseases listed by the OIE (Chapter 1.3.)	Amended names of the listed fish diseases in line with 'Infection with' approach	Reviewed Member comments	Present for adoption	
OIE procedures relevant to the Agreement on the application of sanitary and phytosanitary measures of the World Trade Organisation (Chapter 5.3.)	Reviewed relevant changes to the *Terrestrial Code* adopted in 2017 and amended, as relevant and circulated for comment	Reviewed Member comments	Present for adoption	
Criteria to assess the safety of aquatic animal commodities (Chapter 5.4.)	Reviewed comments and circulated for comment	Reviewed Member comments	Present for adoption	
New chapter for infection with *Batrachochytrium salamandrivorans* (Chapter 8.X.)	Developed a new draft chapter including horizontal changes and circulated comment	Reviewed Member comments	Present for adoption	
Infection with *Batrachochytrium dendrobatidis* (Chapter 8.1.) and Infection with ranavirus (Chapter 8.2.)	Applied horizontal changes to Ch 8.X. and fish disease-specific chapters to ensure that all three amphibian chapters are aligned. Circulated for comment	Reviewed Member comments	Present for adoption	
Infection with infectious hypodermal and haematopoietic necrosis (Chapter 9.4.)	Reviewed the *ad hoc* Group assessment of *M. rosenbergii* and deleted from Article 9.4.2.	Reviewed Member comments	Present for adoption	

Horizontal amendments to fish disease-specific chapter		Reviewed the names used for all listed fish diseases in Article 10.X.1. and made changes in line with the accepted convention: 'infection with pathogenic agent X'. Aligned all chapters to deal with inconsistencies in the use of italics for viral and parasitic diseases. To be applied in all disease-specific chapters for viral diseases in the *Aquatic Code*	Present for adoption	
Epizootic haematopoietic necrosis (Chapter 10.1.)	Reviewed list of susceptible species in Article 10.1.2. after consideration of the work of the *ad hoc* Group; also applied horizontal changes and circulated for comment	Reviewed Member comments	Present for adoption	

Annex 40 (contd)

AQUATIC CODE				
Task	**Sept 2017**	**Feb 2018**	**GS May 2018**	**Sept 2018**
Infection with *Aphanomyces invadans* (epizootic ulcerative syndrome) (Chapter 10.2.)		Review Member comments	Present for adoption	
Infection with *Gyrodactylus salaris* (Chapter 10.3.)	Reviewed list of susceptible species in Article 10.3.2. after consideration of the work of the *ad hoc* Group; applied horizontal changes and circulated for comment	Reviewed Member comments	Present for adoption	
Infection with infectious salmon anaemia virus (Chapter 10.4.)	Reviewed list of susceptible species in Article 10.4.2. after consideration of the work of the *ad hoc* Group; applied horizontal changes and circulated for comment	Reviewed Member comments	Present for adoption	
Model Articles X.X.8., X.X.9., X.X.10. and X.X.11.	Developed model articles showing all horizontal changes and provided for comment	Reviewed Member comments	Present for adoption	
Glossary	Definition for basic biosecurity conditions	Circulated for comment		Review Member comments
Criteria for listing species as susceptible (Chapter 1.5.)	Reviewed Member Country comments and circulated for comment	Reviewed Member comments and recirculated for comment		Review Member comments
Infection with salmonid alphavirus (Chapter 10.5.)	Applied horizontal changes and circulated for comment	Reviewed list of susceptible species in Article 10.5.2. after consideration of the work of the *ad hoc* Group; reviewed Member comments		Review Member comments
Koi herpesvirus disease (Chapter 10.7.)		Reviewed list of susceptible species in Article 10.7.2. after consideration of the work of the *ad hoc* Group; applied horizontal changes and circulated for comment		Review Member comments
Spring viraemia of carp (Chapter 10.9.)		Reviewed list of susceptible species in Article 10.9.2. after consideration of the work of the *ad hoc* Group; applied horizontal changes and circulated for comment		Review Member comments

Annex 40 (contd)

***AD HOC* GROUPS**				
Task	**Sept 2017**	**Feb 2018**	**GS May 2018**	**Sept 2018**
Develop lists of susceptible species – all fish chapters	Reviewed *ad hoc* Group report (April 2017 meeting) and amended relevant chapters of the *Aquatic Code*	Reviewed *ad hoc* Group report (November 2017 meeting) and amended relevant chapters of the *Aquatic Code*		Review *ad hoc* Group report (May 2017 meeting) and amend relevant chapters of the *Aquatic Code*
Ad hoc Group on tilapia lake virus (TiLV)	Convene *ad hoc* Group on TiLV that will report back for the February meeting	Reviewed eAHG report and requested the continuation of the work and report back to the next meeting of the Commission in September 2018		Review *ad hoc* Group report
Develop principles for determining surveillance periods in disease-specific chapters	Requested *ad hoc* Group on Disease freedom meet electronically prior to February 2018 to develop principles for determining surveillance periods in disease-specific chapters	Reviewed eAHG report. The Commission agreed to undertake further work on this issue with a view to providing Member Countries with an approach for determining periods required to demonstrate disease freedom		Review and continue the work done by the previous Commission
New chapter on Biosecurity (4.X.)	Requested a second meeting to finalise the draft chapter prior to the February 2018 meeting of the Aquatic Animals Commission	Reviewed the report and draft chapter on aquatic animal biosecurity for aquaculture establishments. Will work to finalise the draft chapter and provide it for the next Commission meeting to be held in September 2018		Review and continue the work done by the previous Commission
AQUATIC MANUAL				
Task	**Sept 2017**	**Feb 2018**	**GS May 2018**	**Sept 2018**
White spot disease (Chapter 2.2.8.)	Amended Section 2.2.2. *Species with incomplete evidence for susceptibility* after consideration of the work of the *ad hoc* Group; reviewed the chapter and proposed further amendments; circulated for comment	Reviewed Member comments	Present for adoption	
Epizootic haematopoietic necrosis (Chapter 2.3.1.)	Amended Section 2.2.2. *Species with incomplete evidence for susceptibility* after consideration of the work of the *ad hoc* Group; reviewed the chapter and proposed further amendments; circulated for comment	Reviewed Member comments	Present for adoption	
Infection with *Gyrodactylus salaris* (Chapter 2.3.3)	Amended Section 2.2.2. *Species with incomplete evidence for susceptibility* after consideration of the work of the *ad hoc* Group; reviewed the chapter and proposed further amendments; circulated for comment	Reviewed Member comments	Present for adoption	

Annex 40 (contd)

***AD HOC* GROUPS**				
Task	**Sept 2017**	**Feb 2018**	**GS May 2018**	**Sept 2018**
Infection with infectious salmon anaemia virus (Chapter 2.3.5.)	Amended Section 2.2.2. *Species with incomplete evidence for susceptibility* after consideration of the work of the *ad hoc* Group; reviewed the chapter and proposed further amendments; circulated for comment	Reviewed Member comments	Present for adoption	
Infection with infectious hypodermal and haematopoietic necrosis (IHHN) (Chapter 5.5.4., and Sections 2.2.1. and 2.2.2.)	Deleted *Macrobrachium rosenbergii* as a susceptible species and circulated for comment	Reviewed Member comments	Present for adoption	
Develop lists of susceptible species – all fish chapters	Reviewed *ad hoc* Group report (April 2017 meeting) and amended relevant chapters of the *Aquatic Manual*	Reviewed *ad hoc* Group report (November 2017 meeting) and amended relevant chapters of the *Aquatic Manual*		Review *ad hoc* Group report (May 2017 meeting) and amend relevant chapters of the *Aquatic Manual*
Koi herpesvirus disease (Chapter 2.3.7.)		Considered *ad hoc* Group report (revised list of susceptible species) and reviewed whole chapter. Circulated for comment		Review Member comments
Infection with salmonid alphavirus (Chapter 2.3.6)		Considered *ad hoc* Group report (revised list of susceptible species) and reviewed whole chapter. Circulated for comment		Review Member comments
Infection with yellow head virus genotype 1 (Chapter 2.2.9.)		Included PCR positive species in Section 2.2.2.		Review Member comments
OTHER WORK				
Guidelines on application of criteria for listing aquatic animal diseases	Reviewed draft guidelines	Continued to work on the draft guidelines		Review the document and provide the document to Member Countries for information as part of the September 2018 meeting report

Appendix to Annex 17: Susceptible Species and Carrier Species for IHN

Source: Literature and Directive 2006/88/EC, Annex I of Regulation (EC) No 1251/2008

(background highlighted in blue colour = economic and/or epedemiologic importance in Europe)

ORDER	FAMILY	SUSCEPTIBLE SPECIES	CARRIER SPECIES	EVIDENCE COUNTRY/ REGION	NATURAL INFECTION	EXPERIMEN -TAL INFECTION	REFERENCES
Order of salmoniformes *Salmoniformes* Lachsartige	Salmonidae family *Salmonidae* Lachsfische	rainbow trout (*Oncorhynchus mykiss*) = steelhead trout (Salmo gairdneri) Regenbogenforelle		Europe, USA, Canada, Asia	X		RL 2012/31/EU v. 25.10.2012; Amend et al. 1969, Pilcher & Fryer 1980; Hsu et al. 1986; Shors and Winston 1989
		Sockeye salmon (*Oncorhynchus nerka*) Rotlachs		USA, Asia	X		RL 2012/31/EU v. 25.10.2012; Rucker et al. 1953; Williams and Amend, 1976; Sano et al. 1977; Pilcher & Fryer 1980; Hsu et al. 1986; Traxler et al. 1997; Kimura T and Awakura T, 1977
		Atlantic salmon (*Salmo salar*) Atlantischer		Canada	X		RL 2012/31/EU v. 25.10.2012; Mulcahy and Wood 1986; Armstrong et al. 1993; Saksida 2006
		brown trout (*Salmo trutta*) Bachforelle		Asia, Europe	X		Yamazaki and Motonishi, 1992, Knüsel et al. 2007
		Chinook salmon (*Oncorhynchus tshawytscha*) Königslachs		USA	X		RL 2012/31/EU v. 25.10.2012; Ross et al. 1960; Hsu et al. 1986

		coho salmon (*Oncorhynchus kisutch*) Silberlachs		USA Without clinical symptoms but pathogenic for others	X		RL 2012/31/EU v. 25.10.2012; LaPatra et al. 1989
		chum salmon (*Oncorhynchus keta*) Ketalachs		Asia	X		RL 2012/31/EU v. 25.10.2012 Sano et al. 1977, Kimura T and Awakura T, 1977
		pink *(Oncorhynchus gorbuscha)* Buckel-Lachs o. Rosa-Lachs			X		OIE 2009
		Amago salmon (*Oncorhynchus rhodurus)* Amago-Lachs = Biwa-Forelle		Asia	X		RL 2012/31/EU v. 25.10.2012; Sano et al. 1977
		masou salmon (Oncorhynchus masou) Masou-Lachs		Asia	X		RL 2012/31/EU v. 25.10.2012; Park et al. 1993, Sano et al. 1977
		cutthroat trout *(Oncorhynchus clarki)* Cutthroat-Forelle		Canada	X		Parisot and Pelnar 1962; Hsu et al. 1986;
		whitespotted char **(*Salvelinus leucomaenis*) Japanischer Saibling**		Asia	X		Kimura and Awakura, 1977
		brook trout **(*Salvelinus fontinalis*) Bachsaibling**		USA, Japan	X	X Immersion	Pilcher & Fryer 1980; Yamazaki and Motonishi, 1992; Bootland et al. 1994; OIE 2016

		grayling (*Thymallus thymallus*) Äsche		Europe	X		Kolodziejek et al. 2008; OIE 2009
		char, brook trout **(*Salvelinus* sp.) Saibling**				X Immersion	LaPatra et al. 1993; OIE 2016
			Lake trout (*Salvelinus namaycush*) Amerikanischer Seesaibling			X Immersion	Follet et al. 1997
		whitespotted char **(*Salvelinus leucomaenis*) Japanischer Saibling**		Japan	X		Kimura and Awakura 1977; OIE 2016
			arctic char (*Salvelinus alpinus*) Seesaibling			X Immersion	McAllister et al. 2000
Order of acipenserformes Acipenseriformes	Acipenseridae family Acipenseridae Störe		white sturgeon (*Acipenser transmontanus*) Weißer Stör			X Immersion	LaPatra et al. 1995
			Beluga/ European sturgeon *(Huso huso)* Europäischer Hausen				
			Russian sturgeon (*Acipenser gueldenstaedtii*) Russischer Stör				
			sterlet (*Acipenser ruthenus*) Sterlet				
			Sternhausen (*Acipenser stellatus*) starry sturgeon				
			European sea sturgeon (*Acipenser sturio*) Europäischer Stör				

			Siberian sturgeon (*Acipenser baerii*) Sibirischer Stör				
Order of Esociformes (*Esociformes*) Hechtartige	Ecocidae family (*Esocidae*) Hechte	pike (*Esox lucius*) Hecht		Europe	X		Reschova et al. 2008
Order of Clupeiformes (*Clupeiformes*) Heringsartige	Clupeidae family (*Clupeidae*) Heringe	Pacific herring (*Clupea pallasii)* Pazifischer Hering		Canada	X		Kent et al. 1998
Order of Gadiformes (*Gadiformes*) Dorschartige	Gadidae family (*Gadidae*) Dorsche	Atlantic cod (*Gadus morhua*) Dorsch		USA	X		OIE 2016; Enzmann et al. 2005
			haddock (*Melanogrammus aeglefinus)* Schellfisch				
Order of Pleuronectiformes (*Pleuronec-tiformes*) Plattfische	*Scophthalmidae family* (*Scophthalmidae)* Steinbutte		turbot (*Scophthalmus maximus*) Steinbutt			X Injektion	Castric and Jeffroy, 1991
	Pleuronectidae family (*Pleuronectidae*) Schollen		Atlantic halibut (*Hippoglossus hippoglossus*) Heilbutt				
			European flounder (*Platichthys flesus)* Flunder				

Order of Osmeriformes (*Osmeriformes*) Stintartige	Osmeridae family (*Osmeridae*) Stinte	ayu (*Plecoglossus altivelis*) Ayu		Japan	X		Nishizawa et al. 2006; OIE 2016
Order of Perciformes (*Perciformes*) Barschartige	Embiotocidae family (*Embioto-cidae*)	shiner perch (*Cymatogaster) aggregate*) Brandungsbarsch		Canada	X		Kent et al. 1998
	Percidae family (*Percidae*) Echte Barsche		yellow perch (*Perca flavescens*) Amerikanischer Flussbarsch			X Immersion, Injection	Palmer and Emmenegger 2014
			pike perch (*Sander lucioperca*, Syn.:*Stizostedion lucioperca*,*Luciope rca sandra)* Zander				
	Aulorhynchid ae family (*Aulorhyn-chidae*) Stichlings-fische	tube- snout (*Aulorhynchus Flavidus*) Stichlingsartige		Canada	X		Kent et al. 1998
Order of Cypriniformes (Cypriniformes) Karpfenartige	Cyprinidae family (Cyprinidae) Karpfenfische		(*Hypophthalmicht hys nobilis* or. alt *Aristichthys nobilis)* Marmorkarpfen				
			goldfish (*Carassius auratus*) Goldfisch				
			crucian carp (*Carassius carassius*) Europäische Karausche				

			common carp od. European carp (*Cyprinus carpio*) Karpfen				
			silver carp (*Hypophthalmichthys molitrix*) Silberkarpfen				
			Karpenfische der Gattung Leuciscus spp. asp fish (*Leuciscus aspius*) Rapfen, chub (*Leuciscus cephalus*) Döbel, ide (*Leuciscus idus*) Aland, dace (*Leuciscus leuciscus* Hasel), (*Leuciscus souffia*) Strömer				
			common roach (*Rutilus rutilus*) Rotauge				
			common rudd (*Scardinius erythrophthalmus*) Rothasel = Rotfeder				
			tench (*Tinca tinca*) Schleie				
Order of Siluriformes (Siluriformes) Welsartige	Siluridae family (*Siluridae*) Echte Welse		sheatfish (*Silurus glanis*) Wels				
	Clariidae family (*Clariidae*) Kiemensack welse		African sharptooth catfish (*Clarias gariepinus*) Afrikanische Raubwels				

	Ictaluridae family (*Ictaluridae*) Katzenwelse		channel catfish species (*Ictalurus spp.*) Arten der Katzenwelse				
			black bullhead (catfish) (*Ameiurus melas*, Syn.: *Ictalurus nebulosus*) Schwarzer Zwergwels = Schwarzer Katzenwels				
			Getüpfelter Gabelwels (*Ictalurus punctatus*) channel catfish				
	(*Pangasiidae*) Haiwelse		iridescent shark (*Pangasia nodon hypophthalmus*) Pangasius				
Order of Anguilliformes (Anguilliformes) Aalartige	Anguillidae family (*Anguillidae*) Aale	European eel (*Anguilla anguilla*) Europäischer Aal		Europe	X		Bergmann et al 2002; Enzmann et al. 2005
Spariformes	(*Sparidae*) Meerbrassen		gilt-head seabream (*Sparus aurata*) Goldbrassen			X Injection	Castric and Jeffroy, 1991
Order of Decapoda (Decapoda) Zehnfußkrebse	Astacidae family Astacidae		European crayfish, noble crayfish or broad-fingered crayfish (*Astacus astacus*) Edelkrebs o. oder Europäischer Flusskrebs				

			signal crayfish (*Pacifastacus leniusculus*) Signalkrebs				
			red swamp crawfish, red swamp crayfish, Louisiana crawfish, Louisiana crayfish or mudbug (*Procambarus clarkii*) Roter Amerikanischer Sumpfkrebs				
Order of Ephemeroptea (Ephemeroptea) Eintagsfliegen	Baetidae family Baetidae (Blue-Winged Olives)		mayfly (*Callibaetis sp.*) Eintagsfliege	USA	X		Shors and Winston, 1989

Literature:

Amend DF, Yasutake WT and Mead RW, 1969: A haematopoietic virus disease of rainbow trout and sockeye salmon. Trans. Am. Fish Soc. 98, 796-804.

Armstrong RD, Roninson JR, Rymeas C and Needam T, 1993: Infectious haematopoietic necrosis virus in Atlantic salmon in British Columbia. Can Vet J 34, 312- 313.

Bergmann SM, Ariel E, Skall HF, Fichtner D, Schlotfeldt HJ and Olesen NJ, 2002: Vergleich von Methoden zum Nachweis einer Infektion mit verschiedenen Isolaten des Virus der Infektiösen Hämatopoetischen Nekrose (IHNV). Berl Münch Tierärztl Wschr 115, 385-389.

Bootland LM, Lorz HV, Rohovec JS and Leong JC, 1994: Expertimental infection of brook troutr with infectious hematopoietic necrosis virus types 1 and 2. J Aquat Anim Health 6, 144-148.

Castric J and Jeffroy J, 1991: Experimentally induced diseases in marine fish with IHNV and a rhabdovirus of eel. Aquaculture Europe '91, Dublin (Eire), Special Publication of the European Aquaculture Society 14, 54-55.

Enzmann P-J, Kurath G, Fichtner D and Bergmann SM, 2005: Infectious hematopoietic necrosis virus: monophyletic origin of European isolates from

North American Genogroup M. Dis Aquat Org 66, 187-195.

Follet JE, Meyers TR, Burton TO and Geesin JL, 1997: Comparative susceptibilities of several salmonid species in Alaska to infectious hematopoietic necrosis virus (IHNV) and North American viral hemorrhagic septicemia virus (VHSV). J Aquat Anim Health 9, 34-40.

Hsu Y-L, Engelking HM and Leong JC, 1986: Occurrence of different types of injfectious hematopoietic necrosis virus in fish. Appl and Environ Microbiol 52, 1353-1361.

Kent ML, Traxler GS, Kieser D, Richard J, Dawe SC, Shaw RW, Prosperiporta G, Ketcheson J and Evelyn TPT, 1998: Survey of salmonid pathogens in ocean- caught fishes in British Columbia, Canada. J Aquat Anim Health 10, 211-219.

Kimura T and Awakura T, 1977: Current status of disease of cultured salmonids in Hokkaido, Japan. International Symposium on Disease of Cultured Salmonids, Tavolek, pp. 124-160.

Kimura T and Awakura T, 1977: Studies on viral diseases of Japanese fishes. VI. Infectious hematopoietic necrosis (IHN) of salmnonids in the mainland of Japan. J Tokyo Univ Fish 63(2).

Knüsel R, Bergmann SM, Einer-Jensen K, Casey J, Segner H and Wahli T, 2007: Virus isolation vs RT-PCR: which method is more successful in detecting VHSV and IHNV in fish sampled under field conditions? J Fish Dis 30, 559-568.

Kolodziejek J, Schachner O, Dürrwald R, Latif M and Nowotny N, 2008: Mid-G region of the glycoprotein gene of Austrian infectious hematopoietic necrosis virus isolates from two lineages within European isolates and are distinct from American and Asian lineages. J Clin Microbiol 46, 22-30.

LaPatra SE, Fryer JL, Wingfield WH and Hedrick RP, 1989: Infectious hematopoietic necrosis virus in Coho salmon Oncorhynchus kisutch. J Aquat Anim Health 1, 277-280.

LaPatra SE, Jones SE, Lauda KA, McDowell TS, Schneider R and Hedrick RP, 1995: White sturgeon as a potential vector of infectious hematopoietic necrosis virus. J Aquat Anim health 7, 225-230.

LaPatra SE, Parsons JE, Jones GR and McRoberts WO, 1993: Early life stage survival and susceptibility of brook trout, coho salmon, rainbow trout, and their reciprocal hybrids to infectious hematopoietic necrosis virus. J Aquat Anim Health 5, 270-274.

McAllister PE, Bebak J and Wagner BA, 2000: Susceptibility of Arctic char to experimental challenge with infectious hematopoietic necrosis virus (IHNV) and infectious pancreatic necrosis virus (IPNV). J Aquat Anim Health 12, 35-43.

Mulcahy D and Wood J, 1986: A natural epizootic of infectious haematopoietic necrosis in imported Atlantic salmon, Salmo salar L., reared in the enzootic region. J Fish Dis 9, 173-175.

Nishizawa T, Kinoshita S, Kim WS, Higashi S and Yoshimizu M, 2006: Nucleotide diversity of Japanes isolates of infectious hematopoietic necrosis virus (IHNV) based on the glycoprotein gene. Dis Aquat Org 71, 267-272.

OIE 2009 Manual of Diagnostic Tests for Aquatic Animals, Chapter 2.3.4 Infectious haematopoietic necrosis pp. 209-221.

OIE 2016 Manual of Diagnostic Tests for Aquatic Animals, Chapter 2.3.4 Infectious haematopoietic necrosis.

Palmer AD and Emmenegger EJ, 2014: Susceptibility of Koi and yellow perch to infectious hematopoietic necrosis virus by experimental exposure. J Aquat Anim Health 26, 78-83.

Parisot TJ and Pelnar J, 1962: An interim report on Sacramento River Chinook disease a virus-like disease of Chinook salmon. Prog Fish Cult 24, 51-55.

Park MA, Sohn SG, Lee SD, Chun SK, Park JW, Fryer JL and Hah YC, 1993: Infectious hematopoietic necrosis virus from salmonids cultured in Korea. J Fish Dis 16, 471-478.

Pilcher KS and Fryer JL (1980) The viral diseases of fish: a review through 1978. Part I: Diseases of proven viral etiology. CRC Critial Reviews in Microbiology 7, 287-364.

Reschova S, Pokorova D, Hulova J, Kulich P and Vesely T, 2008: Surveillance of viral fish diseases in the Czech Republic over the period January 1999-December 2006. Veterinarni Medicina 53, 86-92.

RL 2012/31/EU v. 25.10.2012

Ross AJ, Pelnar J and Rucker RR, 1960: A virus-like disease of Chinook salmon. Trans Am Fish Soc 89, 160-163.

Rucker RR, Whipple WJ, Parevin JR and Evans CA, 1953: A contagious disease of salmon possibly of virus origin. US Fish Wildlife Serv Bull 54, 35-46.

Saksida SM, 2006: Infectious haematopoietic necrosis epidemic (2001 to 2003) in farmed Atlantic salmon Salmo salar in British Columbia. Dis.Aquat Org 72, 213-223.

Sano T, Nishimura T, Okamoto N, Yamazaki T and Hanada H, 1977: Studies on viral diseases of Japanese fishes. VI Infectious haematopoietic necrosis (IHN) of salmonids in the mainland of Japan. J of the Tokyo University of Fisheries Vol 63, 81-85.

Shors TS and Winston V, 1989: Detection of infectouis hematopoietic necrosis virus in an invertebrate (Callibaetis sp). Am J Vet Res 50, 1307-1309.

Shors ST and Winston V, 1989: Neutralizing antibodies for infectious hematopoietic necrosis virus in eggs of Steelhead trout (Salmo gairdneri). Am J Vet Res 50, 232-234.

Traxler GS, Roome J, Laida K and LaPatra, 1997: Appearance of infectious hematopoietic necrosis virus (IHNV) and neutralizing antibodies in sockey salmon Oncorhynchus nerka during their migration and maturation period. Dis Aquat Org 28, 31-38.

Williams I and Amend D, 1976: A natural epizootic of infectious hematopoietic necrosis in fry of sockey salmon (Oncorhynchus nerka) at Chilko Lake, British Columbia. J Fish Res Board Can 33, 1564-1567.

Yamazaki T and Motonishi A, 1992: Control of infectious hematopoietic necrosis and infectious pancreatic necrosis in salmonid fish in Japan. In: Kimura T (ed.), Proceedings of the Oji International Symposium on Salmonid Diseases, Hokkaido University Press, Sapporo, Japan, pp. 103-110.