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

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

Paris (France), 2–6 March 2015

The OIE Aquatic Animal Health Standards Commission (hereinafter referred to as the Aquatic Animals Commission) met at OIE Headquarters in Paris from 2 to 6 March 2015.

The list of participants and the adopted agenda are presented at Annex 1 and Annex 2.

The Aquatic Animals Commission thanked the following Member Countries for providing written comments on draft texts circulated after the Commission's September 2014 meeting: Australia, Brazil, Canada, Chile, China (People's Rep. of), Chinese Taipei, Costa Rica, Cuba, El Salvador, Guatemala, Honduras, Japan, New Zealand, Nicaragua, Norway, Panama, Switzerland, Thailand, the United States of America (USA), the Member States of the European Union (EU), and the African Union–Interafrican Bureau for Animal Resources (AU-IBAR) on behalf of the OIE Delegates of Africa. The Aquatic Animals Commission acknowledged the large number of comments received and welcomed the receipt of comments from Member Countries that have not submitted comments previously.

The Aquatic Animals Commission reviewed comments that Member Countries had submitted prior to 30 January 2015 and amended texts in the OIE *Aquatic Animal Health Code* (the *Aquatic Code*) where appropriate. The amendments are shown in the usual manner by 'double underline' and '~~strikethrough~~' and may be found in the Annexes to the report. The amendments made at the March 2015 meeting are highlighted with a coloured background in order to distinguish them from those made at the September 2014 meeting.

All Member Countries' comments were considered by the Aquatic Animals Commission. However, the Commission was not able to prepare a detailed explanation of the reasons for accepting or not accepting every proposal received.

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

The table below summarises the texts as presented in the Annexes. Member Countries should note that texts in Annexes 3 to 21 are proposed for adoption at the 83rd General Session in May 2015; Annexes 22 to 25 are presented for Member Countries' comments; Annexes 26 to 28 are presented for information.

The Commission strongly encourages Member Countries to participate in the development of the OIE's international standards by submitting comments on this report. Comments should be submitted as specific proposed text changes, supported by a scientific rationale. Proposed deletions should be indicated in '~~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 22 to 25 of this report must reach OIE Headquarters by **21st August 2015** to be considered at the September 2015 meeting of the Aquatic Animals Commission.

All comments should be sent to the OIE International Trade Department at: trade.dept@oie.int.

Texts proposed for adoption:	**Annex number**
***Aquatic Code*:**	
User's guide	Annex 3
Glossary	Annex 4
Diseases listed by the OIE (Chapter 1.3.)	Annex 5
Import risk analysis (Chapter 2.1.)	Annex 6
Recommendations for disinfection of salmonid eggs (new Chapter 4.X.)	Annex 7
Control of hazards in aquatic animal feed (Chapter 4.7.)	Annex 8
General obligations related to certification (Chapter 5.1.)	Annex 9
Certification procedures (Chapter 5.2.)	Annex 10
Risk analysis for antimicrobial resistance arising from the use of antimicrobial agents in aquatic animals (new Chapter 6.5.)	Annex 11
Infection with *Batrachochytrium dendrobatidis* (Chapter 8.1.)	Annex 12
Infection with ranavirus (Chapter 8.2.)	Annex 13
Articles X.X.7. and X.X.11. of disease-specific chapters	Annex 14
Corrections in Articles 10.4.4. and 10.4.6.	Annex 15
Infection with *Perkinsus olseni* (Article 11.6.2.)	Annex 16
***Aquatic Manual*:**	
Infectious hypodermal and haematopoietic necrosis (Chapter 2.2.2.)	Annex 17
Necrotising hepatopancreatitis (Chapter 2.2.4.)	Annex 18
Taura syndrome (Chapter 2.2.5.)	Annex 19
Yellow head disease (Chapter 2.2.8.)	Annex 20
Infection with *Perkinsus olseni* (Chapter 2.4.7.)	Annex 21
Texts for Member Countries' comment:	**Annex number**
***Aquatic Code*:**	
Notification of diseases and epidemiological information (Chapter 1.1.)	Annex 22
Criteria for listing (Chapter 1.2.)	Annex 23
Infection with yellow head virus (Chapter 9.2.)	Annex 24

***Aquatic Manual*:**	
Acute hepatopancreatic necrosis disease (new draft Chapter X.X.X.)	Annex 25
Annexes for Member Countries' information:	
OIE *ad hoc* Group report on notification of animal diseases and pathogenic agents (January 2015)	Annex 26
OIE *ad hoc* Group report on susceptibility of crustacean species to infection with OIE listed diseases (February 2015)	Annex 27
Aquatic Animal Health Standards Commission Work Plan for 2015/2016	Annex 28

1. OIE *Aquatic Animal Health Code*

1.1. General comments

General comments were received from Chile and Norway. The Aquatic Animals Commission agreed with a Member Country's comment on the need for more detailed recommendations on disease prevention and control such as biosecurity, including measures for the prevention of emerging diseases. The Aquatic Animals Commission recognised the importance of this need and will continue to work on revisions to chapters on general recommendations for disease prevention and control in Section 4 of the *Aquatic Code.*

The Aquatic Animals Commission also recognises the need for improved guidance on compartmentalisation as suggested by a Member Country. This issue is discussed further at Item 6.

1.2. User's guide

Comments on the User's Guide were received from AU-IBAR, Canada, Chile, EU, Japan, Norway, PR China, Switzerland, Thailand and USA. The Aquatic Animals Commission also considered proposed amendments made by the OIE Terrestrial Animal Health Standards Commission (the Code Commission) at its February 2015 meeting to ensure alignment, where relevant.

The Aquatic Animals Commission considered Member Countries' comments and revised the text, as appropriate. In response to a Member Country's comment, the Commission amended text in point 5 in Section A to clarify the application of risk analysis when there are no OIE recommendations on a given pathogenic agent or for specific commodities.

The Aquatic Animals Commission clarified that the reference made to infection with infectious salmon anaemia virus is to illustrate the proposed approach for pathogen differentiation, and provides an example of risk management options based on strain differentiation.

The Aquatic Animals Commission agreed with a Member Country comment to amend text in point 4 (Trade requirements) and point 5 (International health certificates) in Section C to align with the relevant sections of the OIE *Terrestrial Animal Health Code (Terrestrial Code)* User's guide.

The revised User's guide is attached as Annex 3 to be presented for adoption at the 83rd General Session in May 2015.

1.3. Glossary

The Aquatic Animals Commission considered comments provided by Australia, Canada, Chile, Costa Rica, Cuba, EU, PR China, New Zealand and Norway. The Commission also considered proposed amendments to relevant definitions developed by the Code Commission at its February 2015 meeting.

In response to several Member Countries' comments, the Aquatic Animals Commission amended the definition for "biosecurity". In response to a Member Country's comment to include "chemical measures" in the definition, the Commission noted that "physical measures" refers to infrastructure and equipment required to contain pathogenic agents which should not be confused with physical and chemical means of disinfection. The Commission noted the amended definition aligns with that proposed for the *Terrestrial Code*.

The Aquatic Animals Commission wished to remind Member Countries that the revised definitions for "disinfectant" and "disinfection" were provided by the *ad hoc* Group on disinfection. This *ad hoc* Group has been tasked with developing a new draft chapter to provide guidance on disinfection of aquaculture establishments, water and fish eggs for Section 4 of the *Aquatic Code*. The Commission clarified that the proposed definitions are important to ensure a clear understanding of the use of these terms in the new draft chapter.

The Aquatic Animals Commission considered comments from several Member Countries on the types of processes that should fit within the definition of disinfectant. The Commission considered that physical processes such as drying and heating should be included within the definition because these are applied with a similar purpose to chemical disinfectants, i.e. to destroy or inhibit growth of pathogenic agents.

The Aquatic Animals Commission also considered comments from Member Countries on the types of actions disinfectants have on pathogenic agents. The Commission did not accept a Member Country's comment to include "removal" of pathogenic agents within the definition. The Commission agreed that exclusion of pathogenic agents and other material, for example through filtration, may be an important step in the disinfection process but should not be considered a "disinfectant". The Commission requested that the *ad hoc* Group consider exclusion of pathogenic agents within the context of the new draft chapter on disinfection of aquaculture establishments currently under development.

In response to a Member Country's comment that many chemicals not normally considered disinfectants, may inhibit the growth of microorganisms, the Commission agreed to amend the definition to include 'in the course of disinfection'.

The Aquatic Animals Commission considered Member Countries' comments on the types of things that disinfection may be applied to. The Commission agreed that for the purposes of the definition, it is not necessary to specify what is being disinfected and that "items" means anything that may require disinfection.

The Aquatic Animals Commission noted that no comments were received on its proposal to delete the definition for hazard identification.

The Aquatic Animals Commission did not agree with a Member Country's comment to retain the definition of 'Infective period'. Following the proposed amendments to Chapter 1.1, this definition will only appear in Chapter 4.5. and its use in the context of that chapter does not require a specific definition.

The Aquatic Animals Commission noted that only minor comments were provided on the definitions of "risk analysis" and "risk assessment" and agreed that these comments did not justify further amendments to the definitions. The Commission noted that the proposed definitions align with those proposed by the Code Commission.

In response to a Member Country's comment to explicitly mention veterinary public health in the definition of Veterinary Authority, the Aquatic Animals Commission agreed that as this definition is common to both the *Aquatic Code* and the *Terrestrial Code* that this proposal should be reviewed jointly by the two Commissions.

The revised Glossary is attached as Annex 4 to be presented for adoption at the 83rd General Session in May 2015.

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

The Aquatic Animals Commission reviewed comments provided by Member Countries on proposed changes to Article 1.1.5. and also reviewed amendments proposed by the OIE *ad hoc* Group on Notification of animal diseases and pathogenic agents to Article 1.1.4.

The Aquatic Animals Commission noted that comments supported the proposed changes to Article 1.1.5. The Commission agreed to the proposed amendments in Article 1.1.4. and revised the text as appropriate.

The report of the OIE *ad hoc* Group on Notification of animal diseases and pathogenic agents is presented as Annex 26 for Member Countries' information.

The revised Chapter 1.1. Notification of diseases and provision of epidemiological information is presented at Annex 22 for Member Countries' comments.

1.5. Criteria for listing diseases (Chapter 1.2.)

The Aquatic Animals Commission reviewed the report of the *ad hoc* Group on Notification of animal diseases and pathogenic agents. The Commission agreed that it was valuable having representatives of three OIE Specialist Commissions as observers to the meeting of this *ad hoc* Group. They also recommended further strengthening of coordination between Aquatic Animals Commission the Code Commission to ensure consistency of horizontal chapters in both *Codes*, where appropriate.

The terms of reference of the OIE *ad hoc* Group on Notification of animal diseases and pathogenic agents included a review of the listing criteria (i.e. Chapters 1.2.) of the *Terrestrial Code* and *Aquatic Code*.

The Aquatic Animals Commission noted that the *ad hoc* Group proposed a set of simplified criteria for Chapter 1.2. of the *Aquatic Code*. While the Commission noted that the proposed criteria have been simplified, there is still a need to ensure clarity of those criteria. The Commission noted that the removal of the explanatory notes leads to a loss of guidance on how to apply the criteria and the underlying principles. The Commission agreed that it is critical that the concepts underlying the listing criteria should be easily understood. The explanatory notes currently provide such clarity and their removal could result in changes to the underlying principles reflected by the criteria.

The Aquatic Animals Commission discussed some of the underlying principles of the current listing criteria that are reflected in Chapter 1.2. of the *Aquatic Code*. These issues are summarised below.

International spread – this criterion is currently satisfied if "*under international trading practices, the entry and establishment of the disease is likely*". The wording proposed for this criterion by the *ad hoc* Group is "*International spread of the agent … has been proven*". This sets a different standard to the current criterion.

Several countries or countries with zones may be declared free – this criterion is currently satisfied if "*Several countries or countries with zones may be declared free of the disease…*". The wording proposed for this criterion by the *ad hoc* Group is "*At least one country has demonstrated freedom or impending freedom…*". This sets a different standard to the current criterion in terms of the number of countries and the level of action taken to declare freedom. The current explanatory notes indicate an underlying principle (although not explicitly) that notification should be warranted to support disease control efforts. The proposed new criterion may be problematic because it may be difficult to demonstrate freedom if a disease has not been listed.

Repeatable and robust means of detection – this criterion is currently satisfied if "*A repeatable and robust means of detection/diagnosis exists*". The explanatory notes indicate that the diagnostic test should have undergone a formal validation process or a robust case definition should be available. The wording proposed for this criterion by the *ad hoc* Group reflects the need for a case definition but not a need for test validation.

Consequences – the current criteria for consequences are divided between production impacts, impacts on wild aquatic animal populations, and public health impacts. The wording proposed for this criterion by the *ad hoc* Group is divided similarly, although the criterion on production impacts less explicitly refers to farmed populations. The Commission noted that the type of impacts can be separated in different ways; for example, by the values affected (commercial or environmental) or the type of resources affected (farmed or wild), noting that wild aquatic animals may simultaneously have environmental and commercial value.

The report of the OIE *ad hoc* Group on Notification of animal diseases and pathogenic agents is presented at Annex 26 for Member Countries' information.

The Aquatic Animals Commission encourages Member Countries to comment on the issues raised above rather than specific wording proposed by the *ad hoc* Group. This will inform further consideration of the listing criteria by the Commission at its September 2015 meeting.

The revised Chapter 1.2. Criteria for listing diseases is presented at Annex 23 for Member Countries' comments.

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

a) Acute hepatopancreatic necrosis disease (AHPND)

The Aquatic Animals Commission reviewed comments received from Canada, PR China, Chinese Taipei, EU, Norway and Thailand regarding the listing of acute hepatopancreatic necrosis disease **(**AHPND) in accordance with Article 1.2.2. of the *Aquatic Code*. While some Member Countries supported listing, three did not. The Commission noted that comments against listing were based on a recent report that a *Vibrio* species other than *Vibrio parahaemolyticus* was found to be PCR positive for the plasmid that carries the toxin gene necessary to cause AHPND.

While the Aquatic Animals Commission recognised this new development in the knowledge of AHPND, they agreed that this finding must be balanced against a considerable body of scientific information that now exists and which demonstrates that a strain of *V. parahaemolyticus* carrying the pVA-1 plasmid is the cause of AHPND.

In September 2014, the Aquatic Animals Commission recommended that an *ad hoc* Group be convened to develop a draft chapter on AHPND for inclusion in the *Aquatic Manual*. The draft *Aquatic Manual* chapter has been developed (see point 2.6 below) and provides information supporting reliable identification of the aetiological agent of AHPND. Section 1 of the draft *Aquatic Manual* chapter provides a definition for AHPND and Section 7 provides definitions for suspect and confirmed cases.

The Aquatic Animals Commission noted that, as stated in Chapter 1.2. of the *Aquatic Code*, the objective of listing is to support Member Countries' efforts to prevent the transboundary spread of important diseases of aquatic animals through transparent and consistent reporting.

The Aquatic Animals Commission agreed that AHPND should be proposed for listing at the 83rd General Session in May 2015.

See also Item 2.6 regarding development of new draft *Aquatic Manual* chapter on AHPND.

The Aquatic Animals Commission encourages Member Countries to refer to the Commission's assessment of AHPND against the listing criteria (presented in Annex 21 of the September 2014 Aquatic Animals Commission meeting report).

b) Infection with HPR-deleted or HPR0 infectious salmon anaemia virus

In response to a Member Country's comment on the discrepancy between the title of Chapter 10.4. and the name of the disease in Chapter 1.3., the Aquatic Animals Commission clarified that listing in Chapter 1.3. is for the purpose of reporting and notification, and need not necessarily be identical with the title of the disease-specific chapter in the *Aquatic Code*. For infection with infectious salmon anaemia (ISA) virus, the Commission prefers to keep the name as currently listed in Chapter 1.3. to emphasise the fact that infection with both HPR-deleted and HPR0 forms of the ISA virus are notifiable to the OIE.

c) Crayfish plague *(Aphanomyces astaci)*

In response to a Member Country's request to change the name of the disease to 'infection with *Aphanomyces astaci*', the Aquatic Animals Commission agreed that the proposal is in line with the OIE approach to naming diseases. However, the Commission decided not to change the name as currently listed until the corresponding disease chapters in the *Aquatic Code* and *Manual* have been reviewed. The Commission agreed to review the listing names of diseases and the corresponding *Code* and *Manual* Chapter titles when chapters are being amended regarding the lists of susceptible species.

The Aquatic Animals Commission also reminded Member Countries that changes to the name of OIE listed diseases does have implications for the national legislation of Member Countries.

d) Infectious hypodermal and haematopoietic necrosis (IHHN)

The Aquatic Animals Commission did not support a Member Country's comment to delist IHHN, as no rationale based on the criteria of Chapter 1.2. was provided to substantiate the proposal.

The Aquatic Animals Commission reminds Member Countries that to delist a disease, scientific rationale is required to demonstrate that the disease no longer meets the listing criteria as decribed in Chapter 1.2. of the *Aquatic Code.*

e) Infection with ranavirus

The Aquatic Animals Commission considered a Member Country's comment that Ranavirus is a genus that includes several species of virus. The Commission noted that the objective of listing is to support Member Countries' efforts to prevent the transboundary spread of important diseases of aquatic animals through transparent and consistent reporting. The Commission agreed that listing at the genus level may not provide a robust basis for decision making and targeted actions.

The Aquatic Animals Commission noted that as infection with ranavirus was first listed in 2008 a review of the case definition of this disease may now be warranted.

The Aquatic Animals Commission agreed to refer the issue to the OIE designated expert for advice and review this issue at their next meeting.

f) Other diseases of amphibians

The Aquatic Animals Commission considered a Member Country's comment regarding the possible listing of additional diseases of amphibians such as the chytrid fungus *Batrachochytrium salamandrivorans,* which has caused rapid declines in wild amphibians. The Commission agreed to add this issue to its work plan and noted that there is currently no OIE Reference Laboratory for infection with *Batrachochytrium dendrobatidis* that could assist with this task.

The revised Chapter 1.3. Diseases listed by the OIE is attached as Annex 5 to be presented for adoption at the 83rd General Session in May 2015.

1.7. Import risk analysis (Chapter 2.1.)

The Aquatic Animals Commission considered comments provided by Australia, Brazil, Chile, PR China, EU and Norway.

In response to a Member Country's suggestion to add release assessment to the chapter, the Aquatic Animals Commission pointed out that the 2012 edition of the *Aquatic Code* changed the term 'release assessment' to 'entry assessment' in Article 2.1.4. and this change is consistent with the second edition of the OIE *Handbook on Import Risk Analysis for Animals and Animal Products*.

The Aquatic Animals Commission reiterated the importance of ensuring consistency between Chapter 2.1. of the *Aquatic Code* with the changes in Articles 2.1.5. and 2.1.6. recently adopted in Chapter 2.1 of the *Terrestrial Code*.

The revised Chapter 2.1. Import risk analysis is attached as Annex 6 to be presented for adoption at the 83rd General Session in May 2015.

1.8. Recommendations for disinfection of salmonid eggs (new Chapter 4.X.)

The Aquatic Animals Commission considered comments provided by Canada, Chile, PR China, EU, New Zealand, Norway and Switzerland and amended the draft chapter as appropriate.

The new Chapter Recommendations for disinfection of salmonid eggs (Chapter 4.X.) is attached as Annex 7 to be presented for adoption at the 83rd General Session in May 2015.

The Aquatic Animals Commission wished to note that if Chapter 4.X. is adopted at the General Session in May 2015, the *Aquatic Manual* Chapter 1.1.3. 'Methods for disinfection of aquaculture establishments' will be deleted as a consequential change. The Commission wished to remind Member Countries that Chapter 1.1.3. is misplaced in the *Aquatic Manual* and that the *ad hoc* Group on Disinfection is revising Chapter 4.3. of the *Aquatic Code* to provide more detailed recommendations on this subject.

1.9. Control of hazards in aquatic animal feed (Chapter 4.7.)

The Aquatic Animals Commission considered comments provided by Australia, Canada, Chile, EU, Norway, New Zealand, PR China, and Switzerland. The Commission reviewed the comments received, and amended the text of draft chapter as appropriate.

The revised Chapter 4.7. Control of hazards in aquatic animal feed is attached as Annex 8 to be presented for adoption at the 83rd General Session in May 2015.

1.10. General obligations related to certification (Chapter 5.1.)

The Aquatic Animals Commission considered comments provided by Australia, Chile, EU and Japan. The Commission also considered the text in Chapter 5.1. of the *Terrestrial Code* as revised by the Code Commission at its February 2015 meeting. The Commission amended the text based on these considerations.

The revised Chapter 5.1. General obligations related to certification is attached as Annex 9 to be presented for adoption at the 83rd General Session in May 2015.

1.11. Certification procedures (Chapter 5.2.)

The Aquatic Animals Commission considered comments provided by Chile and EU. The Aquatic Animals Commission also considered the text in Chapter 5.2. of the *Terrestrial Code* as revised by the Code Commission at its February 2015 meeting. The Commission amended the text based on these considerations.

The revised Chapter 5.2. Certification procedures is attached as Annex 10 to be presented for adoption at the 83rd General Session in May 2015.

1.12. Risk analysis for antimicrobial resistance arising from the use of antimicrobial agents in aquatic animals (draft Chapter 6.5.)

The Aquatic Animals Commission considered comments provided by Australia, EU, PR China, New Zealand, Norway, Switzerland, Thailand and USA and amended the text as appropriate.

The Aquatic Animals Commission moved text defining the use of the terms for hazard, risk and risk to public health, for the purpose of this chapter, into a new point 3 of Article 6.5.1. to improve readability. The lists of factors to be considered in points on release assessment, exposure assessment and consequence assessment in Articles 6.5.3. and 6.5.4. were expanded and text in the points on risk estimation were simplified to improve clarity. The Commission also replaced the term ‘release assessment’ with the term ‘entry assessment’ throughout the chapter to ensure consistency with Chapter 2.1.

The new Chapter 6.5. Risk analysis for antimicrobial resistance arising from the use of antimicrobial agents in aquatic animals is attached as Annex 11 to be presented for adoption at the 83rd General Session in May 2015.

1.13. Amphibian disease-specific chapters (Chapters 8.1. and 8.2.)

The Aquatic Animals Commission considered comments provided by Australia, Chinese Taipei, El Salvador, EU and New Zealand and amended the text of these chapters as appropriate.

The Aquatic Animals Commission wished to remind Member Countries that, as noted in their September 2014 meeting report, there is an inconsistency between the *Aquatic Code* and *Aquatic Manual* in the recommendations for treatment prior to importation of amphibians intended for the pet trade. Treatment of live animals prior to importation is not considered an adequate risk mitigation measure to prevent spread of infection with *Batrachochytrium dendrobatidis*. The Commission therefore proposed the removal of the provision for treatment of live aquatic animals from Articles 8.1.8. and 8.1.10.

A Member Country commented that crayfish are now known to be effective carriers of *Batrachochytrium dendrobatidis* and thus that the *Aquatic Code* and *Aquatic Manual* chapters may need to be updated. The Commission agreed to review the evidence at its next meeting.

The revised Chapter 8.1. Infection with *Batrachochytrium dendrobatidis* is attached as Annex 12 to be presented for adoption at the 83rd General Session in May 2015.

The revised Chapter 8.2. Infection with ranavirus is attached as Annex 13 to be presented for adoption at the 83rd General Session in May 2015.

1.14. Infection with yellow head virus (Chapter 9.2.)

The Aquatic Animals Commission considered a Member Country's comment to add 'genotype 1' after the name of infection with yellow head virus (YHV) given that genotype 1 is the only known pathogenic agent of yellow head disease. (See also Item 1.18.).

The revised Chapter 9.2. Infection with yellow head virus is attached as Annex 24 for Member Countries' comments.

1.15. Articles X.X.7. and X.X.11. of disease-specific chapters

In its September 2014 meeting report, the Aquatic Animals Commission recognised that the text in Articles X.X.7. and X.X.11. of disease-specific chapters is almost identical and therefore proposed to merge these two articles in all disease-specific chapters of the *Aquatic Code*. The Commission noted that the model articles apply to Article X.X.7. (importation of live aquatic animals) and Article X.X.11. (importation of aquatic animal products) from a country, zone or compartment declared free for all disease-specific chapters except in Chapter 10.4. where this issue applies to Articles 10.4.10., 10.4.11., 10.4.15. and 10.4.16.

The Commission noted that only comments supportive of this proposal were received.

The revised model Articles X.X.7. and X.X.11. is attached as Annex 14 to be presented for adoption at the 83rd General Session in May 2015.

[Note: for Chapter 10.4., this issue applies to Articles 10.4.10., 10.4.11., 10.4.15. and 10.4.16.]

1.16. Corrections in Articles 10.4.4. and 10.4.6.

In its September 2014 meeting report, the Aquatic Animals Commission noted that the text in point 2 of Articles 10.4.4. and 10.4.6. in Chapter 10.4. was incorrect. Only comments supportive of the proposed changes were received.

The revised Articles 10.4.4. and 10.4.6. are attached as Annex 15 to be presented for adoption at the 83rd General Session in May 2015.

1.17. Infection with *Perkinsus olseni* (Article 11.6.2.)

In its September 2014 meeting report, the Aquatic Animals Commission had proposed the deletion of *Crassostrea gigas* as a species susceptible to infection with *Perkinsus olseni* as there is no information to substantiate that this species is susceptible. No objections to this proposal were received.

The revised Article 11.6.2. is attached as Annex 16 to be presented for adoption at the 83rd General Session in May 2015.

1.18. List of susceptible species for OIE listed crustacean diseases

The Aquatic Animals Commission considered the report of the *ad hoc* Group on Susceptibility of crustacean species to infection with OIE listed diseases and commended the members of this *ad hoc* Group for their excellent work.

The Aquatic Animals Commission reviewed the *ad hoc* Group's proposed changes to the list of susceptible species for Chapter 9.2. Infection with yellow head virus. The Commission noted that the application of the new criteria for listing species as susceptible to infection with a specific pathogen (described in Chapter 1.5.) to infection with yellow head virus, by the *ad hoc* Group, resulted in an amended list of susceptible species (see details in the *ad hoc* Group report at Annex 27). The Commission agreed to circulate the proposed changes to the list of susceptible species in Article 9.2.2. for Member Countries' comments.

The Aquatic Animals Commission also requested that authors of the *Aquatic Manual* Chapter 2.2.8. Yellow head disease to amend the list of susceptible species in this chapter to align with the recommendations of the *ad hoc* Group report. The Commission will review the amended *Manual* chapter at its next meeting in September 2015.

The Commission also recommended that the *ad hoc* Group continue its work to review the list of susceptible species for other OIE listed crustacean pathogenic agents.

The report of the OIE *ad hoc* Group on Susceptibility of crustacean species to infection with OIE listed diseases is attached as Annex 27 for Member Countries' information.

The revised Article 9.2.2. Infection with yellow head virus is attached as Annex 24 for Member Countries' comments.

2. OIE *Manual of Diagnostic Tests for Aquatic Animals*

2.1. Chapter 2.2.2. Infectious hypodermal and haematopoietic necrosis

The Aquatic Animals Commission, in consultation with the chapter's authors, reviewed Member Countries' comments and amended the text, as appropriate.

The revised Chapter 2.2.2. Infectious hypodermal and haematopoietic necrosis is attached as Annex 17 to be presented for adoption at the 83rd General Session in May 2015.

2.2. Chapter 2.2.4. Necrotising hepatopancreatitis

The Aquatic Animals Commission, in consultation with the chapter's authors, reviewed Member Countries' comments and amended the text, as appropriate.

The revised Chapter 2.2.4. Necrotising hepatopancreatitis is attached as Annex 18 to be presented for adoption at the 83rd General Session in May 2015.

2.3. Chapter 2.2.5. Taura syndrome

The Aquatic Animals Commission, in consultation with the chapter's authors, reviewed Member Countries' comments and amended the text, as appropriate.

The revised Chapter 2.2.5. Taura syndrome is attached as Annex 19 to be presented for adoption at the 83rd General Session in May 2015.

2.4. Chapter 2.2.8. Infection with yellow head virus

The Aquatic Animals Commission, in consultation with the chapter's authors, reviewed Member Countries' comments and amended the text, as appropriate.

The revised Chapter 2.2.8. Infection with yellow head virus is attached as Annex 20 to be presented for adoption at the 83rd General Session in May 2015.

2.5. Chapter 2.4.6. Infection with *Perkinsus olseni*

No Member Countries' comments had been received on the revised section 2.2.1. of Chapter 2.4.6.

The revised Chapter 2.4.6. Infection with *Perkinsus olseni* is attached as Annex 21 to be presented for adoption at the 83rd General Session in May 2015.

2.6. New draft chapter on acute hepatopancreatic necrosis disease

At their September 2014 meeting, the Aquatic Animals Commission recommended that an *ad hoc* Group be convened to develop a draft chapter on acute hepatopancreatic necrosis disease (AHPND) for inclusion in the *Aquatic Manual,* in light of the proposal to list the disease.

The Aquatic Animals Commission acknowledged the *ad hoc* Group's work and revised the draft chapter. The Commission clarified the definition of AHPND to address the disease caused by unique strains of *Vibrio parahaemolyticus*, carrying a plasmid, designated pVA1, containing genes coding for a deadly binary toxin (Pir^{vp}). The Commission emphasised that the main purpose of listing a disease is to share important information on its diagnosis, distribution and notification. For this purpose, Section 7 of disease-specific chapters in the *Aquatic Manual* provides definitions of a suspect and of a confirmed case of the disease in question. For AHPND, a case is suspicious based on histopathology findings or detection of *V. parahaemolyticus* carrying the plasmid or mortality associated with clinical signs; a case can be confirmed by detection of *V. parahaemolyticus* carrying the plasmid and histopathology findings or mortality associated with clinical signs or bioassay.

The draft new Chapter Acute hepatopancreatic necrosis disease (Chapter X.X.X.) is presented at Annex 25 for Member Countries' comment.

3. OIE Reference Centers

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

The Aquatic Animals Commission noted that there are currently no OIE Reference Laboratories for necrotising hepatopancreatitis or for infection with *Batrachochytrium dendrobatidis* and invites applications from Member Countries where expertise exists for these crustacean and amphibian diseases.

3.2. Annual reports of Reference Centre activities in 2014

The Aquatic Animals Commission was presented with an analysis of the activities of the Reference Laboratories and Collaborating Centres for aquatic animal diseases in 2014. Reports had been received from 35 of the 42 OIE Reference Laboratories and one of the two Collaborating Centres for aquatic animals.

The Aquatic Animals Commission was impressed with the quality of the work carried out by the laboratories and expressed its gratitude to the experts for their valuable work.

The Aquatic Animals Commission was pleased to note that the number of OIE Reference Laboratories that do not have an internationally recognised quality management system in place had decreased. The Commission encourages non-accredited OIE Reference Laboratories to take the necessary steps to achieve this goal.

The Aquatic Animals Commission also noted that a large number of OIE Reference Laboratories are producing diagnostic reagents but none are included in the current list of OIE-approved standard reference reagents (see: http://www.oie.int/en/our-scientific-expertise/veterinary-products/reference-reagents/). The Commission encourages the OIE Reference Laboratories for aquatic animal diseases to consider submitting their reference reagents for approval by the OIE.

4. Twinning projects

The Aquatic Animals Commission was updated on the status of aquatic animal disease twinning projects. As of September 2014, the project on infectious salmon anaemia between Canada and Chile has been completed. The following projects are ongoing: infectious haematopoietic necrosis between USA and PR China, Infectious salmon anaemia between Norway and Brazil, Koi Herpesvirus between Japan and Indonesia and Shrimp diseases between USA and Indonesia.

The Aquatic Animals Commission reviewed a twinning project proposal and provided technical comments.

5. Follow-up on Recommendations from the Third Global Conference of OIE Reference Centers, Seoul (Rep. of Korea), 14–16 October 2014

The Aquatic Animals Commission took note of the Recommendations from the Third Global Conference of OIE Reference Centres.

The Aquatic Animals Commission appreciated the inclusion of a session on aquatic animal diseases, which included valuable presentations focused on diagnostic test validation and quality assurance. The Commission noted that the main recommendations of this session were to improve standards and guidelines for the validation of diagnostic tests for aquatic animal diseases, to improve communication among the Aquatic Reference Laboratories and to continue to encourage OIE Reference Laboratories for aquatic animal diseases to become accredited to ISO 17025 or an equivalent quality management system.

The recommendations are available on the OIE website at http://www.oie.int/eng/refcentre2014/A_Final_Recommendations_Korea_2014.pdf.

6. Follow-up actions from the OIE Global Conference on Aquatic Animal Health: 'Riding the wave to the future' Ho Chi Minh City (Vietnam), 20–22 January 2015

The Aquatic Animals Commission discussed outcomes and recommendation of the OIE Global Conference on Aquatic Animal Health held in Ho Chi Minh City (Vietnam), 20-22 January 2015.

The Aquatic Animals Commission noted strong interest from participants during the surveillance session. The presentations and panel discussions raised several points regarding strengthening recommendations on surveillance in the *Aquatic Code*. In particular, the session highlighted the need to provide guidance on more flexible approaches to surveillance while also addressing the challenges of conducting surveillance for aquatic animal diseases. The Commission recognises the need for guidance on cost effective approaches to surveillance (outcome and risk based surveillance). The Commission also recognises the need to consider underlying epidemiological principles when specifying required surveillance periods in the disease-specific chapters.

The Aquatic Animals Commission also noted strong interest from participants during the compartmentalisation session. The Commission noted that the panel discussion highlighted the need to clarify the concept of compartmentalisation and reminds Member Countries that the purpose of compartmentalisation in the *Aquatic Code* is to facilitate international trade. This may be distinct from biosecurity measures not primarily designed to facilitate international trade.

The Aquatic Animals Commission noted the need to provide updated and separate guidance for zoning and compartmentalisation in the *Aquatic Code*. This revised guidance should support practical application of these concepts by the private sector and Competent Authorities. These revised chapters would require supporting guidance in Section 4 of the *Aquatic Code* on principles of biosecurity and disease control.

The conference emphasised the importance of the PVS pathway to support Member Countries to build capacity of their Veterinary and Aquatic Animal Health Services. The Commission recommended that Member Countries consider requesting an OIE PVS Tool: Aquatic evaluation of their Veterinary Service or Aquatic Animal Health Service with the objective of improving competencies and general compliance with OIE standards for aquatic animals. The Commission noted that many countries expressed an interest in an OIE PVS Tool: Aquatic evaluation and considered this to a positive development.

In line with the outcomes of the Third Global Conference of OIE Reference Centers the conference highlighted ways in which the *Aquatic Manual* can be improved to provide clear guidance on test validation. The Aquatic Animals Commission sees the opportunity to draw on the knowledge and expertise of the OIE Reference Centres network to pursue this work.

The Aquatic Animals Commission expressed its appreciation to donors for financing this event, the Government of Vietnam for hosting the conference, to the speakers for their excellent presentations and to participants for their contribution to the discussions.

An aim of the conference was to set priorities for future work of the Aquatic Animals Commission. The Commission agreed this goal was achieved and the issues noted above should be considered in the formulation of the new Commission's Work Plan at its next meeting in September 2015.

The conference recommendations, presentations and abstracts are available on the OIE website at http://www.oie.int/eng/A_AAHRWF2015/recommandations.htm

7. Aquatic Animals Commission Work Plan for 2015/2016

The Aquatic Animals Commission reviewed and updated its work plan. The work plan provides Member Countries with an overview of current and upcoming activities.

The Aquatic Animals Commission's Work Plan for 2015/16 is presented in Annex 28 for Member Countries' information.

8. Other business

8.1. Notification of new susceptible species to infection with *Perkinsus olseni*

Following the notification by a Member Country of a new species *Haliotis iris* susceptible to infection with *P. olseni*, the Aquatic Animals Commission agreed to request the OIE designated expert to review the scientific evidence supporting listing this new susceptible host species according to the criteria in Chapter 1.5. of the *Aquatic Code*. The Commission noted that the current *Aquatic Manual* indicates a wide range of susceptible species, and explicitly suggests that more susceptible species are yet to be identified. The Aquatic Animals Commission agreed to include this item on its Work Plan.

8.2. Fish-borne Zoonotic Trematodes

The Aquatic Animals Commission held a teleconference with Dr Rohana Subasinghe (FAO) to discuss the issue of Fish-borne Zoonotic Trematodes (FZT) raised during the OIE Global Conference on Aquatic Animal Health in Vietnam.

The Aquatic Animals Commission recognises the importance of FZT which are estimated to infect over 18 million people worldwide, and a number of other mammals (e.g. cats, dogs, pigs, fish-eating birds). The Commission also noted that FZT have recently been added to the WHO list of neglected tropical emerging diseases. In aquatic systems one of the main risk factors for FZT infection and transmission is the contamination of pond environments with FZT eggs from infected hosts (i.e. humans, domestic animals and fish-eating birds). Factors that promote the growth of snail intermediate host populations also increase the risk of FZT. In aquaculture systems, it should be possible to reduce environmental contamination with FZT by mechanically removing the intermediate hosts (snails). This is particularly important as future fish availability will significantly and increasingly depend on aquaculture.

The Aquatic Animals Commission agreed to include this important subject in its Work Plan and consider developing a chapter to provide guidance on managing the risks associated with FZT.

9. Next meeting

September 2015 (specific date to be confirmed).

.../Annexes

Annex 1

MEETING OF THE OIE
AQUATIC ANIMAL HEALTH STANDARDS COMMISSION

Paris, 2–6 March 2015

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Annex 2

MEETING OF THE OIE
AQUATIC ANIMAL HEALTH STANDARDS COMMISSION

Paris, 2–6 March 2015

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Adopted agenda

1. OIE *Aquatic Animal Health Code*
   - 1.1. General comments
   - 1.2. User's guide
   - 1.3. Glossary
   - 1.4. Notification of diseases and provision of epidemiological information (Chapter 1.1.)
   - 1.5. Criteria for listing (Chapter 1.2.)
   - 1.6. Diseases listed by the OIE (Chapter 1.3.)
   - 1.7. Import risk analysis (Chapter 2.1.)
   - 1.8. Recommendations for disinfection of salmonid eggs (new Chapter 4.X.)
   - 1.9. Control of hazards in aquatic animal feed (Chapter 4.7.)
   - 1.10. General obligations related to certification (Chapter 5.1.)
   - 1.11. Certification procedures (Chapter 5.2.)
   - 1.12. Risk analysis for antimicrobial resistance arising from the use of antimicrobial agents in aquatic animals (draft Chapter 6.5.)
   - 1.13. Amphibian disease-specific chapters (8.1. and 8.2.)
   - 1.14. Infection with yellow head virus (Chapter 9.2)
   - 1.15. Articles X.X.7. and X.X.11. of disease-specific chapters
   - 1.16. Corrections in Articles 10.4.4. and 10.4.6.
   - 1.17. Infection with *Perkinsus olseni* (Article 11.6.2.)
   - 1.18. List of susceptible species for OIE listed crustacean diseases
2. OIE *Manual of Diagnostic Tests for Aquatic Animals*
   - 2.1. Chapter 2.2.2. Infectious hypodermal and haematopoietic necrosis
   - 2.2. Chapter 2.2.4. Necrotising hepatopancreatitis
   - 2.3. Chapter 2.2.5. Taura sysdrome
   - 2.4. Chapter 2.2.8. Yellow head disease

Annex 2 (contd)

2.5. Chapter 2.4.7. Infection with *Perkinsus olseni*

2.6. New draft chapter on acute hepatopancreatic necrosis disease

3. OIE Reference Centres

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

3.2. Annual reports of Reference Centre activities in 2014

4. Twinning projects

5. Follow-up on Recommendations from the Third Global Conference of OIE Reference Centres, Seoul (Rep. of Korea), 14–16 October 2014

6. Follow-up actions from the OIE Global Conference on Aquatic Animal Health: 'Riding the wave to the future', Ho Chi Minh City (Vietnam), 20–22 January 2015

7. Aquatic Animals Commission Work Plan for 2015/2016

8. Other business

8.1. Notification new susceptible species to infection with *Perkinsus olseni*

8.2. Fish-borne Zoonotic Trematodes

9. Next meeting

Annex 3

~~GUIDE TO THE~~ USER'S GUIDE ~~OF THE~~ ~~*AQUATIC ANIMAL HEALTH CODE*~~

A. Introduction

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

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

~~3) The *Aquatic Code* currently does not encompass any zoonotic disease, however, veterinary public health is part of the mandate of the OIE, including in the field of aquatic animal health.~~

3~~4~~) The 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 ~~and~~ welfare of farmed fish ~~during production and trade in aquatic animals and aquatic animal product~~s.

4~~5~~) ~~The absence of chapters, articles or~~ When the *Aquatic Code* does not include recommendations on particular pathogenic agents or commodities, ~~does not mean that~~ Veterinary Authorities and other appropriate sanitary measures can still be applied by Competent Authorities ~~may not apply appropriate *aquatic animal* health and welfare measures~~ provided they are based on a risk analysis conducted in accordance with the *Aquatic Code*~~. However, such measures should be based on sound scientific justification according to the principles of the WTO SPS Agreement.~~

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* ~~with a contextual meaning~~ are defined in the Glossary. The reader should be aware of ~~the contextual~~ definitions given in the Glossary when reading and using the *Aquatic Code*. Defined terms appear in italics. In the online ~~on-line~~ 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 ~~diagnosis,~~ surveillance and notification of pathogenic agents. The section ~~standards~~ 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 ~~trade~~ standards. The importing country may also use these standards to justify import measures which ~~are~~ exceed ~~more trade restrictive~~ ~~stricter~~ ~~than~~ existing OIE ~~trade~~ 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 ~~Veterinary Services and Aquatic Animal Health Services~~ of Member Countries to meet their objectives of improving aquatic animal health and welfare of farmed fish, as well as to establish and maintain confidence in their *international aquatic animal health certificate*s.

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, ~~and~~ disposal of aquatic animal waste and control of hazards in aquatic animal feed.

7) The standards in the chapters of Section 5 are designed for the implementation of general sanitary measures for trade. ~~In particular,~~ They ~~chapters~~ address certification and the measures applicable by the exporting, transit and importing countries. ~~Section 5 also includes~~ A~~a~~ range of model *international aquatic animal health certificates* is ~~are~~ provided to facilitate ~~for~~ consistent documentation ~~to be used as a harmonised basis of~~ 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 ~~their welfare~~ during ~~their~~ transport, ~~at the time of~~ stunning and killing for human consumption, and when ~~as well as in the situation of~~ killing for disease control purposes.

10) The standards in each of the chapters of Sections 8 to 11 are designed to prevent the ~~aetiological~~ 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. ~~Chapters include specific measures to prevent and control the infections of global concern.~~

C. Specific issues

1) Notification

Chapter 1.1. describes Member Countries' obligations under the OIE Organic Statutes. Listed ~~as well as emerging~~ diseases, as prescribed in Chapter 1.1., are compulsorily notifiable. Member Countries are encouraged ~~also~~ 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. ~~provides~~ 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) Pathogen differentiation

Some pathogens have one or more variants. Existence of highly pathogenic variants and the need to differentiate them from more benign variants is 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 ~~should be~~ proportionate to the risk posed by the different strains ~~of the pathogenic agent~~ should be applied~~.~~ ~~As an example,~~ Infection with infectious salmon anaemia virus is a ~~T~~the first listed disease for which ~~offering~~risk management options based on strain differentiation are provided ~~for infection with infectious salmon anaemia virus is Infection with infectious salmon anaemia virus~~.

3) Determining the susceptibility of species

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*. This is ~~of particular~~ importan~~ce~~ in the ~~context of~~ aquaculture context, given the large number~~s~~ of existing ~~species,~~ and ~~the number of~~ new aquaculture species ~~existing in being introduced to aquaculture~~.

4) Trade requirements

~~International a~~Aquatic animal health measures related to international trade ~~measures~~ 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 ~~more or less restrictive than~~ those recommended by the *Aquatic Code*. ~~To scientifically justify However, i~~If measures exceed ~~are more trade restrictive than~~ OIE standards ~~measures~~ the importing country should provide scientific justification by conducting 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 (SPS Agreement).

Chapters 5.1. to 5.3. describe the obligations and ~~ethics~~ ethical responsibilities of importing and exporting countries in international trade. ~~Veterinary Authorities and other~~ Competent Authorities and all veterinarians ~~or~~ and certifying officials directly involved in international trade should be familiar with these chapters. ~~These c~~Chapter~~s~~ 5.3. also ~~provide~~ describes ~~guidance~~ the OIE ~~for~~ informal procedure for dispute mediation ~~by the OIE~~.

Disease-specific chapters in the *Aquatic Code* include articles listing the commodities 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 ~~apply measures trade restrictions to~~ the listed ~~commodities~~ with respect to the listed commodities.

5) Trade in aquatic animal commodities

Chapter 5.4. describes the criteria used to assess the safety of aquatic animal commodities ~~that are listed in Articles X.X.3. and X.X.11. (crustacean and mollusc) or X.X.12. (amphibian and fish) disease-specific chapters~~.

Based on assessments using criteria in Article 5.4.1., in all disease chapters, point 1 of Article X.X.3. lists aquatic animal ~~products~~ 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 ~~products~~ commodities in point 1 of Article X.X.3. are based on the absence of the pathogenic agent ~~in the traded aquatic animals~~ commodity ~~and aquatic animal products~~ or inactivation of the pathogenic agent by treatment or processing.

Based on assessments using criteria in Article 5.4.2, in all *disease* chapters, point 1 of Article X.X.12. (for Chapter 10.4. the relevant Article is 10.4.17.) ~~(amphibian and fish disease chapters) and Article X.X.11. (crustacean and mollusc disease chapters)~~ lists *aquatic animal~~s~~* ~~or aquatic animal products~~ 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~~s~~* *commodities* ~~or aquatic animal products~~ in point 1 of Article X.X.12. ~~(amphibian and fish disease chapters) and Article X.X.11. (crustacean and mollusc disease 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.

~~Disease-specific chapters in the *Aquatic Code* reflect the reality of trade and include traded commodities, accounting for their diversity, and propose a list of safe commodities for trade facilitation. The disease-specific chapters of the Aquatic Code include an article listing the commodities that are considered safe for trade without the imposition of sanitary measures, regardless of the status of the country or zone for the agent in question. This is a work in progress and some chapters do not yet contain articles listing safe commodities. Where such a list is present, importing countries should not apply trade restrictions to the listed commodities with respect to the agent in question.~~

Annex 3 (contd)

~~5~~6) International health certificates

An international aquatic animal health certificate is an official document that ~~drawn up by~~ the ~~Veterinary Authority or other~~ *Competent Authority* of the exporting country ~~draws up~~ issues in accordance with Chapter 5.1. and Chapter 5.2. It ~~Certificates~~ lists ~~the~~ aquatic animal health requirements for the exported *commodity*. The quality of the exporting country's ~~Veterinary Services or~~ Aquatic Animal Health Services is essential in providing assurances to trading partners regarding the safety of exported aquatic animal~~s~~ commodities ~~and aquatic animal products~~. This includes the ~~Veterinary Services or~~ 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 animal~~s~~ commodities ~~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 following steps should be taken when drafting *international aquatic animal health certificates*:

a) identify ~~list~~ the diseases, ~~for~~ from which the importing country is justified in seeking protection ~~in regards to~~ 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 ~~or eradication~~ programmes;

b) for commodities capable of transmitting these diseases through international trade, the importing country should apply the relevant articles ~~addressing the commodity in question~~ in the ~~relevant~~ disease-specific chapters. The application of the articles should be adapted to the disease status of the exporting country, zone or compartment. Such status should be established according to 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 aquatic animal health certificates* should be kept as simple as possible and should be clearly worded, to avoid misunderstanding of the importing country's requirements;

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

7~~6~~) Guidance notes for importers and exporters

It is recommended that ~~Veterinary Authorities and other~~ Competent Authorities ~~are recommended to~~ 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.

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Annex 4

GLOSSARY

BIOSECURITY

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

DISINFECTANTS

means chemical compounds or physical processes capable of destroying *pathogenic agents* ~~microorganisms~~ or inhibiting their growth ~~or survival ability~~ in the course of *disinfection*.

DISINFECTION

~~means the application, after thorough cleansing, of procedures intended to destroy the infectious or parasitic agents of *diseases* of *aquatic animals*, including zoonoses; this applies to *aquaculture establishments* (i.e. hatcheries, fish farms, oyster farms, shrimp farms, nurseries, etc.), *vehicles*, and different equipment/objects that may have been directly or indirectly contaminated.~~

means the process of cleaning and applying *disinfectants* to inactivate *pathogenic agents* on potentially contaminated items.

~~***HAZARD IDENTIFICATION***~~

~~means the process of identifying the pathogenic agent(s) which could potentially be introduced in the commodity considered for importation.~~

~~***INFECTIVE PERIOD***~~

~~means the longest period during which an affected *aquatic animal* can be a source of *infection*.~~

RISK ANALYSIS

means the ~~complete~~ process composed of *hazard* ~~*identification*~~ identification, *risk assessment*, *risk management* and *risk communication*.

RISK ASSESSMENT

means the scientific evaluation of the likelihood and the biological and economic consequences of entry, establishment and spread of a *hazard* ~~within the *territory* of an *importing country*.~~

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— Text deleted.

Annex 5

CHAPTER 1.3.

DISEASES LISTED BY THE OIE

Preamble: The following *diseases* are listed by the OIE 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:

– Epizootic haematopoietic necrosis

– 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 haematopoietic necrosis

– Koi herpesvirus disease

– Red sea bream iridoviral disease

– Spring viraemia of carp

– Viral haemorrhagic septicaemia.

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*

– Infection with *Xenohaliotis californiensis*.

Annex 5 (contd)

Article 1.3.3.

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

– Acute hepatopancreatic necrosis disease

– Crayfish plague (*Aphanomyces astaci*)

– Infection with yellow head virus

– Infectious hypodermal and haematopoietic necrosis

– Infectious myonecrosis

– Necrotising hepatopancreatitis

– Taura syndrome

– White spot disease

– White tail disease.

Article 1.3.4.

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

– Infection with *Batrachochytrium dendrobatidis*

– Infection with ranavirus.

Annex 6

CHAPTER 2.1.

IMPORT RISK ANALYSIS

Article 2.1.1.

Introduction

The importation of *aquatic animals* and *aquatic animal products* involves a degree of *disease risk* to the *importing country*. This *risk* may be represented by one or several *diseases* or *infections*.

The principal aim of import *risk analysis* is to provide *importing countries* with an objective and defensible method of assessing the *disease risks* associated with the importation of *aquatic animals*, *aquatic animal products*, *aquatic animal* genetic material, feedstuffs, *biological products* and *pathological material*. The principles and methods are the same whether the *commodities* are derived from aquatic and/or terrestrial animal sources. The analysis should be transparent. This is necessary so that the *exporting country* is provided with clear reasons for the imposition of import conditions or refusal to import.

Transparency is also essential because data are often uncertain or incomplete and, without full documentation, the distinction between facts and the analyst's value judgements may blur.

This chapter provides recommendations and principles for conducting transparent, objective and defensible *risk analyses* for *international trade*. However, it cannot provide details on the means by which a *risk analysis* is carried out as the purpose of the *Aquatic Code* is simply to outline the necessary basic steps. The components of *risk analysis* ~~described in this chapter~~ are *hazard identification*, *risk assessment*, *risk management* and *risk communication* (Figure 1).

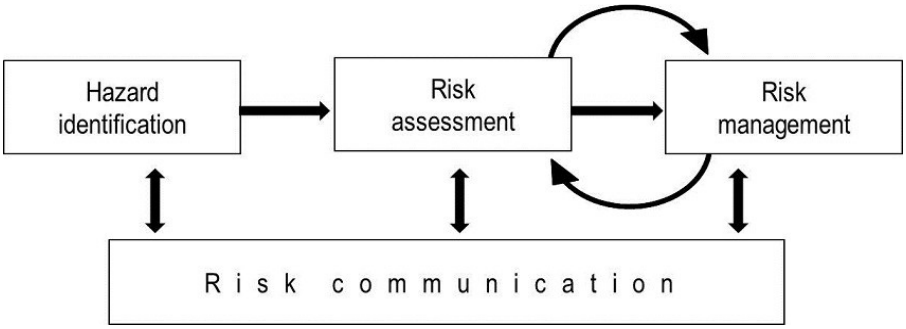

Fig. 1. *The four components of risk analysis*

The *risk assessment* is the component of the analysis that estimates the *risks* associated with a *hazard*. *Risk assessments* may be qualitative or quantitative. For many *diseases*, particularly for those *diseases* listed in the *Aquatic Code* where there are well developed internationally agreed standards, there is broad agreement concerning the likely *risks*. In such cases it is more likely that a qualitative assessment is all that is required. Qualitative assessment does not require mathematical modelling skills to carry out and so is often the type of assessment used for routine decision-making. No single method of import *risk assessment* has proven applicable in all situations, and different methods may be appropriate in different circumstances.

The process of import *risk analysis* on *aquatic animals* and *aquatic animal products* usually needs to take into consideration the results of an evaluation of the *Aquatic Animal Health Services*, zoning and compartmentalisation, and *surveillance* systems that are in place for monitoring *aquatic animal* health in the *exporting country*. These are described in separate chapters in the *Aquatic Code*.

Annex 6 (contd)

Article 2.1.2.

Hazard identification

Hazard identification involves identifying the *pathogenic agents* that could potentially produce adverse consequences associated with the importation of a *commodity*.

The ~~potential~~ *hazards* identified would be those appropriate to the species being imported, or from which the *commodity* is derived, and which may be present in the *exporting country*. It is then necessary to identify whether each *hazard* is already present in the *importing country*, and whether it is a *listed disease* or is subject to control or eradication in that country and to ensure that import measures are not more trade restrictive than those applied within the country.

Hazard identification is a categorisation step, identifying biological agents dichotomously as ~~potential~~ *hazards* or not *hazards*. The *risk assessment* should be concluded if *hazard identification* fails to identify *hazards* associated with the importation.

The evaluation of the *Aquatic Animal Health Services*, *surveillance* and control programmes, and zoning and compartmentalisation systems are important inputs for assessing the likelihood of *hazards* being present in the *aquatic animal* population of the *exporting country*.

An *importing country* may decide to permit the importation using the appropriate sanitary standards recommended in the *Aquatic Code*, thus eliminating the need for a *risk assessment*.

Article 2.1.3.

Principles of risk assessment

1) *Risk assessment* should be flexible in order to deal with the complexity of real-life situations. No single method is applicable in all cases. *Risk assessment* should be able to accommodate the variety of *aquatic animal commodities*, the multiple *hazards* that may be identified with an importation and the specificity of each *disease*, detection and *surveillance* systems, exposure scenarios and types and amounts of data and information.

2) Both qualitative *risk assessment* and quantitative *risk assessment* methods are valid.

3) The *risk assessment* should be based on the best available information that is in accord with current scientific thinking. The assessment should be well documented and supported with references to the scientific literature and other sources, including expert opinion.

4) Consistency in *risk assessment* methods should be encouraged and transparency is essential in order to ensure fairness and rationality, consistency in decision-making and ease of understanding by all the interested parties.

5) *Risk assessments* should document the uncertainties, the assumptions made, and the effect of these on the final *risk* estimate.

6) *Risk* increases with increasing volume of *commodity* imported.

7) The *risk assessment* should be amenable to updating when additional information becomes available.

Article 2.1.4.

Risk assessment steps

1. Entry assessment

Entry assessment consists of describing the biological pathway(s) necessary for an importation activity to introduce a *pathogenic agent* into a particular environment, and estimating the probability of that complete process occurring, either qualitatively (in words) or quantitatively (as a numerical estimate). The entry assessment describes the probability of the entry of each of the ~~potential~~ *hazards* (the *pathogenic agents*) or under each specified set of conditions with respect to amounts and timing, and how these might change as a result of various actions, events or measures. Examples of the kind of inputs that may be required in the entry assessment are:

a) Biological factors

- Species, strain or genotype, and age of *aquatic animal*
- Strain of agent
- Tissue sites of *infection* and/or contamination
- Vaccination, testing, treatment and *quarantine*.

b) Country factors

- *Incidence* or *prevalence*
- Evaluation of *Aquatic Animal Health Services*, *surveillance* and control programmes, and zoning and compartmentalisation systems of the *exporting country*.

c) Commodity factors

- Whether the *commodity* is alive or dead
- Quantity of *commodity* to be imported
- Ease of contamination
- Effect of the various processing methods on the *pathogenic agent* in the *commodity*
- Effect of storage and transport on the *pathogenic agent* in the *commodity*.

If the entry assessment demonstrates no significant *risk*, the *risk assessment* does not need to continue.

2. Exposure assessment

Exposure assessment consists of describing the biological pathway(s) necessary for exposure of animals and humans in the *importing country* to the *hazards* (in this case the *pathogenic agents*) from a given *risk* source, and estimating the probability of these exposure(s) occurring, either qualitatively (in words) or quantitatively (as a numerical estimate).

The probability of exposure to the identified *hazards* is estimated for specified exposure conditions with respect to amounts, timing, frequency, duration of exposure, routes of exposure, and the number, species and other characteristics of the animal and human populations exposed. Examples of the kind of inputs that may be required in the exposure assessment are:

Annex 6 (contd)

a) Biological factors

- Properties of the agent (e.g. virulence, pathogenicity and survival parameters)
- Genotype of host.

b) Country factors

- Presence of potential vectors or intermediate hosts
- *Aquatic animal* demographics (e.g. presence of known susceptible and carrier species, distribution)
- Human and terrestrial animal demographics (e.g. possibility of scavengers, presence of piscivorous birds)
- Customs and cultural practices
- Geographical and environmental characteristics (e.g. hydrographic data, temperature ranges, water courses).

c) Commodity factors

- Whether the *commodity* is alive or dead
- Quantity of *commodity* to be imported
- Intended use of the imported *aquatic animals* or *products* (e.g. domestic consumption, restocking, incorporation in or use as *aquaculture feed* or bait)
- Waste disposal practices.

If the exposure assessment demonstrates no significant *risk*, the *risk assessment* may conclude at this step.

3. Consequence assessment

Consequence assessment consists of describing the relationship between specified exposures to a biological agent and the consequences of those exposures. A causal process should exist by which exposures produce adverse health or environmental consequences, which may in turn lead to socio-economic consequences. The consequence assessment describes the potential consequences of a given exposure and estimates the probability of them occurring. This estimate may be either qualitative (in words) or quantitative (a numerical estimate). Examples of consequences include:

a) Direct consequences

- *Aquatic animal infection*, *disease*, production losses and facility closures
- Public health consequences.

b) Indirect consequences

- *Surveillance* and control costs
- Compensation costs
- Potential trade losses
- Adverse, and possibly irreversible, consequences to the environment.

4. Risk estimation

Risk estimation consists of integrating the results of the entry assessment, exposure assessment, and consequence assessment to produce overall measures of *risks* associated with the *hazards* identified at the outset. Thus *risk* estimation takes into account the whole of the *risk* pathway from *hazard* identified to unwanted outcome.

For a quantitative assessment, the final outputs may include:

– The various populations of *aquatic animals* and/or estimated numbers of *aquaculture establishments* or people likely to experience health impacts of various degrees of severity over time

– Probability distributions, confidence intervals, and other means for expressing the uncertainties in these estimates

– Portrayal of the variance of all model inputs

– A sensitivity analysis to rank the inputs as to their contribution to the variance of the *risk* estimation output

– Analysis of the dependence and correlation between model inputs.

Article 2.1.5.

Principles of risk management

1) *Risk management* is the process of deciding upon and implementing measures to address the *risks* identified in the *risk assessment* ~~achieve the Member Country's appropriate level of protection~~, whilst at the same time ensuring that negative effects on trade are minimised. The objective is to manage *risk* appropriately to ensure that a balance is achieved between a country's desire to minimise the likelihood or frequency of *disease* incursions and their consequences and its desire to import *commodities* and fulfil its obligations under international trade agreements.

2) The international standards of the OIE are the preferred choice of *sanitary measures* for *risk management*. The application of these *sanitary measures* should be in accordance with the intentions of the standards.

Article 2.1.6.

Risk management components

1) *Risk* evaluation - the process of comparing the *risk* estimated in the *risk assessment* with the reduction in *risk* expected from the proposed *risk management* measures ~~Member Country's appropriate level of protection~~.

2) Option evaluation - the process of identifying, evaluating the efficacy and feasibility of, and selecting measures to reduce the *risk* associated with an importation ~~in order to bring in line with the Member Country's appropriate level of protection~~. The efficacy is the degree to which an option reduces the likelihood or magnitude of adverse health and economic consequences. Evaluating the efficacy of the options selected is an iterative process that involves their incorporation into the *risk assessment* and then comparing the resulting level of *risk* with that considered acceptable. The evaluation for feasibility normally focuses on technical, operational and economic factors affecting the implementation of the *risk management* options.

3) Implementation - the process of following through with the *risk management* decision and ensuring that the *risk management* measures are in place.

Annex 6 (contd)

4) Monitoring and review - the ongoing process by which the *risk management* measures are continuously audited to ensure that they are achieving the results intended.

Article 2.1.7.

Principles of risk communication

1) *Risk communication* is the process by which information and opinions regarding *hazards* and *risks* are gathered from potentially affected and interested parties during a *risk analysis*, and by which the results of the *risk assessment* and proposed *risk management* measures are communicated to the decision-makers and interested parties in the *importing* and *exporting countries*. It is a multidimensional and iterative process and should ideally begin at the start of the *risk analysis* process and continue throughout.

2) A *risk communication* strategy should be put in place at the start of each *risk analysis*.

3) The *communication of risk* should be an open, interactive, iterative and transparent exchange of information that may continue after the decision on importation.

4) The principal participants in *risk communication* include the authorities in the *exporting country* and other stakeholders such as domestic aquaculturists, recreational and commercial fishermen, conservation and wildlife groups, consumer groups, and domestic and foreign industry groups.

5) The assumptions and uncertainty in the model, model inputs and the *risk* estimates of the *risk assessment* should be communicated.

6) Peer review of *risk analyses* is an essential component of *risk communication* in order to obtain a scientific critique and to ensure that the data, information, methods and assumptions are the best available.

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Annex 7

CHAPTER 4.X.

RECOMMENDATIONS FOR SURFACE DISINFECTION OF SALMONID EGGS

Article 4.X.1.

Introduction

The practice of disinfecting salmonid *eggs* at hatcheries is an essential part of ensuring that endemic *diseases* are not transferred ~~to~~ between incubators ~~with *eggs*~~ and ~~ultimately~~ between facilities and forms a part of ~~normal~~ routine hatchery hygiene protocols. The *disinfection* process is also important when trading salmonid *eggs* between *compartments*, *zones* or countries to prevent the transfer of some *pathogenic agents*. Although generally effective for *disinfection* of the *egg* surface and reproductive fluids, the use of *disinfectants* will not prevent vertical transmission.

Salmonid *eggs* may be disinfected with a number of chemical agents. However, the most common method used is *disinfection* with the iodine-based product, povidine-iodine. ~~Different protocols should be applied depending on the stage of *egg* development.~~

Iodophores, commonly povidone-iodine solutions, ~~are commonly used *disinfectants* for treating salmonid *eggs*. They~~ have the advantage of providing a neutral pH, being non-irritant and are relatively non-toxic. The neutral pH is important for minimising toxicity and ensuring efficacy. It is recommended to follow manufacturer's instructions~~directions~~ to identify circumstances where pH may be a concern.~~Povodine-iodine solutions are the most commonly used iodophore because of their low toxicity and neutral pH under most circumstances.~~ If other iodine based agents are used for *disinfection* it is essential that they ~~are~~ be adequately buffered.

Article 4.X.2.

Disinfection protocol for salmonid eggs

This *disinfection* protocol may be applied to newly fertilised or eyed salmonid *eggs*. However newly fertilised *eggs* should be allowed to commence hardening prior to undergoing the *disinfection* protocol. Although there is a considerable margin of safety for hardened *eggs*, the *disinfection* protocol is not recommended for unfertilised ova or during fertilisation. It is essential that the pH of the iodophore solution is maintained between 6 and 8.

To disinfect ~~S~~salmonid *eggs* ~~should undergo~~ the ~~f~~ollowing ~~*disinfection*~~ protocol should be applied:

1) rinsed in 0.9% pathogen free saline (30–60 seconds) to remove organic matter; then

2) immersed in a iodophore solution containing 100 ppm available iodine for a minimum of 10 minutes. The iodophore solution should be used only once. The ratio of *eggs* to iodophore solution should be a minimum of 1:4; then

3) rinsed again in 0.9% pathogen free saline for 30–60 seconds; then

4) held in pathogen free water.

Annex 7 (contd)

All rinsing and *disinfection* solutions should be prepared using pathogen free water. Iodophore s~~S~~olutions may be buffered using ~~100 mg~~ sodium bicarbonate ($NaHCO_3$) ~~per litre of diluted iodophore solution~~ if the pH is low.

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Annex 8

CHAPTER 4.7.

CONTROL OF PATHOGENIC AGENTS IN AQUATIC ANIMAL FEED

Article 4.7.1.

Introduction

Feed can be a source of infectious *disease* in *aquatic animals*.

Because *aquatic animals* are often a principle ingredient in *feeds* for *aquatic animals*, and because the use of ~~unprocessed or~~ semi-processed, raw and live *feed* continues to be a common practice, the *risk* of *disease* transmission via *feed* ~~needs to~~ should be addressed.

Article 4.7.2.

Purpose and scope

The purpose of this chapter is to address transmission of infectious *diseases* of *aquatic animals* via *feed* to prevent entry and spread into a country, *zone* or *compartment* free from *pathogenic agents* of concern.

This chapter applies to the production and use of all products destined for *feed* and *feed ingredients* whether produced commercially or on farm.

Risk analysis principles (in accordance with Chapter 2.1.) should be applied to determine the *risks* associated with the production and use of *feed* in *aquatic animals*.

This chapter is complementary to guidance provided by the Codex Code of Practice on Good Animal Feeding (CAC/RCP 54-2004).

Article 4.7.3.

Responsibilities

The responsibilities of the *Competent Authority* include setting and enforcing regulatory requirements related to *animal feed*, and verifying that these requirements are met. This also includes raising awareness ~~on~~ about *risks* related to use of unprocessed or semi-processed *feed* in *aquaculture*.

Feed producers have the responsibility to ensure ~~that~~ production of *feed* ~~meets regulatory requirements~~ is performed in a manner to prevent the spread of ~~infectious~~ *diseases* of *aquatic animals*. Records and contingency plans should be in place, as appropriate, to enable the tracing, recall, or destruction of non-compliant products. All personnel involved in the harvest, manufacture, transport, storage and handling of *feed* and *feed ingredients* should be adequately trained and aware of their role and responsibility in preventing the spread of infectious *diseases* of *aquatic animals*. Equipment for producing, storing and transporting *feed* and *feed ingredients* should be kept clean and maintained in good working order.

Owners and managers of *aquaculture establishments* should adhere to regulatory requirements and implement *biosecurity plans* ~~health programmes~~ on their farms in order to manage *risks* related to the use of ~~unprocessed or~~ semi-processed, raw and live *feed*. This can be done through identification of *disease* free sources and record keeping ~~of sources of *feed*~~ for traceability purposes, implementation of on farm risk mitigation measures, and early detection of infectious *diseases*.

Private veterinarians and other *aquatic animal health professionals* providing specialist services to producers and to the *feed* industry may be required to meet specific regulatory requirements pertaining to the services they provide (e.g. disease reporting, quality standards, transparency).

Annex 8 (contd)

Article 4.7.4.

Hazards associated with aquatic animal feed

Biological hazards that may ~~occur~~ be present in *feed* and *feed ingredients* include *pathogenic agents* such as bacteria, viruses, fungi, and parasites. The scope of these recommendations covers listed *diseases* and other *pathogenic agents* that cause an adverse effect on *aquatic animal* health.

Chemical and physical hazards associated with feed and feed ingredients are not addressed in this Chapter.

Antimicrobial resistance arising from the use of *antimicrobial agents* in *feed* is addressed in Section 6.

Article 4.7.5.

Risk pathways and exposure

Feed may be contaminated with *pathogenic agents* present at the time of harvesting, transport, storage, and processing of *commodities* used as *feed ingredients.* Contamination may also occur during manufacture, transport, storage, and use of *feed*. Poor hygienic practices during processing and manufacture, transport and storage are potential sources of contamination with *pathogenic agents*.

Aquatic animals can be directly exposed to *pathogenic agents* in *feed*. *Aquatic animals* can also be indirectly exposed through contamination of the environment by *feed*.

Article 4.7.6.

Risk management

1. Use of safe feed and feed ingredients ~~from any source~~

   Some *commodities* undergo significant processing such as heat treatment, acidification, extrusion and extraction. There may be a negligible likelihood ~~risk~~ that *pathogenic agents* will survive in such products if they have been produced in accordance with Good Manufacturing Practice.

   Criteria provided in Chapter 5.4. may be used to assess the safety of *commodities* to be used as *feed* or *feed ingredients*.

   Articles X.X.3. of all *disease*-specific chapters in Sections 8 to 11 list~~s~~ *commodities* considered safe for any purpose including use as *feed* or *feed ingredients*.

   Competent Authorities should also consider sourcing *feed* and *feed ingredients* from a country, *zone* or *compartment* free from *pathogenic agents* of concern.

2. Use of feed and feed ingredients from sources that may not be free from pathogenic agents of concern

   When using feed and feed ingredients from sources that may not be free from *pathogenic agents* of concern, *Competent Authorities* should consider the following *risk* mitigation measures:

   a) treatment (e.g. by heating or acidification) of the *commodity* using a method approved by the *Competent Authority* to inactivate *pathogenic agent(s)* as per Articles X.X.10. (for Chapter 10.4. the relevant Article is 10.4.1~~7~~4.) of all disease-specific chapters in Sections 8 to 11; or

   b) confirmation (e.g. by testing) that *pathogenic agents* are not present in the *commodity*; or

   c) use of *feed* only in populations that are not susceptible to the *pathogenic agent(s)* in question and where *susceptible species* will not come into contact with the *feed* or its waste products.

Annex 8 (contd)

3. Feed production

To prevent contamination by *pathogenic agents* during processing, manufacture, storage and transport of *feed* and *feed ingredients*, the following is recommended:

a) flushing, sequencing or physical cleaning-out of manufacturing lines and storage facilities should be performed between batches as appropriate;

b) buildings and equipment for processing and transporting *feed* and *feed ingredients* should be constructed in a manner that facilitates hygienic operation, maintenance and cleaning and prevents contamination;

c) *feed* manufacturing plants should be designed and operated ~~to~~ in a manner that avoids cross-contamination between batches;

d) processed *feed* and *feed ingredients* should be stored separately from unprocessed *feed ingredients*, under appropriate storage conditions;

e) *feed* and *feed ingredients*, manufacturing equipment, storage facilities and their immediate surroundings should be kept clean;

f) measures to inactivate *pathogenic agents*, such as heat treatment, should be used where appropriate;

g) labelling should provide for the identification of *feed* and *feed ingredients* as to the batch, place and date of production to assist in tracing *feed* and *feed ingredients*.

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Annex 9

CHAPTER 5.1.

GENERAL OBLIGATIONS RELATED TO CERTIFICATION

Article 5.1.1.

A combination of factors should be taken into account to facilitate *international trade* in *aquatic animals* and *aquatic animal products*, without incurring unacceptable *risks* to human and *aquatic animal* health.

Because of differences between countries in their *aquatic animal* health situations, various options are offered by the *Aquatic Code*. The *aquatic animal* health situation in the *exporting country*, in the *transit country* or *countries* and in the *importing country* should be considered before determining the requirements for trade. To maximise harmonisation of the *aquatic animal* health aspects of *international trade*, *Competent Authorities* of Member Countries should base their import requirements on the OIE standards.

These requirements should be included in the certificates drawn up in accordance with the model *international aquatic animal health certificates* provided for in Chapter 5.11.

Certificat~~ion~~es should be exact and concise, and should clearly address the requirements of the *importing country*. For this purpose, prior consultation between *Competent Authorities* of *importing* and *exporting countries* may be necessary. This consultation

helps to determine the exact requirements of the certification.

Certificates should be issued and signed by a ~~single competent~~ certifying official authorised by the *Competent Authority* to perform inspections, and endorsed through signature and/or official stamp of the *Competent Authority*. The certification requirements should not include conditions for *diseases* that are not transmitted by the *commodity* concerned. The certificate should be signed in accordance with the provisions of Chapter 5.2.

When officials of a *Competent Authority* wish to visit another country for matters of professional interest to the *Competent Authority* of the other country, the latter should be informed prior to any such visit. This visit should be mutually agreed upon between *Competent Authorities*.

Article 5.1.2.

Responsibilities of the importing country

1) The import requirements included in the *international veterinary certificate* should assure that *commodities* introduced into the *importing country* comply with the standards of the OIE. *Importing countries* should align ~~restrict~~ their requirements with ~~to~~ ~~those~~ ~~recommended~~ the recommendations in the relevant standards of the OIE ~~necessary to achieve the national appropriate level of protection~~. If there are no such ~~standards~~ recommendations or if the country chooses a level of protection requiring measures ~~these are~~ that exceed ~~stricter than~~ the standards of the OIE, these ~~they~~ should be based on an import *risk analysis* conducted in accordance with Chapter 2.1.

2) The *international aquatic animal health certificate* should not include requirements for the exclusion of *pathogenic agents* or *aquatic animal diseases* that are present in the *importing country* and are not subject to any official control programme~~, except when the strain of the *pathogenic agent* in the *exporting country* is of significantly higher pathogenicity and/or has a larger host range~~. The measures imposed on imports to manage the *risks* posed by a *pathogenic agent* or *aquatic animal disease* should not exceed ~~be stricter~~ ~~require a higher level of protection~~ ~~than~~ those ~~that provided by measures~~ applied as part of the official control programme operating within the *importing country*.

Annex 9 (contd)

3) The *international aquatic animal health certificate* should not include measures against *pathogenic agents* or *diseases* that are not OIE listed, unless the *importing country* has demonstrated through an import *risk analysis*, carried out in accordance with Section 2, that the *pathogenic agent* or *disease* poses a significant *risk* to the *importing country*.

4) The transmission of the requirements of the *importing country* or certificates from the *Competent Authority* of the *importing country* and the communication of import requirements to persons other than the *Competent Authority* of another country necessitates that copies of these documents be also sent to the *Competent Authority* of the *exporting country*. This important procedure avoids delays and difficulties that may arise between traders and *Competent Authorities* when the authenticity of the certificates or permits is not established.

   The transmission of this information is the responsibility of *Competent Authorities* of the *exporting country*. However, it can be issued by private sector *veterinarians* at the place of origin of the *commodities* when this practice is the subject of appropriate approval and authentication by *Competent Authorities*.

5) Situations may arise that result in changes to the consignee, identification of the means of transportation, or *frontier post* after a certificate is issued. If it is determined that these do not change the *aquatic animal* health or public health status of the consignment, then they should not prevent the acceptance of the certificate.

Article 5.1.3.

Responsibilities of the exporting country

1) An *exporting country* should, on request, supply the following to *importing countries*:

   a) information on the aquatic animal health situation and national aquatic animal health information systems to determine whether that country is free or has *zones* or *compartments* free from *listed diseases*, and on the pathway followed to achieve *disease* freedom e.g. historical freedom, absence of *susceptible species* or *targeted surveillance*, including the regulations and procedures in force to maintain the free status;

   b) regular and prompt information on the occurrence of *listed diseases*;

   c) details of the country's ability to apply measures to control and prevent *listed diseases*;

   d) information on the structure of the *Competent Authority* and the authority that they exercise;

   e) technical information, particularly on biological tests and vaccines applied in all or part of the country.

2) *Competent Authorities* of *exporting countries* should:

   a) have official procedures for the authorisation of *certifying officials*, defining their functions and duties as well as conditions of oversight and accountability, including possible suspension and termination of the authorisation;

   b) ensure that relevant instructions and training are provided to *certifying officials*;

   c) monitor the activities of the *certifying officials* to verify their integrity and impartiality.

3) The *Competent Authority* of the *exporting country* is ultimately accountable for certification used in *international trade*.

Article 5.1.4.

Responsibilities in case of an incident related to importation

1) *International trade* involves a continuing ethical responsibility. Therefore, if within a reasonable period subsequent to an export taking place, the *Competent Authority* becomes aware of the appearance or reappearance of a *disease* that has been specifically included in the *international aquatic animal health certificate* or other *disease* of potential epidemiological importance to the *importing country* there is an obligation for the *Competent Authority* to notify the *importing country*, so that the imported *commodities* may be inspected or tested and appropriate action be taken to limit the spread of the *disease* should it have been inadvertently introduced.

2) If a *disease* condition appears in imported *aquatic animals* within a reasonable period after importation, the *Competent Authority* of the *exporting country* should be informed so as to enable an investigation to be made, because this may be the first available information on the occurrence of the *disease* in a previously free *aquatic animal* population. The *Competent Authority* of the *importing country* should be informed of the result of the investigation because the source of *infection* may not be in the *exporting country*.

3) ~~If, after importation of *commodities*, a *disease* condition appears, within a reasonable period after importation, in *aquatic animals* in the *importing country*, the *Competent Authority* of the *exporting country* should be informed so as to enable an investigation to be made, because this may be the first available information on the occurrence of the *disease* in a previously free *aquatic animal* population. The *Competent Authority* of the *importing country* should conduct trace back investigations because the source of *disease* may not be in the *exporting country*.~~

If a *disease* appears in *aquatic animals* in an *importing country* and is associated with importation of *commodities*, the *Competent Authority* of the *exporting country* should be informed. This will enable the *exporting country* to investigate as this may be the first available information on the occurrence of the *disease* in a previously free *aquatic animal* population.

4) In case of suspicion, on reasonable grounds, that an *international aquatic animal health certificate* may be fraudulent, the *Competent Authority* of the *importing country* and *exporting country* should conduct an investigation. Consideration should also be given to notifying any third country(ies) that may have been implicated. All associated consignments should be kept under official control, pending the outcome of the investigation. *Competent Authorities* of all countries involved should fully cooperate with the investigation. If the *international aquatic animal health certificate* is found to be fraudulent, every effort should be made to identify those responsible so that appropriate action can be taken according to the relevant legislation.

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Annex 10

CHAPTER 5.2.

CERTIFICATION PROCEDURES

Article 5.2.1.

Protection of the professional integrity of the certifying official

Certification should be based on the highest possible ethical standards, the most important of which is that the professional integrity of the *certifying official* should be respected and safeguarded.

It is essential to include in the certificate only those specific statements that can be accurately and honestly signed by a *certifying official*. For example, these requirements should not include certification of an area as being free from *diseases* that are not notifiable in that country, or the occurrence of which the signing *certifying official* is not necessarily informed about. It is unacceptable to ask for certification for events that will take place after the document is signed when these events are not under the direct control and supervision of the signing *certifying official*.

Article 5.2.2.

Certifying officials

Certifying officials should:

1) be authorised by the *Competent Authority* of the *exporting* country to sign *international aquatic animal health certificates*;

2) only certify matters that are within their own knowledge at the time of signing the certificate, or that have been separately attested by another ~~competent~~ party authorised by the *Competent Authority*;

3) sign only at the appropriate time certificates that have been completed fully and correctly; where a certificate is signed on the basis of supporting documentation, the *certifying official* should have verified or be in possession of that documentation before signing;

4) have no conflict of interest in the commercial aspects of the *aquatic animals* or *aquatic animal products* being certified and be independent from the commercial parties.

Article 5.2.3.

Preparation of *international aquatic animal health certificates*

Certificates should be drawn up in accordance with the following principles:

1) Certificates should be designed so as to minimise the potential for fraud including use of a unique identification number, or other appropriate means to ensure security. Paper certificates should bear the signature of the *certifying official* and the official identifier (stamp) of the issuing *Competent Authority*. Each page of a multiple page certificate should bear the unique certificate number and a number indicating the number of the page out of the total number of pages. Electronic certification procedures should include equivalent safeguards.

2) Certificates should be written using terms that are simple, unambiguous and as easy to understand as possible, without losing their legal meaning.

3) If so required, certificates should be written in the language of the *importing country*. In such circumstances, they should also be written in a language understood by the *certifying official*.

Annex 10 (contd)

4) Certificates should require appropriate identification of *aquatic animals* and *aquatic animal products* except where this is impractical (e.g. eyed eggs).

5) Certificates should not require a *certifying official* to certify matters that are outside his/her knowledge or that he/she cannot ascertain and verify.

6) Where appropriate, when presented to the *certifying official*, certificates should be accompanied by notes of guidance indicating the extent of enquiries, tests or examinations expected to be carried out before the certificate is signed.

7) The text of a certificate should not be amended except by deletions that should be signed and stamped by the *certifying official*.

8) The signature and stamp should be in a colour different to that of the printing of the certificate. The stamp may be embossed instead of being a different colour.

9) Only original certificates should be accepted by the *importing country*.

10) Replacement certificates may be issued by a *Competent Authority* to replace original certificates that have been, for example, lost, damaged, contain errors, or where the original information is no longer correct. These replacements should be provided by the issuing authority and be clearly marked to indicate that they are replacing the original certificate. A replacement certificate should reference the number and the issue date of the certificate that it supersedes. The superseded certificate should be cancelled and where possible, returned to the issuing authority.

Article 5.2.4.

Electronic certification

1) Certification may be provided by electronic exchange of data ~~documentation~~ sent directly from the *Competent Authority* of the *exporting country* to the *Competent Authority* of the *importing country.*

   a) Systems providing electronic certificates normally provide an interface with the commercial organisation marketing the commodity for provision of information to the certifying authority. The certifying official should have access to all necessary information such as origin of aquatic animals and laboratory results.

   b) When exchanging electronic certificates and in order to fully utilise electronic data exchange the Competent Authorities should use internationally standardised language, message structure and exchange protocols. Guidance for electronic certification in standardised ~~World Wide Web Consortium (WC3)~~ Extensible Markup Language (XML ~~schemas~~) as well as secure exchange mechanisms between Competent Authorities is provided by the United Nations Centre for Trade Facilitation and Electronic Business (UN/CEFACT).

   c) A secure method of electronic data exchange should be ensured by digital authentication of the certificates, encryption, non-repudiation mechanisms, controlled and audited access and firewalls.

2) Electronic certificates should carry the same information as conventional certificates.

3) The *Competent Authority* should have in place systems for the security of electronic certificates against access by unauthorised persons or organisations.

4) The *certifying official* should be officially responsible for the secure use of his/her electronic signature.

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Annex 11

CHAPTER 6.5.

RISK ANALYSIS FOR ANTIMICROBIAL RESISTANCE ARISING FROM THE USE OF ANTIMICROBIAL AGENTS IN AQUATIC ANIMALS

Article 6.5.1.

Recommendations for analysing the risks to aquatic animal health and public ~~human~~ health from antimicrobial resistant microorganisms of aquatic animal origin

1. Introduction

Antimicrobial resistance is a naturally occurring phenomenon influenced by many factors. However, ~~the main driving force for the selection of antimicrobial resistance is the use of *antimicrobial agents* in any environment, including human, animal and other usages (under study).~~ problems related to antimicrobial resistance are inherently related to *antimicrobial agent* use in any environment, including human and non-human uses.

Antimicrobial resistance associated with the use of *antimicrobial agents* for therapeutic and non-therapeutic purposes has led to the selection and dissemination of antimicrobial resistant~~ce~~ microorganisms, with a resulting loss of therapeutic efficacy in animal and human medicine of ~~one or several~~ *antimicrobial agents*.

2. Objective

For the purpose~~s~~ of this chapter, the principal aim of *risk analysis* is to provide Member Countries with a transparent, objective and scientifically defensible method of assessing and managing the human and *aquatic animal* health associated with the selection and dissemination of resistance arising from the use of *antimicrobial agents* in *aquatic animals*.

Guidance on the issue of foodborne antimicrobial resistance related to the non-human use of *antimicrobial agents* is covered by the Codex Guidelines for risk analysis of foodborne antimicrobial resistance (CAC/GL77-2011).

3. Definitions

For the purpose of this chapter, the hazard is the resistant microorganism or resistance determinant that emerges as a result of the use of a specific *antimicrobial agent* in *aquatic animals*. This definition reflects the potential for resistant microorganisms to cause adverse health effects, as well as the potential for horizontal transfer of genetic determinants between microorganisms. The conditions under which the hazard might produce adverse consequences include any scenarios through which humans or *aquatic animals* could become exposed to an antimicrobial resistant pathogen, fall ill and then be treated with an *antimicrobial agent* that is no longer effective.

For the purposes of this chapter, risk to *aquatic animal* health relates to the *infection* of *aquatic animals* with microorganisms in which resistance has emerged due to *antimicrobial agent* usage in *aquaculture*, and resulting in the loss of benefit of antimicrobial therapy used to manage *aquatic animal diseases*.

For the purpose of this chapter, risk to public health relates to the *infection* of humans with microorganisms in which resistance has emerged due to *antimicrobial agent* usage in *aquatic animals*, and resulting in the loss of benefit of antimicrobial therapy used to manage the human *infection*.

Annex 11 (contd)

~~3~~4. The risk analysis process

The components of *risk analysis* described in this chapter are hazard identification, *risk assessment*, *risk management* and *risk communication*.

The chapter includes factors to be considered at various steps of the *risk analysis* process. These factors are not intended to be exhaustive and not all elements may be applicable in all situations.

~~4. Hazard identification~~

~~For the purpose of this chapter, the *hazard* is the resistant microorganism or resistance determinant that emerges as a result of the use of a specific *antimicrobial agent* in *aquatic animals*. This definition reflects the potential for resistant microorganisms to cause adverse health effects, as well as the potential for horizontal transfer of genetic determinants between microorganisms. The conditions under which the *hazard* might produce adverse consequences include any scenarios through which humans or *aquatic animals* could become exposed to an antimicrobial resistant pathogen, fall ill and then be treated with an *antimicrobial agent* that is no longer effective.~~

5. Risk assessment

The assessment of the risk to human and *aquatic animal* health from antimicrobial resistant microorganisms resulting from the use of *antimicrobial agents* in *aquatic animals* should examine:

a) the likelihood of emergence of resistant microorganisms arising from the use of an *antimicrobial agent*, or more particularly, dissemination of the resistance determinants if transmission is possible between microorganisms;

b) all pathways and their ~~importance by which~~ contribution to the likelihood of humans and *aquatic animals* ~~could be~~ being exposed to these resistant microorganisms or resistance determinants~~, together with the likelihood of exposure~~;

c) the consequences of exposure in terms of risks to human and *aquatic animal* health.

The general principles of *risk assessment* as defined in Article ~~Chapter~~ 2.1.3 apply equally to both qualitative and quantitative *risk assessment*. ~~At a minimum, a qualitative *risk assessment* should be undertaken.~~

Article 6.5.2.

Special considerations for conducting antimicrobial resistance risk analysis in aquaculture

1. Introduction

Antimicrobial resistance (AMR) *risk analysis* in *aquaculture* is challenged by a variety of factors that impact both *risk assessment* and *risk management*, including the diversity of *aquaculture*, relative lack of methods for culture and antimicrobial susceptibility testing (AST), relative lack of ~~approved~~ information on use of drugs, and potential for the development of a reservoir of resistant microorganisms and resistance determinants with a potential for horizontal transmission.

Nevertheless, the fundamental principles of *risk analysis* (*risk assessment*, *risk management*, *risk communication*) provide a framework just as valuable for *aquaculture* as for terrestrial animal production.

2. Data needs ~~Definition of the risk~~

~~The definitions of *risk* used in this chapter are those associated with the use of *antimicrobial agents* within *aquaculture*.~~

Special care is required in the design of data collection programmes for *risk assessment* to take account of possible confounding factors.

Because many types of *aquaculture* operations (in particular, open systems) intersect with terrestrial animal production and human environments, it is especially important to clearly identify the *risk* to be assessed. The selection and dissemination of resistant microorganisms or resistant determinants may be associated with the use of *antimicrobial agents* on *aquatic animals* or it may be the result of antimicrobial use in nearby terrestrial animal production operations or the presence of *antimicrobial agents* in human waste water.

~~Special care is, therefore, required in design of data collection programmes for *risk assessment* to take account of these confounding factors.~~

3. Diversity of aquaculture

The range of species under culture, the number and type of different culture systems, and the range of *antimicrobial agents* and their routes of administration impact elements of the *risk assessment*, particularly the release assessment. Therefore, careful attention should be used when grouping seemingly similar sectors of the *aquaculture* industry.

Identification, selection and monitoring of *risk management* options are also influenced by the diversity of *aquaculture*.

4. Lack of standardised methods for antimicrobial susceptibility testing (AST)

~~The c~~Currently, ~~situation in *aquaculture* is that~~ standardised methods for antimicrobial susceptibility testing for many relevant *aquaculture* species are ~~generally~~ lacking resulting in ~~a loss in the~~ inability to quantify specific *risks* ~~and an increase in attendant uncertainty~~. Standardised AST methods should be used where available; or when standardised methods are not available well-described, scientifically sound approaches should be applied.

5. Lack of approved drugs

The small number of approved *antimicrobial agents* for use in *aquaculture* challenges *risk analysis*, both in terms of *risk assessment* and *risk management*.

The collection and use of thorough information on the types and quantities of *antimicrobial agents* that are in use in *aquaculture* and relevant to the *risk assessment* is important. In some circumstances legal extra-~~/~~or off-label and illegal uses may also need to be considered. See Chapter 6.3.

For *risk management*, the small number of approved drugs in combination with a range of regulatory and *aquatic animal* health infrastructure in countries engaged in *aquaculture* presents additional challenges. *Risk management* options should be practical and take into account the ability for enforcement and compliance.

For monitoring and *surveillance* programmes, a lack of approved drugs means systems for collection of data and information on the quantities of *antimicrobial agents* used may need to consider not only licensed distribution of approved drugs, but information on the use of unapproved drugs.

6. Potential for development of a reservoir (horizontal transmission)

Microorganisms inhabiting the environment represent the fundamental reservoir of resistant determinants in the biosphere. This reservoir represents the basic origin of all *antimicrobial agent* resistance determinants encountered in human and veterinary medicine. The frequency of resistance determinants in environmental microorganisms is maintained by intrinsic, non-anthropogenic factors; all human uses of *antimicrobial agents*, including in *aquaculture*, have the potential to increase the size of the reservoir.

Annex 11 (contd)

There is a *risk* that the use of *antimicrobial agents* in *aquaculture* will result in a rise in the frequency of resistance determinants in the environmental microbiome. ~~and that t~~This may result in an increase in the frequency with which determinants are transferred to microorganisms capable of infecting humans, animals or *aquatic animals*.The assessment and management of this *risk* is extremely complex. The biological pathways both for the release assessment and the exposure assessment are myriad and at present no specific guidelines can be offered.

Article 6.5.3.

Analysis of risks to human health

1. Definition of the risk

The *infection* of humans with microorganisms in which ~~that have acquired~~ resistance has emerged due to *antimicrobial agent* usage in *aquatic animals*, and resulting in the loss of benefit of antimicrobial therapy used to manage the human *infection*.

2. Hazard identification

– Microorganisms that have acquired resistance~~,~~ (including multiple resistance), arising from the use of an *antimicrobial agent* in *aquatic animals*.

– Microorganisms having obtained a resistance determinant from other microorganisms which have acquired resistance arising from the use of an *antimicrobial agent* in *aquatic animals*.

The identification of the hazard should include consideration of the class or subclass of the *antimicrobial agent*. This definition should be read in conjunction with point 4 of Article 6.5.1.

3. ~~Release~~ Entry assessment

A~~n release~~ entry assessment describes the biological pathways ~~necessary for~~ by which the use of a specific *antimicrobial agent* in *aquatic animals* ~~to~~ leads to the release of resistant microorganisms or resistance determinants into a particular environment. ~~,~~ This assessment includes ~~and~~ estimating ~~either~~ qualitatively or quantitatively the probability of that complete process occurring. The ~~release~~ entry assessment describes the probability of the entry of each of the hazards under each specified set of conditions with respect to amounts and timing~~, and how these might change as a result of various actions, events or measures~~.

The following factors should be considered in the ~~release~~ entry assessment:

– species of *aquatic animals* treated with the *antimicrobial agent*(s) in question;

– *aquaculture* production system (intensive~~/~~ or ~~-~~extensive, net pens, tanks, raceways, ponds, other);

– number of *aquatic animals* treated, their age and their geographical distribution;

– prevalence of *disease* for which the *antimicrobial agent* is indicated or is used in the target *aquatic animal* population;

– data on trends in *antimicrobial agent* use and changes in *aquaculture* production systems;

– data on potential extra-label or off-label use;

– methods and routes of administration of the *antimicrobial agent*;

– dosage regimen (dose, dosing interval and duration of the treatment);

– pharmacokinetics and relevant pharmacodynamics of the *antimicrobial agent*;

– site and type of *infection*;

– development of resistant microorganisms;

– prevalence of *pathogenic agents* that are likely to develop resistance in an *aquatic animal* species;

– mechanisms and pathways of direct or indirect transfer of resistance;

– potential linkage of virulence attributes and resistance;

– cross-resistance or co-resistance with other *antimicrobial agents*;

– data on trends and occurrence of resistant microorganisms obtained through *surveillance* of *aquatic animals* and *aquatic animal products* and waste products.

The following confounding factors should be considered in the release assessment:

– resistant microorganisms or resistant determinants associated with *aquatic animals* or *aquatic animal products* that are a result of terrestrial contamination of the aquatic environment, *feed* contamination or contamination during post-harvest processing.

4. Exposure assessment

An exposure assessment describes the biological pathways necessary for exposure of humans to the resistant microorganisms or resistance determinants released from a given *antimicrobial agent's* use in *aquatic animals*, and estimates the probability of exposures occurring. The probability of exposure to the identified hazards is estimated for specified exposure conditions with respect to amounts, timing, frequency, duration of exposure, routes of exposure, ~~species~~ and other characteristics of the human populations exposed.

The following factors should be considered in the exposure assessment:

– human demographics, including population subgroups, ~~and~~ food consumption patterns, ~~including~~ and traditions and cultural practices with respect to the preparation and storage of food;

– prevalence of resistant microorganisms in food at the point of consumption;

– microbial load in contaminated food at the point of consumption;

– environmental contamination with resistant microorganisms;

– transfer of resistant microorganisms and their resistance determinants between humans, *aquatic animals,* and the *environment*;

– measures taken for microbial decontamination of food;

– survival capacity and dissemination of resistant microorganisms during the food production process (including slaughtering, processing, storage, transportation and retailing);

– disposal practices for waste products and the likelihood for human exposure to resistant microorganisms or resistance determinants through those waste products;

– capacity of resistant microorganisms to become established in humans;

– human-to-human transmission of the microorganisms under consideration;

- capacity of resistant microorganisms to transfer resistance to human commensal microorganisms and zoonotic agents;
- amount and type of *antimicrobial agents* used to treat humans;
- pharmacokinetics, such as metabolism, bioavailability, distribution to the gastrointestinal flora;
- level of direct contact of workers in the *aquaculture* or processing industries to the antimicrobial resistant organisms.

5. Consequence assessment

A consequence assessment describes the relationship between specified exposures to resistant microorganisms or resistance determinants and the consequences of those exposures. A causal process should exist by which exposures produce adverse health or environmental consequences, which may in turn lead to socio-economic consequences. The consequence assessment describes the potential consequences of a given exposure and estimates the probability of them occurring.

The following factors should be considered in the consequence assessment:

- microbial dose and subsequent host response interactions;
- variation in susceptibility of exposed populations or subgroups of the population;
- variation and frequency of human health effects resulting from loss of efficacy of *antimicrobial agents* and associated costs (e.g. illness and hospitalisation);
- potential linkage of virulence attributes and resistance;
- changes in food consumption patterns due to loss of confidence in the safety of food products and any associated secondary *risks*;
- interference with antimicrobial therapy in humans;
- importance of the *antimicrobial agent* ~~in human medicine~~ in animal health and public health (see OIE list of *antimicrobial agents* of veterinary importance and WHO list of Critically Important Antimicrobials);
- prevalence of resistance in human bacterial pathogens under consideration.

6. Risk estimation

A risk estimation integrates the results from the ~~*release*~~ entry *assessment*, exposure assessment and consequence assessment to produce overall estimates of risks associated with the hazards. Thus, risk estimation takes into account the whole of the risk pathway from hazard identification to the unwanted consequences.

~~The following factors should be considered in the *risk* estimation:~~

- ~~number of people falling ill and the proportion of that number infected with antimicrobial resistant microorganisms;~~
- ~~adverse effects on vulnerable human sub-population (children, immuno-compromised persons, elderly, pregnant, etc.);~~
- ~~increased severity or duration of infectious *disease*;~~
- ~~number of person/days of illness per year;~~

- deaths (total per year; probability per year or reduced life expectancy for a random member of the population or a member of a specific more exposed sub-population) linked to antimicrobial resistant microorganisms when compared with deaths linked to sensitive microorganisms of the same species;

- severity of the *disease* caused by the target resistant microorganisms;

- availability of alternative antimicrobial therapy;

- potential impact of switching to an alternative *antimicrobial agent* (e.g. alternatives with potential increased toxicity);

- occurrence of antimicrobial resistance in target pathogens observed in humans.

7. Risk management

The OIE defines r*R*isk *management* as consists ing of the steps described below.

a) Risk evaluation

Risk evaluation - the process of comparing the *risk* estimated in the *risk assessment* with the reduction in *risk* expected from the proposed *risk management* measures.

b) Option evaluation

A range of *risk management* options is available to minimise the emergence and dissemination of antimicrobial resistance and these include both regulatory and non-regulatory options, such as the development of codes of practice for the use of *antimicrobial agents* in *aquaculture* animal husbandry.

Risk management decisions need to consider fully the implications of these different options for human health and *aquatic animal* health and welfare and also take into account economic considerations and any associated environmental issues. Effective control of *aquatic animal diseases* can have the dual benefits of reducing the *risks* to human health associated with both the bacterial pathogen under consideration and antimicrobial resistance.

c) Implementation

Risk managers should develop an implementation plan that describes how the decision will be implemented, by whom and when. *Competent Authorities* should ensure an appropriate regulatory framework and infrastructure.

d) Monitoring and review

Risk management options should be continuously monitored and reviewed in order to ensure that the objectives are being achieved.

8. Risk communication

Communication with all interested parties should be promoted at the earliest opportunity and integrated into all phases of *risk analysis*. This will provide all interested parties, including *risk* managers, with a better understanding of *risk management* approaches. *Risk communication* should be also well documented.

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Article 6.5.4.

Analysis of risks to aquatic animal health

1. Definition of the risk

The *infection* of *aquatic animals* with microorganisms in which ~~that have acquired~~ resistance has emerged due to antimicrobial usage in *aquatic animals*, and resulting in the loss of benefit of antimicrobial therapy used to manage the *aquatic animal infection.*

2. Hazard identification

– Microorganisms that have acquired resistance, (including multiple resistance) arising from the use of an *antimicrobial agent* in *aquatic animals*.

– Microorganisms having obtained a resistance determinant from another micro-organism which has acquired resistance arising from the use of an *antimicrobial agent* in *aquatic animals*.

The *identification of the hazard* should include considerations of the class or subclass of the *antimicrobial agent*. This definition should be read in conjunction with point 4 of Article 6.5.1.

3. ~~Release~~Entry assessment

The following factors should be considered in the ~~release~~ entry assessment:

– *aquatic animal* species treated with the *antimicrobial agent* in question;

– *aquaculture* production system (intensive~~/~~ or extensive, net pens, tanks, raceways, ponds, other);

– number of *aquatic animals* treated, and their age, geographical distribution, and where appropriate, sex;

– prevalence of *disease* for which the *antimicrobial agent* is indicated or is used in the target *aquatic animal* population;

– data on trends in *antimicrobial agent* use or sales and changes in *aquaculture* production systems;

– data on potential extra-label or off-label use;

– methods and routes of administration of the *antimicrobial agent*;

– dosage regimen (dose, dosing interval and duration of the treatment);

– ~~methods and routes of administration of the *antimicrobial agent*;~~

– the pharmacokinetics and ~~relevant~~ pharmacodynamics of the *antimicrobial agent*;

– ~~site and~~ type and site of *infection*;

– development of resistant microorganisms;

– prevalence of *pathogenic agents* that are likely to develop resistance in an *aquatic animal* species;

– mechanisms and pathways of direct or indirect transfer of resistance ~~transfer~~;

– cross-resistance or co-resistance with other *antimicrobial agents*;

– data on trends and occurrence of resistant microorganisms obtained through *surveillance* of *aquatic animals*, *aquatic animal products* and waste products.

The following confounding factors should be considered in the release assessment:

– resistant microorganisms or resistant determinants associated with *aquatic animals* or their products that are a result of terrestrial contamination of the aquatic environment, *feed* contamination or contamination during post-harvest processing.

4. Exposure assessment

The following factors should be considered in the exposure assessment:

– prevalence and trends of resistant microorganisms in clinically ill and clinically unaffected *aquatic animals*;

– prevalence of resistant microorganisms in *feed* and in the *aquatic animal* environment;

– animal-to-animal transmission of the resistant microorganisms and their resistance determinants (*aquatic animal* husbandry practices, movement of *aquatic animals*);

– number or percentage of *aquatic animals* treated;

– quantity and trends of *antimicrobial agent* used in *aquatic animals*;

– survival capacity and spread of resistant micro-organisms;

– exposure of wildlife to resistant microorganisms;

– disposal practices for waste products and the likelihood for *aquatic animal* exposure to resistant microorganisms or resistance determinants through those products;

– capacity of resistant microorganisms to become established in *aquatic animals*;

– exposure to resistance determinants from other sources such as water, effluent, waste pollution, etc.;

– pharmacokinetics, such as metabolism, bioavailability, distribution to relevant flora - considering the gastrointestinal flora of many aquatic species may be transient;

– transfer of resistant microorganisms and resistance determinants between humans, *aquatic animals*, and the environment.

5. Consequence assessment

The following factors should be considered in the consequence assessment:

– microbial dose and subsequent host response interactions;

– variation in *disease* susceptibility of exposed populations and subgroups of the populations;

– variation and frequency of *aquatic animal* health effects resulting from loss of efficacy of *antimicrobial agents* and associated costs;

Annex 11 (contd)

- potential linkage of virulence attributes and resistance;
- importance of the *antimicrobial agent* in *aquatic animal* health and public health (see OIE list of *antimicrobial agents* of veterinary importance and WHO list of Critically Important Antimicrobials);
- additional burden of *disease* due to antimicrobial resistant microorganisms;
- number of therapeutic failures due to antimicrobial resistant microorganisms;
- increased severity and duration of infectious *disease*;
- impact on *aquatic animal* welfare;
- estimation of the economic impact and cost on *aquatic animal* health and production;
- deaths (total per year; probability per year for a random member of the population or a member of a specific more exposed sub-population) linked to antimicrobial resistant microorganisms when compared with deaths linked to sensitive microorganisms of the same species;
- availability of alternative antimicrobial therapy;
- potential impact of switching to an alternative *antimicrobial agent* e.g. alternatives with potential increased toxicity.

6. Risk estimation

A *risk* estimation integrates the results from the entry assessment, exposure assessment and consequence assessment to produce overall estimates of *risks* associated with the *hazards*. Thus, *risk* estimation takes into account the whole of the *risk* pathway from *hazard identification* to the unwanted consequences.

~~The following factors should be considered in the *risk* estimation:~~

- ~~additional burden of *disease* due to antimicrobial resistant microorganisms;~~
- ~~number of therapeutic failures due to antimicrobial resistant microorganisms;~~
- ~~increased severity and duration of infectious *disease*;~~
- ~~impact on aquatic animal welfare;~~
- ~~estimation of the economic impact and cost on *aquatic animal* health and production;~~
- ~~deaths (total per year; probability per year for a random member of the population or a member of a specific more exposed sub-population) linked to antimicrobial resistant microorganisms when compared with deaths linked to sensitive microorganisms of the same species;~~
- ~~availability of alternative antimicrobial therapy;~~
- ~~potential impact of switching to an alternative *antimicrobial agent* e.g. alternatives with potential increased toxicity.~~

7. Risk management

The relevant provisions in point 7 of Article 6.5.3. apply.

8. Risk communication

The relevant provisions in point 8 of Article 6.5.3. apply.

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— Text deleted.

Annex 12

CHAPTER 8.1.

INFECTION WITH *BATRACHOCHYTRIUM DENDROBATIDIS*

[…]

Article 8.1.8.

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

1) When importing live *aquatic animals* 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~~:~~

~~a) require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* attesting that the *aquatic animals* of the species referred to in Article 8.1.2. have been appropriately treated to eradicate *infection* and have been subsequently tested to confirm absence of the *disease* according to specifications provided in the relevant chapter in the *Aquatic Manual*;~~

~~OR~~

~~b)~~ assess the *risk* and apply *risk* mitigation measures such as:

a) the direct delivery to and lifelong holding of the consignment in biosecure facilities for continuous isolation from the local environment;

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

2) If the intention of the introduction is the establishment of a new stock, relevant aspects of the Code of Practice on the Introductions and Transfers of Marine Organisms of the International Council for the Exploration of the Seas (ICES) should be considered.

3) For the purposes of the *Aquatic Code*, relevant aspects of the ICES Code (full version see: http://www.ices.dk/publications/our-publications/Pages/Miscellaneous.aspx) may be summarised to the following points:

a) identify stock of interest (cultured or wild) in its current location;

b) evaluate stock health~~/~~ and disease history;

c) take and test samples for *B. dendrobatidis*, pests and general health/*disease* status;

d import of a founder (F-0) population and quarantine in a secure facility;

e) produce F-1 generation from the F-0 stock in *quarantine*;

f) culture F-1 stock and at critical times in its development (life cycle) sample and test for *infection* with *B. dendrobatidis* and perform general examinations for pests and general health/*disease* status;

Annex 12 (contd)

g) if *infection* with *B. dendrobatidis* is not detected, pests are not present, and the general health/*disease* status of the stock is considered to meet the *basic biosecurity conditions* of the *importing country*, *zone* or *compartment*, the F-1 stock may be defined as *infection* with *B. dendrobatidis* free or specific pathogen free (SPF) for *infection* with *B. dendrobatidis*;

h) release SPF F-1 stock from *quarantine* for *aquaculture* or stocking purposes in the country, *zone* or *compartment*.

4) With respect to point 3 e), *quarantine* conditions should be conducive to multiplication of the pathogen and eventually to clinical expression. If *quarantine* conditions are not suitable for pathogen multiplication and development, the recommended diagnostic approach might not be sensitive enough to detect low *infection* level.

This Article does not apply to *aquatic animals* referred to in point 1 of Article 8.1.3.

[…]

Article 8.1.10.

Importation of live aquatic animals intended for use in animal feed, or for agricultural~~, laboratory, zoo, pet trade,~~ industrial 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, live *aquatic animals* of the 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 require that:

1) the consignment be ~~is~~ delivered directly to, and held in, *quarantine* facilities for slaughter and processing into products authorised by the *Competent Authority*; and

2) water and equipment used in transport and all effluent and waste materials from the processing facility ~~are~~ be treated in a manner that ~~ensures~~ inactivates~~ion of~~ *B. dendrobatidis*.

~~When importing live *aquatic animals* of species referred to in Article 8.1.2. from a country, *zone* or *compartment* not declared free from *B. dendrobatidis*, the *Competent Authority* of the *importing country* should:~~

~~1) require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* attesting that the *aquatic animals* have been appropriately treated to eradicate *infection* and have been subsequently tested to confirm absence of the *disease* according to specifications provided in the relevant chapter in the *Aquatic Manual*;~~

~~OR~~

~~2) assess the *risk* and apply *risk* mitigation measures such as:~~

~~a) the direct delivery to and lifelong holding of the consignment in biosecure facilities for continuous isolation from the local environment;~~

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

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

Annex 12 (contd)

[…]

Article 8.1.13.

Importation of live 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 and zoos, live *aquatic animals* 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 ensure:

1) the direct delivery to and lifelong holding of the consignment in *quarantine* facilities authorised by the *Competent Authority;* 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) the disposal of carcasses in accordance with Chapter 4.6.

— Text deleted.

Annex 13

CHAPTER 8.2.

INFECTION WITH RANAVIRUS

[…]

Article 8.2.10.

Importation of live aquatic animals intended for use in animal feed, or for agricultural, ~~laboratory, zoo, pet trade,~~ industrial or pharmaceutical use, from a country, zone or compartment not declared free from infection with ranavirus

When importing, for use in animal *feed*, or for agricultural, industrial or pharmaceutical use, live *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 be ~~is~~ delivered directly to and held in *quarantine* facilities for slaughter and processing to products authorised by the *Competent Authority*; and

2) water and equipment used in transport and all effluent and waste materials from the processing facility ~~are~~ be treated in a manner that ~~ensures~~ inactivates~~ion of~~ ranavirus.

~~When importing live *aquatic animals* of species referred to in Article 8.2.2. from a country, *zone* or *compartment* not declared free from ranavirus, the *Competent Authority* of the *importing country* should assess the *risk* and apply *risk* mitigation measures such as:~~

~~1) the direct delivery to and lifelong holding of the consignment in biosecure facilities for continuous isolation from the local environment;~~

~~2) the treatment of all effluent and waste materials in a manner that inactivates ranavirus.~~

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

[…]

Article 8.2.13.

Importation of live 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 laboratory and zoo, live *aquatic animals* 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 ensure:

1) the direct delivery to and lifelong holding of the consignment in *quarantine* facilities authorised by the *Competent Authority;* and

2) the treatment of water and equipment used in transport and of all effluent and waste materials in a manner that inactivates ranavirus; and

3) the disposal of carcasses in accordance with Chapter 4.6.

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— Text deleted.

Annex 14

Articles X.X.7. and X.X.11.

(Note: In Chapter 10.4. these amendments apply to Articles 10.4.10., 10.4.11., 10.4.15. and 10.4.16.)

Article X.X.7.

Importation of ~~live~~ aquatic animals and aquatic animal products from a country, zone or compartment declared free from disease X

When importing ~~live~~ *aquatic animals* and *aquatic animal products* of species referred to in Article X.X.2. from a country, *zone* or *compartment* declared free from disease X, the *Competent Authority* of the *importing country* should require that the consignment be accompanied by an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* certifying that, on the basis of the procedures described in Articles X.XX. or X.X.X. (as applicable) and X.X.X., the place of production of the ~~live~~ *aquatic animals* and *aquatic animal products* is a country, *zone* or *compartment* declared free from disease X.

The *certificate* should be in accordance with the Model Certificate in Chapter 5.11.

This Article does not apply to *commodities* referred to in point 1 of Article X.X.3.

~~Article X.X.11.~~

~~**Importation of aquatic animal products from a country, zone or compartment declared free from disease X**~~

~~When importing *aquatic animal products* of species referred to in Article X.X.2. from a country, *zone* or *compartment* declared free from disease X, the *Competent Authority* of the *importing country* should require that the consignment be accompanied by an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* certifying that, on the basis of the procedures described in Articles X.X.X. or X.X.X. (as applicable) and X.X.X., the place of production of the *aquatic animal products* is a country, *zone* or *compartment* declared free from disease X.~~

~~The *certificate* should be in accordance with the Model Certificate in Chapter 5.11.~~

~~This Article does not apply to *commodities* referred to in point 1 of Article X.X.3.~~

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— Text deleted.

Annex 15

CHAPTER 10.4.

INFECTION WITH INFECTIOUS SALMON ANAEMIA VIRUS

[…]

Article 10.4.4.

Country free from infection with 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* status prior to *targeted surveillance* is unknown ~~any of the *susceptible species* referred to in Article 10.4.2. are present and there has been no detectable occurrence of *infection* with ISAV~~ 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 *disease*, the affected area was declared an *infected zone* and a *protection zone* was established; and

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

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

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

   In the meantime, part or all of the non-affected area may be declared a free *zone* provided that such a part meets the conditions in point 3 of Article 10.4.6.

Annex 15 (contd)

The pathway for *self-declaration of freedom* from *infection* with ISAV HPR0 based on absence of clinical disease (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 10.4.6.

Zone or compartment free from infection with 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 10.5.2. are present in the *zone* or *compartment* and *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

2) the *disease* status prior to *targeted surveillance* is unknown ~~any of the *susceptible species* referred to in Article 10.4.2. are present and there has been no detectable occurrence of *infection* with ISAV~~ 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 have been destroyed or removed from the *infected zone* by means that minimise the *risk* of further spread of the *disease*, and the appropriate *disinfection* procedures (as described in the *Aquatic Manual*) have been completed; and

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

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

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— Text deleted.

Annex 16

CHAPTER 11.6.

INFECTION WITH *PERKINSUS OLSENI*

Article 11.6.1.

For the purposes of the *Aquatic Code*, infection with *Perkinsus olseni* means *infection* with *P. olseni*.

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

Article 11.6.2.

Scope

The recommendations in this chapter apply to: primarily venerid clams (*Austrovenus stutchburyi*, *Venerupis pullastra*, *V. aurea*, *Ruditapes decussatus* and *R. philippinarum*), abalone (*Haliotis rubra*, *H. laevigata*, *H. Cyclobates* and *H. scalaris*) and other species (*Anadara trapezia*, *Barbatianovaezelandiae*, *Macomonaliliana*, *Paphies australis*, *Crassostrea ~~gigas and C.~~ ariakensis*). These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

[…]

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— Text deleted.

Annex 17

CHAPTER 2.2.2.

INFECTIOUS HYPODERMAL AND HAEMATOPOIETIC NECROSIS

1. Scope

Infectious hypodermal and haematopoietic necrosis (IHHN) disease is caused by infection with infectious hypodermal and haematopoietic necrosis virus (IHHNV) (Bonami & Lightner, 1991; Bonami *et al.,* 1990; Lightner, 1996a; 2011; Lightner *et al.,* 1983a; 1983b; Lotz *et al.,* 1995; Tang & Lightner, 2002). ~~A large portion of the IHHNV genome has been found to be inserted in the genome of some genetic lines of *Penaeus monodon*. There is no evidence that this variant of IHHNV is infectious (Tang & Lightner, 2002; 2006).~~

Synonyms: the International Committee on Taxonomy of Viruses has assigned IHHNV (a parvovirus) as a tentative species in the genus *Brevidensovirus*, family *Parvoviridae* with the species name of PstDNV (for *Penaeus stylirostris* densovirus) (Fauquet *et al.,* 2005). For the purpose of this *Aquatic Manual*, most references to the viral agent of IHHN will be as IHHNV.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent, agent strains

IHHNV is the smallest of the known penaeid shrimp viruses. The IHHN virion is a 20–22 nm, non-enveloped icosahedron, with a density of 1.40 g ml^{-1} in CsCl, contains linear single-stranded DNA with an estimated size of 3.9 kb, and has a capsid with four polypeptides of molecular weight 74, 47, 39, and 37.5 kD (Bonami *et al.,* 1990; Nunan *et al.,* 2000; GenBank AF218266).

At least two ~~three~~ distinct genotypes of IHHNV have been identified (Tang & Lightner, 2002; Tang *et al.,* 2003b): Type 1) from the Americas and East Asia (principally the Philippines). Type 2 is from South-East Asia. These genotypes are infectious to *P. vannamei* and *P. monodon*. Two putative related sequences are found embedded in the genome of penaeids Type 3A~~)~~ from East Africa, India and Australia, and Type 3B~~)~~ from the western Indo-Pacific region including Madagascar, Mauritius and Tanzania (Tang & Lightner, 2006; Tang *et al.,* 2007). ~~The first two genotypes are infectious to the representative penaeids, *P. vannamei* and *P. monodon*~~ There is evidence that these sequences are not infectious to *P. vannamei* and *P. monodon* ~~two genetic variants are not infectious to these species~~ (Tang & Lightner, 2002; Tang *et al.,* 2003b; 2007). IHHNV type 3A and type 3B related sequences have been found inserted into the genome of *P. monodon* from East Africa, Australia, and the western Indo-Pacific region (Tang & Lightner, 2006; Tang *et al.,* 2007). The putative IHHNV sequences in the *P. monodon* genome are not infectious to the representative host species *P. vannamei* and *P. monodon* (Lightner *et al.,* 2009; Tang & Lightner, 2006; Tang *et al.,* 2007). Primer sets 309F/309R can distinguish the infectious forms of IHHNV from non-infectious forms. Primer sets MG831F/MG831R will distinguish the non-infectious forms of IHHNV.

2.1.2. Survival outside the host

No data.

2.1.3. Stability of the agent (effective inactivation methods)

IHHNV is believed to be the most stable virus of the known penaeid shrimp viruses. Infected tissues remain infectious after repeated cycles of freeze–thawing and after storage in 50% glycerine (Lightner, 1996a; Lightner *et al.,* 1987; 2009).

2.1.4. Life cycle

Not applicable.

2.2. Host factors

2.2.1. Susceptible host species

Most penaeid species can be infected with IHHNV, including the principal cultured species, *P. monodon* (black tiger shrimp/prawn), *P. vannamei* (Pacific white shrimp), and *P. stylirostris* (Pacific blue shrimp).

IHHNV infections are most severe in the Pacific blue shrimp, *P. stylirostris*, where the virus can cause acute epizootics and mass mortality (> 90%). In *P. stylirostris,* the juvenile and subadult life stages are the most severely affected (Bell & Lightner, 1984; 1987; Brock & Lightner 1990; Brock *et al.,* 1983; Lightner, 1996a; Lightner & Redman, 1998a; Lightner *et al.,* 1983a).

IHHNV causes the chronic disease runt-deformity syndrome (RDS) in *P. vanname*i in which reduced, irregular growth and cuticular deformities, rather than mortalities, are the principal effects (Bray *et al.,* 1994; Browdy *et al.,* 1993; Castille *et al.,* 1993; Kalagayan *et al.,* 1991; Lightner, 1996a; 1996b; Motte *et al.,* 2003). IHHNV infection in *P. monodon* is usually subclinical, but RDS, reduced growth rates and reduced culture performance have been reported in IHHNV-infected stocks (Chayaburakul *et al.,* 2004; Primavera & Quinitio, 2000).

2.2.2. Susceptible stages of the host

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).

2.2.3. Species or subpopulation predilection (probability of detection)

See Sections 2.2.1 and 2.2.2.

2.2.4. Target organs and infected tissue

IHHNV infects and has been shown to replicate (using *in-situ* hybridisation [ISH] with specific DNA probes) in tissues of ectodermal and mesodermal origin from the embryo. Thus, the principal target organs include: the gills, cuticular epithelium (or hypodermis), all connective tissues, the haematopoietic tissues, the lymphoid organ, antennal gland, and the ventral nerve cord, its branches and its ganglia. The enteric organs (endoderm-derived hepatopancreas, midgut and midgut caeca mucosal epithelia) and smooth, cardiac, and striated muscle show no histological signs of infection by IHHNV and are usually negative for IHHNV by ISH (Lightner, 1993; 1996a; 2011; Lightner *et al.,* 1992b).

2.2.5. Persistent infection with lifelong carriers

Some members of *P. stylirostris* and *P. vannamei* populations that survive IHHNV infections and/or epizootics, may carry the virus for life and pass the virus on to their progeny and other populations by vertical and horizontal transmission (Bell & Lightner 1984; Lightner, 1996a; 1996b; Morales-Covarrubias & Chavez-Sanchez, 1999; Motte *et al.,* 2003).

2.2.6. Vectors

No vectors are known in natural infections.

2.2.7. Known or suspected wild aquatic animal carriers

IHHNV is common in wild penaeid shrimp in South-East Asia (*P. monodon*) and in the Americas (*P. vannamei, P. stylirostris* and other Pacific side wild penaeid species) (Fegan & Clifford, 2001; Lightner, 1996a; Lightner *et al.,* 2009; Morales-Covarrubias *et al.,* 1999; Nunan *et al.,* 2001).

2.3. Disease pattern

2.3.1. Transmission mechanisms

Transmission of IHHNV can be by horizontal or vertical routes. Horizontal transmission by cannibalism or by contaminated water (Lightner, 1996a; Lightner *et al.,* 1983a; 1983b; 1985), and vertical transmission via infected eggs (Motte *et al.,* 2003) have been demonstrated.

2.3.2. Prevalence

In regions where the virus is enzootic in wild stocks, the prevalence of IHHNV has been found in various surveys to range from 0 to 100%. Some reported mean values for IHHNV prevalence in wild stocks are: 26% and 46% in *P. stylirostris* in the lower and upper Gulf of California, respectively (Pantoja *et al.,* 1999); 100% and 57%, respectively, in adult female and adult male *P. stylirostris* from the mid-region of the Gulf of California (Morales-Covarrubias *et al.,* 1999); 28% in wild *P. vannamei* collected from the Pacific coast of Panama (Nunan *et al.,* 2001); and from 51 to 63% in *P. vannamei* collected from the Pacific coasts of Ecuador, Colombia and Panama (Motte *et al.,* 2003). Other penaeids collected during some of these surveys and found to be IHHNV positive included the brown shrimp, *P. californiensis* and the Western white shrimp *P. occidentalis*. In farms where IHHNV is present, its prevalence can range from very low to 100%, but high prevalence, approaching 100%, is typical (Chayaburakul *et al.,* 2004; Lightner, 1988; 1996a; 1996b; Lightner *et al.,* 1992a; 1983a; Martinez-Cordova, 1992).

2.3.3. Geographical distribution

IHHNV appears to have a world-wide distribution in both wild and cultured penaeid shrimp (Brock & Lightner, 1990; Lightner, 1996a; 1996b; Owens *et al.,* 1992). In the Western Hemisphere, IHHNV is commonly found in wild penaeid shrimp in the eastern Pacific from Peru to Mexico. Although IHHNV has been reported from cultured *P. vannamei* and *P. stylirostris* in most of the shrimp-culturing regions of the Western Hemisphere and in wild penaeids throughout their range along the Pacific coast of the Americas (Peru to northern Mexico), the virus has not been reported in wild penaeid shrimp on the Atlantic coast of the Americas (Bondad-Reantaso *et al.,* 2001; Brock & Main, 1994; Lightner, 1996a, 1996b; Lightner *et al.*, 1992a; Lightner & Redman, 1998a). IHHNV has also been reported in cultured penaeid shrimp from Pacific islands including the Hawaiian Islands, French Polynesia, Guam, and New Caledonia. In the Indo-Pacific region, the virus has been reported from cultured and wild penaeid shrimp in East Asia, South-East Asia, and the Middle East (Bondad-Reantaso *et al.,* 2001; Lightner, 1996a).

Infectious IHHNV was detected for the first time in farmed prawns in Australia in 2008. Additionally an IHHN-like virus sequence has been reported from Australia (Krabsetsve *et al.*, 2004; Owens *et al.*, 1992), and the presence of IHHN in farmed prawns in Australia was reported to the OIE in 2008. As discussed in Section 2.1.1, IHHNV-related sequences have been found inserted into the genome of *P. monodon* from East Africa, Australia, and the western Indo-Pacific region (Tang & Lightner, 2006; Tang *et al.*, 2007).

2.3.4. Mortality and morbidity

Depending on the host species and the genotype of the virus, IHHN may take three distinct forms: in unselected *P. stylirostris*, infection by IHHNV results in an acute, usually catastrophic disease with mortalities approaching 100%. In contrast, in *P. vannamei*, some selected lines of *P. stylirostris*, and in *P. monodon* under some conditions, infection by IHHNV results in a more subtle, chronic disease, RDS, in which high mortalities are unusual, but significant growth suppression and cuticular deformities are common. In the third situation, a large portion of the IHHNV genome has been found to be inserted in the genome of some genetic lines *of P. monodon*. There is ~~no~~ evidence that in *P. monodon*, this ~~variant of~~ inserted IHHNV sequence is not infectious to other penaeids (Tang & Lightner, 2002; 2006).

IHHVV interferes with normal egg and larval development: poor hatching success of eggs, and poor survival and culture performance of the larval and PL stages is observed when broodstock are used from wild or farmed stocks where IHHNV is enzootic (Motte *et al.,* 2003).

Farmed stocks of *P. stylirostris,* juveniles, subadults and adults may show persistently high mortality rates. In *P. vannamei, P. stylirostris,* and possibly *P. monodon,* IHHNV-infected stocks may show poor and highly disparate growth, poor overall culture performance, and cuticular deformities, including especially bent rostrums and deformed sixth abdominal segments.

Demonstration of eosinophilic to pale basophilic intranuclear inclusion bodies in the typical target tissues for IHHNV, as IHHNV intranuclear inclusion bodies are nearly identical in appearance to those occurring in the early stages of WSSV infections, their presence in tissue sections should be considered as a presumptive diagnosis of IHHNV until confirmed with a second test method, such as dot-blot or ISH with IHHNV-specific DNA probes or positive PCR test results for IHHNV.

2.3.5. Environmental factors

The replication rate of IHHNV at high water temperatures was significantly reduced in a study in which viral replication was compared in *P. vannamei* experimentally infected and held at 24°C and 32°C. After a suitable incubation period, shrimp held at 32°C had approximately 10^2 lower viral load than shrimp held at 24°C. However, even at the higher temperature, significant (up to 10^5 virus copies 50 ng^{-1} of shrimp DNA) IHHNV replication still occurred in shrimp held at 32°C (Montgomery-Brock *et al.,* 2007).

2.4. Control and prevention

2.4.1. Vaccination

No effective vaccination methods for IHHNV have been developed.

2.4.2. Chemotherapy

No scientifically confirmed reports of effective chemotherapy treatments.

2.4.3. Immunostimulation

No scientifically confirmed reports of effective immunostimulation treatments.

2.4.4. Resistance breeding

Selected stocks of *P. stylirostris* that are resistant to IHHN disease have been developed and these have had some successful application in shrimp farms (Clifford, 1998; Lightner, 1996a; 1996b; Weppe 1992; Zarian-Herzberg & Ascencio-Valle, 2001). Some selected lines of *P. stylirostris* bred for IHHN disease resistance were found to be refractory to infection (Tang *et al.,* 2000). However, such stocks do not have increased resistance to diseases such as white spot syndrome virus (WSSV), and, hence, their use has been limited. In some stocks a genetic basis for IHHN susceptibility in *P. vannamei* has been reported (Alcivar-Warren *et al.,* 1997).

2.4.5. Restocking with resistant species

There has been some limited application and success with IHHNV-resistant *P. stylirostris* (Clifford, 1998; Lightner, 1996a; Weppe, 1992; Zarin-Herzberg & Ascencio 2001). The relative resistance of *P. vannamei* to IHHN disease, despite infection by IHHNV, is considered to be among the principal factors that led to *P.vannamei* being the principal shrimp species farmed in the Western Hemisphere and, since 2004, globally (Lightner, 2005; Lightner *et al.,* 2009; Rosenberry, 2004).

2.4.6. Blocking agents

There are reports of shrimp with high viral loads of IHHNV being resistant to infection by WSSV (Bonnichon *et al.,* 2006; Tang *et al.,* 2003a). However, there are no reports to date for IHHNV blocking agents.

2.4.7. Disinfection of eggs and larvae

IHHNV is transmitted vertically by the transovarian route (Motte *et al.,* 2003). Hence, while disinfection of eggs and larvae is good management practice (Chen *et al.,* 1992) and is recommended for its potential to reduce IHHNV contamination of spawned eggs and larvae produced from them (and contamination by other disease agents), the method is not effective for preventing transmission of IHHNV (Motte *et al.,* 2003).

2.4.8. General husbandry practices

Some husbandry practices have been successfully applied to the prevention of IHHNV infections and disease. Among these has been the application of polymerase chain reaction (PCR) pre-screening of wild or pond-reared broodstock and/or their spawned eggs/nauplii, and discarding those that test positive for the virus (Fegan & Clifford, 2001; Motte *et al.,* 2003), as well as the development of specific pathogen free (SPF) shrimp stocks of *P. vannamei* and *P. stylirostris* (Lightner, 1996b; 2005; Lotz *et al.,* 1995; Pruder *et al.,* 1995; Wyban 1992). The latter has proven to be the most successful husbandry practice for the prevention and control of IHHN (Jaenike *et al.,* 1992; Lightner, 2005; Pruder *et al.,* 1995). Unfortunately, there is a misconception in the industry that SPF is a genetic trait rather than a condition of health status (Lightner *et al.,* 2009). The development of SPF *P. vannamei* that were free not only of IHHNV, but also of all the major known pathogens of penaeid shrimp, has resulted in the introduction of the species to Asia and to its surpassing *P. monodon* in 2005 as the dominant farmed shrimp species in Asia as well as the Americas where the SPF stocks were developed (FAO, 2006; Lightner, 2005; Lightner *et al.,* 2009; Rosenberry, 2004).

3. Sampling

3.1. Selection of individual specimens

Suitable specimens for testing for infection by IHHNV are all life stages (eggs, larvae, PL, juveniles and adults) (Motte *et al.,* 2003). While IHHNV may infect all life stages, infection severity, and hence virus load, may be below detection limits in spawned eggs and in the larval stages, so these life stages may not be suitable samples for IHHNV detection or certification for IHHN disease freedom.

3.2. Preservation of samples for submission

For routine histology or molecular assays, and guidance on preservation of samples for the intended test method see Chapter 2.2.0.

3.3. Pooling of samples

Samples taken for molecular tests may be combined as pooled samples representing no more than five specimens per pooled sample of juveniles, subadults and adults. However, for eggs, larvae and PL, pooling of larger numbers (e.g. ~150 or more eggs or larvae or 50–150 PL depending on their size/age) may be necessary to obtain sufficient sample material (extracted nucleic acid) to run a diagnostic assay. See also Chapter 2.2.0.

3.4. Best organs and tissues

IHHNV infects tissues of ectodermal and mesodermal origin. The principal target tissues for IHHNV include connective tissue cells, the gills, haematopoietic nodules and haemocytes, ventral nerve cord and ganglia, antennal gland tubule epithelial cells, and lymphoid organ parenchymal cells (Lightner, 1996a; Lightner & Redman, 1998a). Hence, whole shrimp (e.g. larvae or PLs) or tissue samples containing the aforementioned target tissues are suitable for most tests using molecular methods.

Haemolymph or excised pleopods may be collected and used for testing (usually for PCR, or dot-blot hybridisation with specific probes) when non-lethal testing of valuable broodstock is necessary (Lightner, 1996a; Lightner & Redman, 1998a).

3.5. Samples/tissues that are not suitable

IHHNV is a systemic virus, and it does not replicate in enteric tissues (e.g. the hepatopancreas, the midgut, or its caeca). Hence, enteric tissues are inappropriate samples for detection of infection by IHHNV (Lightner, 1996a; 2011; Lightner & Redman, 1998a).

4. Diagnostic methods

4.1. Field diagnostic methods

4.1.1. Clinical signs

Certain cuticular deformities, specifically a deformed rostrum bent to the left or right, which may be presented by *P. vannamei* and *P. stylirostris* with RDS, are pathognomonic for infection by IHHNV (see Section 4.2.1.2). However, this clinical sign is not always apparent in shrimp populations chronically infected with IHHNV. As *P. vannamei, P. stylirostris,* and *P. monodon* can be infected by IHHNV and not present obvious signs of infection (e.g. they may show markedly reduced growth rates or ‘runting’), molecular tests are recommended when evidence of freedom from IHHN disease is required.

4.1.2. Behavioural changes

In acute IHHN disease, *P. stylirostris* may present behavioural changes (see Section 4.2.1.1) but with RDS, no consistent behavioural changes have been reported for affected shrimp.

4.2. Clinical methods

4.2.1. Gross pathology

4.2.1.1. IHHN disease in *Penaeus stylirostris*

IHHNV often causes an acute disease with very high mortalities in juveniles of this species. Vertically infected larvae and early PL do not become diseased, but in approximately 35-day-old or older juveniles, gross signs of the disease may be observed, followed by mass mortalities. In horizontally infected juveniles, the incubation period and severity of the disease is somewhat size and/or age dependent, with young juveniles always being the most severely affected. Infected adults seldom show signs of the disease or mortalities (Bell & Lightner, 1984; 1987; Bondad-Reantaso *et al.,* 2001; Brock *et al.,* 1983; Brock & Main, 1994; Lightner, 1983; 1988; 1993; 1996a; 2011; Lightner *et al.,* 1983a, 1983b). Gross signs are not IHHN specific, but juvenile *P. stylirostris* with acute IHHN show a marked reduction in food consumption, followed by changes in behaviour and appearance. Shrimp of this species with acute IHHN have been observed to rise slowly in culture tanks to the water surface, where they become motionless and then roll-over and slowly sink (ventral side up) to the tank bottom. Shrimp exhibiting this behaviour may repeat the process for several hours until they become too weak to continue, or until they are attacked and cannibalised by their healthier siblings. *Penaeus stylirostris* at this stage of infection often have white or buff-coloured spots (which differ in appearance and location from the white spots that sometimes occur in shrimp with WSSV infections) in the cuticular epidermis, especially at the junction of the tergal plates of the abdomen, giving such shrimp a mottled appearance. This mottling later fades in moribund *P. stylirostris* as such individuals become more bluish. In *P. stylirostris* and *P. monodon* with terminal-phase IHHNV infections, moribund shrimp are often distinctly bluish in colour, with opaque abdominal musculature (Bondad-Reantaso *et al.,* 2001; Lightner, 1983; 1988; 1993; 1996a; 2011; Lightner *et al.,* 1983a; 1983b).

4.2.1.2. IHHN disease in *Penaeus vannamei*

RDS, a chronic form of IHHN disease, occurs in *P. vannamei* as a result of IHHNV infection. The severity and prevalence of RDS in infected populations of juvenile or older *P. vannamei* may be related to infection during the larval or early PL stages. RDS has also been reported in cultured stocks of *P. stylirostris* and *P. monodon*. Juvenile shrimp with RDS may display a bent (45° to 90° bend to left or right) or otherwise deformed rostrum, a deformed sixth abdominal segment, wrinkled antennal flagella, cuticular roughness, 'bubble-heads', and other cuticular deformities. Populations of juvenile shrimp with RDS display disparate growth with a wide distribution of sizes and many smaller than expected ('runted') shrimp. The coefficient of variation (CV = the standard deviation divided by the mean of different size groups within a population) for populations with RDS is typically greater than 30% and may approach 90%, while IHHNV-free (and thus RDS-free) populations of juvenile *P. vannamei* and *P. stylirostris* usually show CVs of 10–30% (Bray *et al.,* 1994; Brock & Lightner, 1990; Brock *et al.,* 1983; Brock & Main, 1994; Browdy *et al.,* 1993; Carr *et al.,* 1996; Lightner, 1996a; Primavera & Quinitio, 2000; Pruder *et al.,* 1995).

4.2.2. Clinical chemistry

Not applicable.

4.2.3. Microscopic pathology

Acute IHHNV infections in *P. stylirostris* can be readily diagnosed using routine haematoxylin and eosin (H&E) stained histological methods (see Section 4.2.6). Chronic IHHNV infections and RDS are much more difficult to diagnose using routine H&E histological methods. For diagnosis of chronic infections, the use of molecular methods are recommended for IHHNV detection (e.g. by PCR or application of IHHNV-specific DNA probes to dot-blot hybridisation tests or ISH of histological sections).

Histological demonstration of prominent intranuclear, Cowdry type A inclusion bodies provides a provisional diagnosis of IHHNV infection. These characteristic IHHN inclusion bodies are eosinophilic and often haloed (with H&E stains of tissues preserved with fixatives that contain acetic acid, such as Davidson's AFA and Bouin's solution) (Bell & Lightner, 1988; Lightner, 1996a), intranuclear inclusion bodies within chromatin-marginated, hypertrophied nuclei of cells in tissues of ectodermal (epidermis, hypodermal epithelium of fore- and hindgut, nerve cord and nerve ganglia) and mesodermal origin (haematopoietic organs, antennal gland, gonads, lymphoid organ, and connective tissue). Intranuclear inclusion bodies caused by IHHNV may be easily confused with developing intranuclear inclusion bodies caused by WSSV infection. ISH assay (see Section 4.3.1.2.3 of this chapter) of such sections with a DNA probe specific to IHHNV provides a definitive diagnosis of IHHNV infection (Lightner, 1996a; 2011; Lightner & Redman, 1998a).

4.2.4. Wet mounts

No reliable methods have been developed for direct microscopic pathology.

4.2.5. Smears

Not applicable.

4.2.6. Fixed sections

Histopathology: histology may be used to provide a definitive diagnosis of IHHNV infection. Because 10% buffered formalin and other fixatives provide, at best, only fair fixation of the shrimp, the use of Davidson's fixative (containing 33% ethyl alcohol [95%], 22% formalin [approximately 37% formaldehyde], 11.5% glacial acetic acid and 33.5% distilled or tap water) is highly recommended for all routine histological studies of shrimp (Bell & Lightner, 1988; Lightner, 1996a). To obtain the best results, dead shrimp should not be used. Only live, moribund, or compromised shrimp should be selected for fixation and histological examination. Selected shrimp are killed by injection of fixative directly into the hepatopancreas; the cuticle over the cephalothorax and abdomen just lateral to the dorsal midline is opened with fine-pointed surgical scissors to enhance fixative penetration (the abdomen may be removed and discarded), the whole shrimp (or cephalothorax less the abdomen) is immersed in fixative for from 24 to no more than 48 hours, and then transferred to 70% ethyl alcohol for storage. After transfer to 70% ethyl alcohol, fixed specimens may be transported (via post or courier to the diagnostic laboratory) by wrapping in cloth or a paper towel saturated with 70% ethyl alcohol and packed in leak-proof plastic bags (see Section 4.2.3).

In-situ hybridisation (see Section 4.3.1.2.3 below).

4.2.7. Electron microscopy/cytopathology

Electron microscopy is not recommended for routine diagnosis of IHHNV.

4.3. Agent detection and identification methods

4.3.1. Direct detection methods

4.3.1.1. Microscopic methods

4.3.1.1.1. Wet mounts

See Section 4.2.4.

4.3.1.1.2. Smears

See Section 4.2.5.

4.3.1.1.3. Fixed sections

See section 4.2.6.

4.3.1.2. Agent isolation and identification

4.3.1.2.1. Cell culture/artificial media

IHHNV has not been grown *in vitro*. No crustacean cell lines exist (Lightner, 1996a; Lightner & Redman, 1998a: 1998b).

4.3.1.2.2. Antibody-based antigen detection methods

None has been successfully developed.

Annex 17 (contd)

4.3.1.2.3. Molecular techniques

Direct detection methods using DNA probes specific for IHHNV are available in dot-blot and ISH formats. PCR tests for IHHNV have been developed and a number of methods and commercial products using these methods are readily available.

DNA probes for dot-blot and ISH applications: gene probe and PCR methods provide greater diagnostic sensitivity than do more traditional techniques for IHHN diagnosis that employ classic histological approaches. Furthermore, these methods have the added advantage of being applicable to non-lethal testing of valuable broodstock shrimp. A haemolymph sample may be taken with a tuberculin syringe, or an appendage (a pleopod for example) may be biopsied (Bell *et al.,* 1990), and used as the sample for a direct dot-blot test.

Dot-blot hybridisation procedure for IHHNV: the probe is labelled with a non-radioactive label, digoxigenin-11-dUTP (DIG-11-dUTP). The system using DIG to label nucleic acid probes was developed by Boehringer Mannheim Biochemicals (this company is now owned by Roche Diagnostic Corporation), which is described in the Roche *DIG Nonradioactive Labeling and Detection Product Selection Guide* and *DIG Application Manual for Filter Hybridization™ System User's Guide for Membrane Hybridization* and from Boehringer Mannheim's *Nonradioactive In Situ Hybridization Application Manual* (Roche Applied Science, 2006a; 2006b). The protocols given below use a DIG-labelled probe to IHHNV produced by one of several methods. Probes may be produced using a fragment of cloned IHHNV DNA as the template by the random primed labelling method (Lightner, 1996a; Mari *et al.,* 1993). An alternative method for producing DIG-labelled probes uses specific primers from the cloned IHHNV DNA and the Roche PCR DIG Probe Synthesis Kit™.

Dot-blot hybridisation procedure: the dot-blot hybridisation method given below uses a DIG-labelled DNA probe for IHHNV and generally follows the methods outlined in Mari *et al.* (1993) and Lightner (1996a). Formulas for the required reagents are given after the protocols.

i) Prepare a positively charged nylon membrane (Roche Diagnostics Cat. No. 1-209-299 or equivalent[1]): cut pieces to fit samples and controls and mark with soft-lead pencil making 1 cm squares for each sample. Include a positive and a negative control on each filter. Lay out on to a piece of filter paper (Whatman 3MM).

ii) If necessary, dilute samples to be assayed in TE (Tris/EDTA [ethylene diamine tetra-acetic acid]) buffer plus 50 µg ml^{-1} salmon sperm DNA, using 1 µl sample in 9 µl buffer in 1.5 ml microcentrifuge tubes. Samples for dot-blots can be haemolymph, tissues homogenised in TN (Tris/NaCl: 0.4 M NaCl and 20 mM Tris/HCl, pH 7.4) buffer, or extracted DNA in 10 mM Tris/HCl.

iii) Boil samples for 10 minutes and quench on ice for 5 minutes. Briefly microfuge samples in the cold to bring down all liquid and to pellet any coagulated protein. Keep on ice until samples are dotted on to the membrane.

iv) Dot 1–3 µl of each sample on to an appropriate place on the filters. Allow to air-dry and then fix samples on to the membrane by baking at 80°C for 30 minutes or by UV cross-linking using a DNA transilluminator for 3 minutes.

v) Adjust a water bath to 68°C and prepare the prehybridisation solution. For a 10 × 15 cm membrane, prepare 8 ml per membrane. Set a stirring hot plate to 'low' and stir while warming the solution for 30 minutes until the blocking agent has dissolved and the solution is cloudy. Also, prepare some heat-seal bags that are slightly larger in size than the membrane: five to six bags will be needed per membrane.

vi) Remove membranes from the oven or transilluminator and put into a heat-seal bag with 4 ml per membrane of prehybridisation solution. Seal the bags and put into a 68°C water bath for 30 minutes 1 hour.

vii) Boil the DIG-labelled probe for 10 minutes, quench on ice and then microfuge in the cold to bring all the liquid down in the microcentrifuge tube. Keep on ice. Remove the prehybridisation solution from the bags. Add 2 ml of fresh prehybridisation solution to each bag and then add the correct, predetermined amount of DIG-labelled probe to each, mixing well as it is being added. Seal the bags, place back in the 68°C water bath and incubate for 8–12 hours.

[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*.

viii) Wash membranes well with:

2 × standard saline citrate (SSC)/0.1% sodium dodecyl sulphate (SDS)	2 ×	5 minutes at room temperature
0.1 × SSC/0.1% SDS (use 4 ml/filter and seal in bags)	3 ×	15 minutes at 68°C
Buffer I	1 ×	5 minutes at room temperature
Buffer II	1 ×	30 minutes at room temperature
Buffer I	1 ×	5 minutes at room temperature

(Buffers are prepared ahead of time).

ix) React the membrane in bags with anti-DIG AP conjugate (Roche Diagnostics 1-093-274) diluted 1/5000 in Buffer I. Use 3 ml per membrane; incubate for 30–45 minutes at room temperature on a shaker platform.

x) Wash membrane well with:

Buffer I	2 ×	15 minutes at room temperature
Buffer III	1 ×	5 minutes at room temperature

xi) Develop the membranes in bags using 3 ml per membrane of development solution (nitroblue tetrazolium salt [NBT]/X-phosphate in Buffer III) made up just prior to use. React in the dark at room temperature for 1–2 hours. Stop the reactions in Buffer IV and dry the membranes on 3MM filter paper.

xii) Photograph the results (colour fades over time).

xiii) Store dry membranes in heat-seal bags.

In-situ *hybridisation* (*ISH*) *procedure:* the ISH method given below uses a DIG-labelled DNA probe for IHHNV and generally follows the methods outlined in Mari *et al.* (1993) and Lightner (1996a). Formulas for the required reagents are given after the protocols.

i) Embed tissue in paraffin and cut sections at 4–6 µm thickness. Place sections on to positively charged microscope slides (do not put gelatine in water to float sections; just use water).

ii) Put slides in a slide rack, such as a Tissue-Tek rack. Heat the slides in an oven for 45 minutes at 60°C. In the staining centre, rehydrate the tissue as follows:

Xylene (or suitable substitute)	3 ×	5 minutes each
Absolute alcohol	2 ×	1 minute each
95% alcohol	2 ×	10 dips each
80% alcohol	2 ×	10 dips each
50% alcohol	1 ×	10 dips
Distilled water	six rinses (do not let slides dry out)	

iii) Wash the slides for 5 minutes in phosphate buffered saline (PBS or Tris/NaCl/EDTA [TNE] buffer). Prepare fresh proteinase K at 100 µg ml^{-1} in PBS (or TNE). Place slides flat in a humid chamber, pipette on 500 µl of the proteinase K solution and incubate for 10–15 minutes at 37°C. Drain fluid onto blotting paper.

iv) Return slides to slide rack. Fix sections in 0.4% cold formaldehyde for 5 minutes at room temperature.

v) Incubate slides in 2 × SSC for 5 minutes at room temperature.

vi) With slides flat, add 0.5–1 ml prehybridisation buffer and incubate in a humid chamber for 15–30 minutes at 37°C.

vii) Boil the DIG-labelled probe for 10 minutes and quench on ice; spin briefly in the cold and keep on ice. Dilute the probe to 25 ng ml^{-1} in prehybridisation solution and cover the tissue with 250 µl of the solution. Incubate the slides for 2–4 hours at 42°C or overnight at 37°C in a humid chamber. Drain fluid onto blotting paper. During this incubation, pre-warm the wash buffers at 37°C.

viii) Place slides in slide rack. Wash the slides as follows:

2 × SSC	2 ×	5–30 minutes at 37°C
1 × SSC	2 ×	5 minutes at 37°C
0.5 × SSC	2 ×	5 minutes at 37°C

Annex 17 (contd)

ix) Wash the slides for 5 minutes in Buffer I at room temperature. Put the slides flat in a humid chamber and block with 0.5 ml per slide of Buffer II. Incubate for 15 minutes at 37°C. Drain the fluid on to blotting paper.

x) Dilute the anti-DIG AP conjugate (Roche Applied Science cat. 10686322) 1/1000 in Buffer II (1 µl anti-DIG AP per 1 ml buffer). Cover tissue with 500 µl of diluted conjugate and incubate in a humid chamber for 30 minutes at 37°C.

xi) Place the slides in a slide rack. Wash in Buffer I twice for 5–10 minutes each time at room temperature. Wash once with Buffer III for 5–10 minutes.

xii) Prepare the development solution by first adding 4.5 µl NBT per 1 ml buffer III. Mix well. Then add 3.5 µl X-phosphate per ml of solution and mix well. Pipette on 500 µl per slide and incubate in a humid chamber in the dark for 2–3 hours at room temperature.

xiii) Stop the reaction by returning the slides to a slide rack and washing in Buffer IV for 15 minutes at room temperature.

xiv) Counterstain the slides by dipping for 5 minutes in 0.5% aqueous Bismarck brown Y.

xv) Dehydrate the slides in the staining centre as follows:

95% alcohol	3 ×	10 dips each
Absolute alcohol	3 ×	10 dips each
Xylene (or suitable substitute)	4 ×	10 dips each

Do not allow the slides to dry out – leave them in the last xylene (or xylene substitute) container until ready for cover-slips.

xvi) Mount with cover-slips and mounting medium (Permount).

xvii) Examine the slides under bright-field for a dark-blue or black precipitate that marks sites where IHHNV DNA is present. Pathodiagnostic intranuclear Cowdry type A inclusions are well marked with the probe. Also often marked are host cell nuclei without obvious inclusions, cytoplasmic inclusions, and accumulation of free virus in the tissue spaces and haemolymph.

NOTE: Always run a known positive and negative control.

Reagent formulas for ISH method:

i) 10 × phosphate buffered saline

NaCl	160 g
KH_2PO_4	4 g
Na_2HPO_4	23 g
KCl	4 g
DD H_2O	1950 ml (qs to 2 litres)

pH to 8.2 with NaOH; autoclave to sterilise; store at room temperature. To make 1 × PBS, dilute 100 ml 10 × PBS in 900 ml DD H_2O; Filter 1 × solution through a 0.45 µm filter; store at 4°C.

ii) 10 × Tris/NaCl/EDTA (TNE) buffer

Tris base	60.57 g
NaCl	5.84 g
EDTA	3.72 g
DD H_2O	900 ml (qs to 1 litre)

pH to 7.4 with concentrated or 5 M HCl. To make 1 × TNE, dilute 100 ml 10 × TNE in 900 ml DD H_2O; Filter 1 × solution through a 0.45 µm filter; store at 4°C.

iii) Proteinase K, 100 µg ml^{-1} (prepare just prior to use)

PBS	10 ml 1 × PBS
Proteinase K	1 mg

iv) 0.4% formaldehyde

37% formaldehyde	5.4 ml
DD H_2O	500 ml

Store at 4°C; can be reused up to four times before discarding.

v) Prehybridisation buffer (50 ml final volume)

4 × SSC	10 ml 20 × SSC
50% formamide	25 ml 100% formamide
1 × Denhardt's	2.5 ml 20 × Denhardt's
5% dextran sulphate	10 ml 25% dextran sulphate

Warm to 60°C

Boil 2.5 ml of 10 mg ml^{-1} salmon sperm DNA and add to buffer for final concentration of 0.5 mg ml^{-1} salmon sperm DNA; store at 4°C.

vi) 20 × SSC buffer

3M NaCl	175.32 g NaCl
0.3 M $Na_3C_6H_5O_7.2H_2O$	88.23 g Na citrate.$2H_2O$
DD H_2O	1000 ml (qs)

pH to 7.0; autoclave; store at 4°C.

To make 2 × SSC, dilute 100 ml 20 × SSC in 900 ml DD H_2O; To make 1 × SSC, dilute 50 ml 20 × SSC in 950 ml DD H_2O; To make 0.5 × SSC, dilute 50 ml 20 × SSC in 1950 ml DD H_2O. Filter solutions through a 0.45 µm filter; store at 4°C.

vii) 20 × Denhardt's solution

BSA (Fraction V)	0.4 g bovine serum albumin
Ficoll 400	0.4 g Ficoll
PVP 360	0.4 g polyvinylpyrollidine
DD H_2O	100 ml

Filter solutions through a 0.45 µm filter; store at 4°C. Aliquot 2.5 ml into small tubes and store frozen.

viii) 25% dextran sulphate

Dextran sulphate	25 g
DD H_2O	100 ml

Mix to dissolve; store frozen in 10 ml aliquots.

ix) Salmon sperm DNA (10 mg ml^{-1})

Salmon sperm DNA	0.25 g
DD H_2O	25 ml

To prepare, warm the water and slowly add the DNA with stirring until completely dissolved; boil for 10 minutes; shear the DNA by pushing through an 18-gauge needle several times; aliquot 2.5 ml into small tubes and store frozen; boil for 10 minutes just before using to facilitate mixing in the buffer.

x) 10 × Buffer I

1 M Tris/HCl	121.1 g Tris base
1.5 M NaCl	87.7 g NaCl
DD H_2O	1000 ml (qs)

pH to 7.5 with HCl. Autoclave; store at 4°C.

To make 1 × Buffer I, dilute 100 ml of 10 × stock in 900 ml DD H_2O. Filter through a 0.45 µm filter; store at 4°C.

xi) Buffer II (blocking buffer)

Blocking reagent	0.25 g Blocking reagent (Roche Diagnostics 1-096-176)
Buffer I	50 ml 1 × Buffer I

Store at 4°C for up to 2 weeks.

Annex 17 (contd)

xii) Buffer III

100 mM Tris/HCl	1.21 g Tris base
100 mM NaCl	0.58 g NaCl
DD H_2O	100 ml (qs)
pH to 9.5 with HCl	
Then add:	
50 mM $MgCl_2$	1.02 g $MgCl_2.6H_2O$

Filter through a 0.45 µm filter; store at 4°C.

xiii) 10% polyvinyl alcohol (PVA)

Polyvinyl alcohol	10 g
DD H_2O	100 ml

To prepare, slowly add PVA to water while stirring on low heat. (It takes 2–3 hours for PVA to go into solution.) Dispense 10 ml per tube and store frozen at –20°C.

xiv) Development solution

Mix 90 ml Buffer III with 10 ml of 10% PVA. Store at 4°C. Just prior to use, for each 1 ml of Buffer III with PVA add:

4.5 µl NBT	75 mg NBT ml^{-1} in 70% dimethylformamide (Roche Diagnostics 1-383-213)
3.5 µl X-phosphate	5-bromo-4-chloro-3-indoyl phosphate, toluidine salt (50 mg ml^{-1} in dimethylformamide) (Roche Diagnostics 1-383-221)

xv) Buffer IV

10 mM Tris/HCl	1.21 g Tris base
1 mM EDTA	0.37 g EDTA.$2H_2O$ (disodium salt)
DD H_2O	1000 ml

pH to 8.0 with HCl. Filter through a 0.45 µm filter; store at 4°C.

xvi) 0.5% Bismarck Brown Y

Bismarck Brown Y	2.5 g
DD H_2O	500 ml

Dissolve the stain in water. Filter through a Whatman No. 1 filter; store at room temperature.

Polymerase chain reaction for IHHNV: several single-step PCR methods (Krabsetsve *et al.,* 2004; Nunan *at al.,* 2000; Shike *et al.,* 2000; Tang *et al.,* 2000; 2007; Tang & Lightner 2001), and a number of commercial PCR kits are available for IHHNV detection. Nested methods are also available from commercial sources.

There are multiple geographical variants of IHHNV, some of which are not detected by all of the available methods for IHHNV. Two primer sets, 392F/R and 389F/R, are the most suitable for detecting all the known genetic variants of IHHNV (Krabsetsve *et al.,* 2004; Tang & Lightner, 2002). However these tests also detect IHHNV-related sequences called ~~including~~ types 3A and 3B, which are inserted into the genome of certain geographic stocks of *P. monodon* from the western Indo-Pacific, East Africa, Australia and India (Duda & Palumbi, 1999; Tang & Lightner, 2006; Tang *et al.,* 2007; Saksmerprome *et al.,* 2011). ~~New~~ PCR primers have been developed that can detect the IHHN viral sequence but do not react with IHHNV-related sequences present in the *P. monodon* stocks from Africa, Australia (Tang *et al.,* 2007), or Thailand (Saksmerprome *et al.,* 2011). Primer set 309F/R amplifies only a segment from IHHNV types 1 and 2 (the infectious forms of IHHNV), but not types 3A and 3B, which are non-infectious and part of the *P. monodon* genome (Tang & Lightner, 2006; Tang *et al.,* 2007). Primer set MG831F/R reacts only with types 3A and 3B, which are non-infectious and part of the *P. monodon* genome (Tang *et al.,* 2007). Hence, confirmation of unexpected positive and/or negative PCR results for IHHNV with a second primer set, or use of another diagnostic method (i.e. PCR using primers from another region of the genome, real-time PCR, bioassay, ISH) is highly recommended.

Table 4.1. *Recommended primer sets for one-step PCR detection of IHHNV*

Primer	Product	Sequence	G+C%/Temp.	GenBank & References
389F	389 bp	5'-CGG-AAC-ACA-ACC-CGA-CTT-TA-3'	50%/72°C	AF218266
389R		5'-GGC-CAA-GAC-CAA-AAT-ACG-AA-3'	45%/71°C	(Tang *et al.,* 2000)
77012F	356 bp	5'-ATC-GGT-GCA-CTA-CTC-GGA-3'	50%/68°C	AF218266
77353R		5'-TCG-TAC-TGG-CTG-TTC-ATC-3'	55%/63°C	(Nunan *et al.,* 2000 ~~2001~~)
392F	392 bp	5'-GGG-CGA-ACC-AGA-ATC-ACT-TA-3'	50%/68°C	AF218266
392R		5'-ATC-CGG-AGG-AAT-CTG-ATG-TG-3'	50%/71°C	(Tang *et al.,* 2000; 2007)
309F	309 bp	5'-TCC-AAC-ACT-TAG-TCA-AAA-CCA-A-3'	36%/68°C	AF218266
309R		5'-TGT-CTG-CTA-CGA-TGA-TTA-TCC-A-3'	40%/69°C	(Tang *et al.,* 2007)
MG831F	831 bp	5'-TTG-GGG-ATG-CAG-CAA-TAT-CT-3'	45%/58°C	DQ228358
MG831R		5'-GTC-CAT-CCA-CTG-ATC-GGA-CT-3'	55%/62°C	(Tang *et al.,* 2007)

NOTE: Primers 389F/R and 392F/R described above are from the nonstructural protein-coding region (ORF 1) of the IHHNV genome. Primers 77012F/77353R ~~77353/77012~~ are from a region in between the nonstructural and the structural (coat protein) protein-coding regions of the genome. In the event that results are ambiguous using the 389F/R 'universal' primer set, it is recommended to use primers from a different region of the genome for confirmatory testing. In this case, that would mean using primers 77012F/77353R or the 392F/R primer sets and follow up with sequencing of PCR amplicons for confirmation.

General PCR method for IHHNV: the PCR method described below for IHHNV generally follows the methods outlined in Nunan *et al.* (2000). Cumulative experience with the technique has led to modifications with respect to template (DNA extraction of clinical specimens), choice of primers (Table 4.1), and volume of reaction.

i) Use as a template, the DNA extracted from ground tissue homogenate (TN buffer, 0.4 M NaCl, 20 mM Tris, pH 7.4) or haemolymph (collected with a small amount of 10% sodium citrate) or from tissue or haemolymph that was fixed in 95% ethanol and then dried. A control consisting of tissue or haemolymph from known negative animals should be included during the DNA extraction step. The DNA can be extracted by a variety of methods, but excellent results have been obtained using kits from Roche Diagnostics (Cat. No. 1-796-828) or Qiagen (Cat. No. 51304). Other DNA extraction kits include QIAamp DNA Mini Kit (Qiagen), MagMax™ Nucleic Acid kits (Life Technologies), or Maxwell® 16 Cell LEV DNA Purification Kit (Promega), or ~~reagents from Gibco Life Sciences~~ DNazol ~~Cat. No. 10503-027~~ (Life Technologies). Spectrophotometric readings of the final DNA will indicate the purity of the DNA and the amount of total DNA extracted from the sample. Use 1–5 µl of extracted DNA per 50 µl reaction volume.

ii) The following controls should be included in every PCR assay for IHHNV: a) DNA from a known negative tissue sample; b) DNA from a known positive sample (either from tissue or haemolymph or from a plasmid clone that contains the fragment that the specific set of primers amplifies; and c) a 'no template' control.

iii) Use as primers, primers 389F and 389R, which elicit a band 389 bp in size from IHHNV-infected material, or primers 77012F and 77353R, which elicit a band 356 bp in size from IHHNV-infected material. Prepare primers at 100 ng μl^{-1} in distilled water. Keep frozen at –70°C.

iv) Use a 'hot start' method for the polymerase: if Applied Biosystem's AmpliTaq Gold is used, this involves a 5-minute step at 95°C to denature DNA prior to the primers binding and activation of the enzyme. This programme is then linked to the cycling programme (35 cycles) and an extension programme. The programme is set as follows:

Hot start	Programme 1	5 minutes 95°C	
Linked to	Programme 2	30 seconds 95°C	
		30 seconds 55°C	35 cycles
		1 minute 72°C	
Linked to	Programme 3	7 minutes 72°C	
Linked to	Programme 4	4°C until off	

v) Prepare a 'master mix' consisting of water, 10 × PCR buffer, the four dNTPs, the two primers, $MgCl_2$, AmpliTaq Gold and water (assume use of 1 µl of template; if using more, adjust water accordingly). Add mix to each tube. Use thin-walled tubes designed for PCR. Always run a positive and a negative control.

'Master Mix':

DD H_2O	32.5 µl × number of samples
10 × PCR buffer	5 µl × number of samples
10 mM dTTP	1 µl × number of samples
10 mM dATP	1 µl × number of samples
10 mM dCTP	1 µl × number of samples
10 mM dGTP	1 µl × number of samples
25 mM $MgCl_2$	4 µl × number of samples
Forward primer (100 ng $µl^{-1}$)	1.5 µl × number of samples
Reverse primer (100 ng $µl^{-1}$)	1.5 µl × number of samples
AmpliTaq Gold	0.5 µl × number of samples

Vortex this solution to mix all reagents well; keep on ice.

NOTE: The volume of the PCR reaction may be modified. Previously, the PCR reactions for IHHNV were run in 100 µl volumes, but it is not necessary to use that amount of reagents, therefore 50 µl volumes are described in this procedure. Likewise, the PCR reactions can also be run in volumes as small as 25 µl. To do this, increase or decrease the volume of the reagents accordingly.

vi) For a 50 µl reaction mix, add 49 µl Master Mix to each tube and then add 1 µl of the sample to be tested.

vii) Vortex each tube, spin quickly to bring down all liquid. If the thermal cycler does not have a heated lid to prevent condensation, then carefully overlay the top of each sample with 25–50 µl mineral oil and re-cap the tubes. Insert tubes into the thermal cycler and start programme 1 ('hot start'), which is linked to cycling, extension and soak cycles.

viii) If mineral oil was used, recover samples from under the mineral oil using a pipette set at 50 µl and transfer to a fresh tube. Using the long-tipped pipette tips (designed for loading gels) results in less oil being carried over with the sample.

ix) Run 10 µl of the sample in a 1.5% agarose gel (containing 0.5 µg ml^{-1} ethidium bromide to stain the DNA). Look for the 389 bp band (if using primers 389F and 389R) or for the 356 bp band (if using primers 77012F and 77353R). Bands are not always seen, as it is necessary to have at least 10 ng DNA $µl^{-1}$ to see DNA in a gel. A Southern transfer of the gel or a dot-blot can be run for more sensitive detection. The DNA can also be precipitated (0.3 M sodium acetate and 2.5 volumes 100% ethanol, –70°C, for 1–3 hours, centrifuge for 20 minutes) and resuspended in 1/10th volume (i.e. 4 µl) TE (10 mM Tris, 1 mM EDTA, pH 7.5) or water and either re-run in the gel or tested in a dot-blot.

Real-time PCR (*qPCR*) *method for IHHNV:* qPCR methods have been developed for the detection of IHHNV. These methods offer extraordinary sensitivity that can detect a single copy of the target sequence from the IHHNV genome (Dhar *et al.,* 2001; Tang & Lightner, 2001). Using primers 309F/309R, it is possible to distinguish infectious forms of IHHNV from non-infectious forms. Using MG831F/MG831R it is possible to distinguish the non-infectious forms.

The qPCR method using TaqMan chemistry described below for IHHNV generally follows the method used in Tang & Lightner (2001).

i) The PCR primers and TaqMan probe are selected from a region of the IHHNV genomic sequence (GenBank AF218266) that encodes for non-structural protein. The primers and TaqMan probe are designed by the Primer Express software (Applied Biosystems). The upstream (IHHNV1608F) and downstream (IHHNV1688R) primer sequences are: 5'-TAC-TCC-GGA-CAC-CCA-ACC-A-3' and 5'-GGC-TCT-GGC-AGC-AAA-GGT-AA-3', respectively. The TaqMan probe (5'-ACC-AGA-CAT-AGA-GCT-ACA-ATC-CTC-GCC-TAT-TTG-3'), which corresponds to the region from nucleotide 1632 to 1644, is synthesised and labelled with fluorescent dyes 5-carboxyfluoroscein (FAM) on the 5' end and N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA) on the 3' end (Applied Biosystems, part no. 450025).

ii) Preparation of DNA template: the extraction and purification of DNA template is the same as that described in the section of traditional PCR.

iii) The qPCR reaction mixture contains: TaqMan Universal PCR Master Mix (Applied Biosystems, part no. 4324018), 0.3 µM of each primers, 0.15 µM of TaqMan probe, 5–50 ng DNA, and water in a reaction volume of 25 µl. For optimal results, the reaction mixture should be vortexed and mixed well.

iv) Amplification is performed with the GeneAmp 5700 Sequence Detection System (Applied Biosystems; ABI PRISM 7000, 7300, or 7500 or equivalent can also be used). The cycling profile is: activation of AmpliTaq Gold for 10 minutes at 95°C, followed by 40 cycles of denaturation at 95°C for 15 seconds and annealing/extension at 60°C for 1 minute. The levels of fluorescence are measured at the end of the annealing and extension step.

v) At the end of the reaction, real-time fluorescence measurements will be taken with a built in charge-coupled device (CCD) camera. A threshold will be set to be above the baseline that begins to detect the increase in signal associated with an exponential increase of PCR product. ~~Samples will be defined as negative if there is no Ct (threshold cycle) value after 40 cycles the Ct (threshold cycle) values exceed 40 cycles.~~ Samples with a Ct value lower than 40 cycles are considered to be positive. ~~To confirm the real-time PCR results, an aliquot of PCR product can be subjected to electrophoresis on a 4% ethidium bromide-agarose gel and photographed. An 81-bp DNA fragment can be visualised in the samples that are positive for IHHNV.~~

vi) It is necessary to include a 'no template' control in each reaction run. This is to rule out the presence of fluorescence contaminants in the reaction mixture or in the heat block of the thermal cycler. A positive control should also be included, and it can be a plasmid containing the target sequence, or purified virions, or DNA from IHHNV-infected tissue.

Sequencing: PCR products may be cloned and sequenced when necessary to confirm infection with IHHNV, to identify false positives or nonspecific amplification, or to distinguish the amplified product from the infectious form of the virus and demonstrate the presence of the insertion of non-infectious IHHNV genome in host DNA (Tang & Lighter, 2002; 2006).

Through PCR, IHHNV was detected in *P. monodon* from South-East Asia. Most of these IHHNV PCR assays also detected IHHNV-related sequences in *P. monodon* populations in Africa, Australia and Thailand (Tang & Lightner, 2006; Saksmerprome *et al.,* 2011). To discriminate the IHHNV-related sequences from the actual virus, PCR assays using primers that detect the IHHN viral sequence and do not react with IHHNV-related sequences present in the *P. monodon* stocks from Africa or Australia (Tang *et al.,* 2007), or Thailand (e.g. Saksmerprome *et al.,* 2011) have been developed.

PCR commercial kits are available for IHHNV diagnosis and can be acceptable provided they have been validated as fit for such purpose. The OIE validation procedure is described in Chapter 1.1.2 Principles and methods of validation of diagnostic assays for infectious diseases.

4.3.2. Serological methods

Shrimp are invertebrate animals and do not produce antibodies. Therefore, serological methods for IHHN are not available.

Annex 17 (contd)

5. Rating of tests against purpose of use

The methods currently available for surveillance, detection, and diagnosis of IHHNV 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 and/or not available 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. *IHHNV surveillance, detection and diagnostic methods*

Method	Surveillance				Presumptive diagnosis	Confirmatory diagnosis
	Larvae	PLs	Juveniles	Adults		
Gross signs	d	d	d	d	d	d
Bioassay	d	d	d	d	c	c
Direct LM	d	d	d	d	d	d
Histopathology	d	d	c	c	a	b
Transmission EM	d	d	d	d	c	c
Antibody-based assays	d	d	d	c	d	d
DNA probes – *in situ*	d	d	b	b	a	a
PCR, qPCR	a	a	a	a	a	a
Sequence	d	d	d	d	d	a

PLs = postlarvae; LM = light microscopy; EM = electron microscopy; qPCR = real-time polymerase chain reaction.

6. Test(s) recommended for targeted surveillance to declare freedom from infectious hypodermal and haematopoietic necrosis

As indicated in Table 5.1, PCR is the recommended method for targeted surveillance for reasons of availability, utility, and diagnostic specificity and sensitivity.

When investigating acute mortality episodes as part of a targeted surveillance programme, demonstration of pathognomonic IHHNV-induced lesions in the cuticular epithelium by histology (with or without confirmation by ISH with IHHNV-specific DNA probes) is a suitable method (Table 5.1).

7. Corroborative diagnostic criteria

7.1. Definition of suspect case

IHHN shall be suspected if at least one of the following criteria is met:

i) Clinical signs indicative of IHHN and a positive result by *in-situ* hybridisation

or

ii) Histopathology indicative of IHHN and a positive result by *in-situ* hybridisation.

~~Poor hatching success of eggs, and poor survival and culture performance of the larval and PL stages when broodstock are used from wild or farmed stocks where IHHNV is enzootic.~~

~~Farmed stocks of *P. stylirostris*, juveniles, subadults and adults may show persistently high mortality rates. In *P. vannamei, P. stylirostris,* and possibly *P. monodon,* IHHNV infected stocks may show poor and highly disparate growth, poor overall culture performance, and cuticular deformities, including especially bent rostrums and deformed sixth abdominal segments.~~

~~Demonstration of eosinophilic to pale basophilic intranuclear inclusion bodies in the typical target tissues for IHHNV, as IHHNV intranuclear inclusion bodies are nearly identical in appearance to those occurring in the early stages of WSSV infections, their presence in tissue sections should be considered as a presumptive diagnosis of IHHNV until confirmed with a second test method, such as dot-blot or ISH with IHHNV specific DNA probes or positive PCR test results for IHHNV.~~

7.2. Definition of confirmed case

IHHN is considered to be confirmed if two of the following criteria are met:

i) Positive result by *in-situ* hybridisation

ii) Positive result by PCR (always genotype specific)

iii) Sequence analysis to confirm IHHNV nucleic acid sequence.

The two methods must target different areas of the genome.

~~Any combination of at least two of the following four methods (with positive results):~~

- ~~Positive dot-blot hybridisation test results for IHHNV.~~
- ~~ISH positive histological signal to IHHNV-type lesions.~~
- ~~PCR positive results for IHHNV.~~
- ~~Sequencing of PCR specific products may be required when the purpose is to determine the genotype of IHHNV.~~

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NB: There are OIE Reference Laboratories for Infectious hypodermal and haematopoietic necrosis (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
Infectious hypodermal and haematopoietic necrosis

Annex 18

CHAPTER 2.2.4.

NECROTISING HEPATOPANCREATITIS

1. Scope

Necrotising hepatopancreatitis (NHP) disease is caused by infection with a Gram-negative, pleomorphic intracellular alpha-proteobacterium (Frelier *et al.,* 1992; Lightner & Redman, 1994; Lightner *et al*., 1992; Loy *et al.,* 1996a; 1996b) preliminarily called Candidatus *Hepatobacter penaei*. The principal host species in which necrotising hepatobacterium (NHPB) can cause significant disease outbreaks and mortalities are *Penaeus vannamei* and *P. stylirostris* (Del Río-Rodríguez *et al.,* 2006; Frelier *et al.,* 1993; Ibarra-Gámez *et al.,* 2007; Lightner & Redman, 1994; Morales-Covarrubias *et al.*, 2011).

NHP has four distinct phases: initial, acute, transition and chronic. In acute and transition-phase disease, pathognomonic lesions are typically present in histological sections of the hepatopancreas, while in the initial and chronic phases of the disease, there are no pathognomonic lesions, and molecular and antibody-based methods for NHPB detection are necessary for diagnosis (Morales-Covarrubias, 2010; Morales-Covarrubias *et al.*, 2010; 2012; Vincent & Lotz, 2005).

Synonyms: necrotising hepatobacterium (NHPB) or NHP bacterium (NHPB); rickettsial-like organism (RLO).

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent, agent strains

NHPB is a pleomorphic, Gram-negative, intracytoplasmic bacterium preliminarily called Candidatus *Hepatobacter penaei*. It is a member of the α-subclass of proteobacteria ~~and remains unclassified~~ (Frelier *et al.,* 1992; Lightner & Redman, 1994; Loy *et al.,* 1996a; 1996b). The predominant form is a rod-shaped rickettsial-like organism (0.25 × 0.9 µm), whereas the helical form (0.25 × 2–3.5 µm) possesses eight flagella at the basal apex (Frelier *et al.,* 1992; Lightner & Redman, 1994; Loy *et al.,* 1996a; 1996b). Genetic analysis of the NHPB associated with North and South American outbreaks of NHP suggest that the isolates are either identical or very closely related subspecies (Loy *et al.,* 1996a; 1996b).

2.1.2. Survival outside the host

No data.

2.1.3. Stability of the agent

NHPB-infected tissues remain infectious after repeated cycles of freeze–thawing and after storage in 50% glycerine. NHPB frozen at –20°C –70°C and –80°C have been shown to retain infectivity in experimental transmission trials with *Penaeus vannamei* (Crabtree *et al.,* 2006; Frelier *et al.,* 1992).

2.1.4. Life cycle

Not applicable.

2.2. Host factors

2.2.1. Susceptible host species

Most penaeid species can be infected with NHPB, including the principal cultured species in Latin American, *P. vannamei* (Pacific white shrimp) and *P. stylirostris* (Pacific blue shrimp).

NHPB infections are most severe in *P. vannamei* where the intracellular bacterium can cause acute epizootics and mass mortality (>90%). In *P. vannamei,* the juvenile, subadult and broodstock life stages are the most severely affected (Johnson, 1990; Jory, 1997; Lightner, 1996; Morales-Covarrubias, 2010).

NHPB causes chronic disease in *P. vannamei,* the main effects of which are slow growth, a soft cuticle and a flaccid body (Morales-Covarrubias, 2010; Morales-Covarrubias *et al.*, 2012).

Outbreaks of NHP disease have been reported in *P. aztecus* (Johnson, 1990; Jory, 1997; Lightner, 1996; Morales-Covarrubias, 2010). NHP has also been seen in *P.californiensis* and *P. setiferus* (Frelier *et al.,* 1995; Lightner, 1996). *Penaeus setiferus* is reportedly less susceptible to disease than *P. vannamei* (Frelier *et al.,* 1995).

In an NHP survey of the Gulf of Mexico, *P.setiferus* and *P.duorarum* in the vicinity of coastal prawn farms along the Yucatan and Campeche coast revealed no histological evidence of NHP (Del Río-Rodríguez *et al.,* 2006).

2.2.2. Susceptible stages of the host

NHPB has been demonstrated in juveniles, adults and broodstock of *P. vannamei*.

2.2.3. Species or sub-population predilection

See Sections 2.2.1 and 2.2.2.

2.2.4. Target organs and infected tissue

The target tissue is the hepatopancreas, with NHPB infection reported in all hepatopancreatic cell types.

2.2.5. Persistent infection with lifelong carriers

Some members of *P. vannamei* populations that survive NHPB infections and/or epizootics may carry the intracellular bacteria for life and pass it on to other populations by horizontal transmission (Aranguren *et al.,* 2006; Lightner, 2005; Morales-Covarrubias, 2008; 2010; Vincent & Lotz, 2005).

Natural transmission of NHPB is thought to occur *per os* by cannibalism (Frelier *et al.,* 1993; 1995; Johnson, 1990; Lightner, 2005; Morales-Covarrubias, 2010), although cohabitation and dissemination of NHPB via the water column may also play a role (Frelier *et al.,* 1993; 1995). NHPB in faeces shed into pond water has also been suggested as a possible means of transmission (Aranguren *et al.,* 2006; Briñez *et al.,* 2003; Morales-Covarrubias *et al.*, 2006). Outbreaks of disease are often preceded by prolonged periods of high water temperature (approximately 30°C) and salinity (up to 40 parts per thousand [ppt]) (Frelier *et al.,* 1995; Lightner & Redman, 1994; Morales-Covarrubias, 2010; Morales-Covarrubias *et al.*, 2010; 2011; Vincent & Lotz, 2005).

2.2.6. Vectors

No vectors are known in natural infections.

2.2.7. Known or suspected wild aquatic animal carriers

NHPB is common in wild penaeid shrimp in Peru (*P. vannamei*) and Laguna Madre of Tamaulipas, Mexico (*P. aztecus, P. duorarum* and *P. setiferus*) (Aguirre-Guzman *et al.,* 2010; Lightner & Redman, 1994).

2.3. Disease pattern

2.3.1. Transmission mechanisms

Transmission of NHPB can be horizontal by cannibalism; transmission by contaminated water has been demonstrated (Aranguren *et al.,* 2006; 2010; Frelier *et al.,* 1993; Gracia-Valenzuela *et al.*, 2011; Morales-Covarrubias *et al.*, 2012; Vincent *et al.*, 2004).

2.3.2. Prevalence

Some reported mean values for NHPB prevalence in wild stocks are between 5.6 and 15% in *P. duorarum,* and between 5 and 17% in *P. aztecus* collected from Carrizal and Carbonera, Laguna Madre of Tamaulipas, Mexico (Aguirre-Guzman *et al.,* 2010); 0.77% in *P. vannamei*, and 0.43% in *P. stylirostris* collected from Tumbes Region, Peru (Lightner & Redman, 1994).

Some reported mean values for NHPB prevalence in shrimp farms are between 0.6% and 1.3% in *P. vannamei* collected from shrimp farms in Belize, Brazil, Guatemala, Honduras, Mexico, Nicaragua and Venezuela (Morales-Covarrubias *et al.*, 2011).

2.3.3. Geographical distribution

NHPB appears to have a Western hemisphere distribution in both wild and cultured penaeid shrimp (Aguirre-Guzman *et al.,* 2010; Del Río-Rodríguez *et al.,* 2006). In the Western Hemisphere, NHPB is commonly found in cultured penaeid shrimp in Belize, Brazil, Colombia, Costa Rica, Ecuador, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Panama, Peru, United States of America, and Venezuela (Frelier *et al.,* 1992; Ibarra-Gámez *et al.,* 2007; Lightner, 1996; Morales-Covarrubias, 2010; Morales-Covarrubias *et al.*, 2011).

2.3.4. Mortality and morbidity

In *P. vannamei*, infection by NHPB results in an acute, usually catastrophic disease with mortalities approaching 100%.

2.3.5. Environmental factors

The replication rate of NHPB increases at lengthy periods of high temperatures (>29°C) and salinity changes (20–38%). In Mexico, NHPB has been detected at a low prevalence (<7%) in shrimp farms in the months of April, May, July and August. However, in the months of September and October when temperatures are high during the day and low at night, high prevalence and mortality (>20%) are observed (Morales-Covarrubias, 2010).

2.4. Control and prevention

Control

The use of the antibiotics, oxytetracycline and florfenicol 50%, in medicated feeds every 8 hours for 10 days is probably the best NHP treatment currently available, particularly if disease is detected in the initial phase (Frelier *et al.,* 1995; Morales-Covarrubias *et al.*, 2012).

Prevention

a) Early detection (initial phase) of clinical NHP is important for successful treatment because of the potential for cannibalism to amplify and transmit the disease.

b) Shrimp starvation and cannibalism of shrimps with NHPB, as well as positive conditions for NHPB cultivation, are important factors for NHPB propagation in *P. vannamei*.

c) The use of quick hydrated lime ($Ca(OH)_2$) to treat pond bottoms during pond preparation before stocking can help reduce NHP incidence.

d) Preventive measures can include raking, tilling and removing sediments from the bottom of the ponds, prolonged sun drying of ponds and water distribution canals for several weeks, disinfection of fishing gear and other farm equipment using calcium hypochlorite, and drying and extensive liming of ponds.

e) The use of specific pathogen-free (SPF) ~~and female~~ broodstock is an effective preventive measure.

2.4.1. Vaccination

No scientifically confirmed reports.

2.4.2. Chemotherapy

No scientifically confirmed reports.

2.4.3. Immunostimulation

No scientifically confirmed reports.

2.4.4. Resistance breeding

No scientifically confirmed reports.

2.4.5. Restocking with resistant species

No scientifically confirmed reports.

2.4.6. Blocking agents

No scientifically confirmed reports.

2.4.7. Disinfection of eggs and larvae

~~NHPB has been demonstrated to be transmitted horizontally by cannibalism (Frelier *et al.*, 1993; Gracia-Valenzuela *et al.*, 2011; Johnson, 1990; Jory, 1997; Lightner, 1996; Lightner & Redman, 1994; Loy *et al.*, 1996b; Morales-Covarrubias *et al.*, 2011b; Vincent & Lotz, 2005; 2007).~~ Disinfection of eggs and larvae is~~, therefore,~~ a good management practice (Lee & O'Bryen, 2003) and is recommended for its potential to reduce NHPB contamination of spawned eggs and larvae (and contamination by other disease agents).

2.4.8. General husbandry practices

Some husbandry practices have been successfully applied to the prevention of NHPB infections and disease. Among these has been the application of polymerase chain reaction (PCR) to pre-screening of wild or pond-reared broodstock.

3. Sampling

3.1. Selection of individual specimens

Suitable specimens for testing for infection by NHPB are life stages (postlarvae [PL], juveniles and adults).

3.2. Preservation of samples for submission

For routine histology or molecular assays, and guidance on preservation of samples for the intended test method, see Chapter 2.2.0.

3.3. Pooling of samples

Samples taken for molecular tests may be combined as pooled samples representing no more than five specimens per pooled sample of juveniles, sub adults and adults. However, for eggs, larvae and PL, pooling of larger numbers (e.g. ~150 or more eggs or larvae or 50–150 PL depending on their size/age) may be necessary to obtain sufficient sample material (extracted nucleic acid) to run a diagnostic assay. See also Chapter 2.2.0.

3.4. Best organs or tissues

NHPB infects most enteric tissue. The principal target tissue for NHPB is hepatopancreas. Faeces may be collected and used for testing (usually by PCR, or dot-blot hybridisation with specific probes) when non-lethal testing of valuable broodstock is necessary (Bondad-Reantaso *et al.,* 2001; Bradley-Dunlop *et al.,* 2004; Briñez *et al.,* 2003; Frelier *et al.,* 1993; Lightner, 1996; Morales-Covarrubias *et al.*, 2012).

3.5. Samples/tissues those are not suitable

NHPB ~~are enteric bacteria and~~ do not replicate in the midgut ~~(enteric tissues)~~, caeca ~~(enteric tissues)~~, connective tissue cells, the gills, haematopoietic nodules and haemocytes, ventral nerve cord and ganglia, antennal gland tubule epithelial cells, and lymphoid organ parenchymal cells.

4. Diagnostic methods

4.1. Field diagnostic methods

The prevalence and severity of NHPB infections may be 'enhanced' in a contained population by rearing shrimps in relatively crowded or stressful conditions. The 'crowding stress' factors may include high stocking densities, ablated, and marginal water quality (i.e. low dissolved oxygen, elevated water temperature, or elevated ammonia or nitrite) in the holding tank water. These conditions may encourage expression of low-grade NHPB infections and the transmission of the agent from carriers to previously uninfected hosts in the population. This results in increased prevalence and severity of infections that can be more easily detected using the available diagnostic and detection methods for NHPB.

4.1.1. Clinical signs

A wide range of gross signs can be used to indicate the possible presence of NHP. These include: lethargy, reduced food intake, atrophied hepatopancreas, anorexia and empty guts, noticeably reduced growth and poor length weight ratios ('thin tails'); soft shells and flaccid bodies; black or darkened gills; heavy surface fouling by epicommensal organisms; bacterial shell disease, including ulcerative cuticle lesions or melanised appendage erosion; and expanded chromatophores resulting in the appearance of darkened edges in uropods and pleopods. None of these signs are pathognomonic.

4.1.2. Behavioural changes

In acute NHP disease, *P. vannamei* may present behavioural changes including lethargy and reduced feeding activity.

4.2. Clinical methods

4.2.1. Gross pathology

NHPB often causes an acute disease with very high mortalities in young juveniles, adults and broodstock. In horizontally infected young juveniles, adult and broodstock, the incubation period and severity of the disease are somewhat size or age dependent. Infected adults seldom show signs of the disease or mortalities (Aranguren *et al.,* 2006; 2010; Bastos Gomes *et al.,* 2010, Brock & Main, 1994; Morales-Covarrubias *et al.*, 2012). Gross signs are not NHP specific, but acute NHP shows a marked reduction in food consumption, followed by changes in behaviour and appearance (see Section 4.1.1).

4.2.2. Clinical chemistry

Not applicable.

4.2.3. Microscopic pathology

Acute and chronic NHP in *P. vannamei* can be readily diagnosed using routine haematoxylin and eosin (H&E) stain histological methods (see Section 4.2.6).

4.2.3.1. Initial phase of necrotising hepatopancreatitis

Initial NHPB infection is more difficult to diagnose using routine H&E histological methods. For diagnosis of initial infections, molecular methods are recommended for NHPB detection (e.g. by PCR or application of NHPB-specific DNA probes to dot-blot hybridisation tests or *in-situ* hybridisation (ISH) of histological sections).

4.2.3.2. The acute phase of necrotising hepatopancreatitis

The acute NHP disease is characterised by atrophied hepatopancreas with moderate atrophy of the tubule epithelia, presence of bacterial ~~form~~ cells and infiltrating haemocytes involving one or more of the tubules (multifocal encapsulations). Hypertrophic cells, individual epithelial cells appeared to be separated from adjacent cells, undergo necrosis and desquamation in to the tubular lumen. The tubular epithelial cell lipid content is variable.

4.2.3.3. Transition phase of necrotising hepatopancreatitis

The transitional phase of NHP disease is characterised by haemocytic inflammation of the intertubular spaces in response to necrosis, cytolysis, and sloughing of hepatopancreas tubule epithelial cells. The hepatopancreas tubule epithelium is markedly atrophied, resulting in the formation of large oedematous (fluid filled or 'watery') areas in the hepatopancreas. Tubule epithelial cells within multifocal encapsulation are typically atrophied and reduced from simple columnar to cuboidal in morphology. They contain little or no stored lipid vacuoles, markedly reduced or no secretory vacuoles and masses of bacteria. At this phase haemocyte nodules were observed in the presence of masses of bacteria in the centre of the nodule

4.2.3.4. Chronic phase of necrotising hepatopancreatitis

In the chronic phase of NHP, tubular lesions, multifocal encapsulation and oedematous areas decline in abundance and severity and are replaced by infiltration and accumulation of haemocytes at the sites of necrosis. There are areas with fibrosis, few melanised and necrotic tubules and very low presence of hypertrophied cells with masses of bacteria in the cytoplasm and low numbers of haemocyte nodules.

4.2.4. Wet mounts

Wet-mount squash examination of hepatopancreas (HP) tissue is generally conducted to detect presumptive NHP disease. The hepatopancreas may be atrophied and have any of the following characteristics: soft and watery; fluid filled centre; pale with black stripes (melanised tubules); pale centre instead of the normal orange coloration. ~~Elevated mortality rates reaching over 90% can occur within 30 days of onset of clinical signs if not treated.~~ For wet mount analysis the shrimp must be in the intermolt stage, and have not undergone a treatment that could alter the tubules. This technique uses ~~is based on the~~ tubular deformation or ~~tubular~~ atrophy, mainly of the apical region to indicate early stages of NHP.

NHP disease has four phases (a semiquantitative scale):

Initial phase: low presence of tubular deformation (1–5 field^{-1} organism^{-1}) and cell detachment.

Acute phase: infiltration of haemocytes, increased numbers of deformed tubules (6–10 field^{-1} organism^{-1}), encapsulation present in different regions of the sample, which is atrophied tubules surrounded by multiple layers of haemocytes.

Transition phase: infiltration of haemocytes, increased numbers of deformed tubules (11–15 field^{-1} organism^{-1}), melanised tubules, necrotic tubules and a high level of encapsulation present in different regions of the sample. At this stage haemocyte nodules were observed with masses of bacteria in the centre of the nodule.

Chronic phase: areas with fibrosis, few melanised and necrotic tubules and very low presence of hypertrophied cells with masses of bacteria in the cytoplasm.

4.2.5. Smears

Not applicable.

4.2.6. Electron microscopy/cytopathology

Not currently applicable for diagnostic purposes

4.3. Agent detection and identification methods

4.3.1. Direct detection methods

4.3.1.1. Microscopic methods

4.3.1.1.1. Wet mounts

See section 4.2.4

4.3.1.1.2. Smears

Not applicable

4.3.1.1.3. Fixed sections

See section 4.2.3.

4.3.1.1.4. Bioassay method

Confirmation of NHPB infection may be accomplished by bioassay of NHPB-suspect animals with SPF juvenile *P. vannamei* serving as the indicator of the intracellular bacteria (Cock *et al.,* 2009; Johnson, 1990; Lee & O'Bryen, 2003; Lightner, 2005). Oral protocols may be used. The oral method is relatively simple to perform and is accomplished by feeding chopped hepatopancreas of suspect shrimp to SPF juvenile *P. vannamei* in small tanks. The use of a negative control tank of indicator shrimp, which receive only a normal feed, is required. When the hepatopancreas feeding (*per os*) protocol is used to bioassay for NHPB, NHP-positive indicator shrimp (by gross signs and histopathology) are typically apparent within 3–4 days of initial exposure, and significant mortalities occur by 3–8 days after initial exposure. The negative control shrimp must remain negative (for at least 10–15 days) for gross or histological signs of NHP disease and unusual mortalities.

4.3.1.2. Agent isolation and identification

4.3.1.2.1. Cell culture/artificial media

NHPB has not been grown *in vitro*. No crustacean cell lines exist (Morales-Covarrubias *et al.*, 2010; Vincent & Lotz, 2007).

4.3.1.2.2. Antibody-based antigen detection methods

Immunohistochemistry (IHC) tests using monoclonal antibodies (MAbs) ~~specific cDNA probes~~ to NHP according to the methods described in Bradley-Dunlop *et al.* (2004) ~~and Loy & Frelier (1996)~~.

4.3.1.2.3. Molecular techniques

ISH and ~~reverse transcription (RT)-~~PCR tests for NHPB have been developed, and ~~RT-~~PCR kits for NHPB are commercially available. PCR tests for NHP have been developed and a number of methods and commercial products using these methods are available (Loy & Frelier, 1996; Loy *et al.,* 1996b). Gene probes and PCR methods provide greater diagnostic sensitivity than do classic histological approaches to NHP diagnosis. Furthermore, these methods have the added advantage of being applicable to non-lethal testing of valuable broodstock shrimp.

4.3.1.2.3.1. DNA probes for ISH applications with non-radioactive cDNA probes

Non-radioactive, DIG-labelled ~~cDNA~~ probes for NHPB may be produced in the laboratory. The ISH method of Loy & Frelier (1996) and Lightner (1996) provides greater diagnostic sensitivity than do more traditional methods for NHPB detection and diagnosis that employ classical histological methods (Johnson, 1990; Lightner, 1996; Morales-Covarrubias, 2010; Morales-Covarrubias *et al.*, 2012). The ISH assay of routine histological sections of acute, transition and chronic phase lesions in hepatopancreas with a specific DIG-labelled cDNA probe to NHPB, provides a definitive diagnosis of NHPB infection (Lightner, 1996; Loy & Frelier, 1996; Morales-Covarrubias *et al.*, 2006). Pathognomonic NHPB-positive lesions display prominent blue to blue-black areas in the cytoplasm of affected cells when reacted with the cDNA probes. (See Chapter 2.2.2 IHHN for details of the ISH method, and Chapter 2.2.0 Section B.5.3.ii for detailed information on the use of Davidson's AFA fixative.)

4.3.1.2.3.2. ~~Reverse transcription (RT)~~ PCR method

Hepatopancreas and faeces may be assayed for NHPB using PCR. Primers designated as NHPF2: 5'-CGT-TGG-AGG-TTC-GTC-CTT-CAGT-3' and NHPR2: 5'-GCC-ATG-AGG-ACC-TGA-CAT-CAT-C-3', amplify a 379 base pair (bp) designed against the GenBank accession number corresponding to the ~~ribosomal~~ 16S rRNA of NHPB~~, which amplify a 379 bp fragment~~ (Nunan *et al.*, 2008). ~~The primer concentration (F2/R2) used for each is 0.31 µM. The cycling parameters are: Step 1: 95°C for 2 minutes, 1 cycle; Step 2: 60°C for 30 seconds, 72°C for 30 seconds and 95°C for 30 seconds, 25 cycles; Step 3: 60°C for 1 minute, 72°C for 2 minutes, 1 cycle; 4°C infinite hold.~~ The ~~RT-~~PCR method outlined below ~~for NHPB~~ generally follows the method ~~used~~ described in ~~Nunan *et al.* (2008)~~ Aranguren *et al.* (2010) with modifications by an OIE Reference Laboratory in the USA.

i) *Preparation of ~~RNA~~ DNA template:* ~~RNA~~ DNA can be extracted from 25–50 mg of fresh, frozen and ethanol-preserved hepatopancreas. Extraction of ~~RNA~~ DNA should be performed using commercially available ~~RNA~~ DNA tissue extraction kits~~, such as the High Pure RNA Tissue Kit (Roche, Germany) and~~ following the manufacturer's procedures for production of quality DNA ~~RNA~~ templates. Other DNA extraction kits include QIAamp DNA Mini Kit (Qiagen), MagMax™ Nucelic Acid kits (Life Technologies), or Maxwell® 16 Cell LEV DNA Purification Kit (Promega)[2].

~~ii) The RT-PCR assay is carried out in solution, using final RNA concentration must be 10–1000 ng ml^{-1}.~~

ii) The following controls should be included ~~in every~~ when perfomring the ~~RT-~~PCR assay for NHPB: a) known NHPB negative tissue sample; b) a known NHPB-positive sample (hepatopancreas); and c) a 'no template' control.

iii) The ~~GeneAmp® EZ rTth RNA PCR kit (Applied Bioscience, USA)~~ PuReTaq™ Ready-To-Go PCR Bead (RTG beads, GE Healthcare) is used for all amplification reactions described here.

[2] 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*.

iv) The optimised ~~RT-~~PCR conditions (5–50 ng DNA) (final concentrations in ~~50~~ 25 µl total volume) for detection of NHPB in shrimp hepatopancreas samples are: primers (~~0.46~~ 0.2 µM each), dNTPs (~~300~~ 200 µM each), ~~rTth DNA~~ Taq polymerase (~~2.5 U 50~~ 0.1 U μl^{-1}), magnesium ~~acetate~~ chloride (~~2.5~~ 1.5 mM~~, in 5 × EZ buffer (25 mM Bicine, 57.5 mM potassium acetate, 40% [w/v] glycerol, pH 8.2)~~ in 10 mM Tris-HCl, pH 9.0, 50 mM KCl.

v) If the thermal cycler does not have a heated lid, then light mineral oil (50 µl) is overlaid on the top of the ~~50~~ 25 µl reaction mixtures to prevent condensation or evaporation during thermal cycling.

~~vii) The RNA template and all the reagents are combined and reverse transcription was allowed to proceed at 60°C for 30 minutes, followed for 2 minutes.~~

vi) The cycling parameters are: Step 1: 95°C for ~~2~~ 5 minutes, 1 cycle; Step 2: ~~60~~ 95°C for 30 seconds, ~~72~~ 60°C for 30 seconds and ~~95~~ 72°C for 30 seconds, ~~25~~ 35 cycles; Step 3: 60°C for 1 minute, 72°C for 2 minutes, 1 cycle; 4°C infinite hold.

Note: The conditions should be optimised for each thermal cycler using known positive controls.

~~ix) Details of the composition of the reagents and buffers used here may be found in Chapter 2.2.2 IHHN.~~

4.3.1.2.3.3. Real-time PCR method

Real-time PCR methods have been developed for the detection of NHPB. These methods have the advantages of speed, specificity and sensitivity. The sensitivity of real-time PCR is ~100 copies of the target sequence from the NHPB genome (Aranguren *et al.,* 2010; Vincent & Lotz, 2005).

The real-time PCR method using TaqMan chemistry described below for NHPB generally follows the method used in Aranguren *et al* (2010).

i) The PCR primers and TaqMan probe were selected from the 16S, rRNA gene of NHPB (GenBank U65509) (Loy & Frelier*.,* 1996). The primers and TaqMan probe were designed by the Primer Express software version 2.0 (Applied Biosystems). The upstream (NHP1300F) and downstream (NHP1366R) primer sequences are: 5'-CGT-TCA-CGG-GCC-TTG-TACAC-3' and 5'-GCT-CAT-CGC-CTT-AAA-GAA-AAG-ATA-A-3', respectively. The TaqMan probe NHP: 5'-CCG-CCC-GTC-AAG-CCA-TGG-AA-3', which corresponds to the region from nucleotides 1321–1340, is synthesised and labelled with fluorescent dyes 6-carboxyfluorescein (FAM) on the 5' and N,N,N,Ntetramethyl- 6-carboxyrhodamine (TAMRA) on the 3' end.

ii) *Preparation of ~~RNA~~ DNA template:* the extraction and purification of ~~*RNA*~~ *DNA* template from hepatopancreas, is the same as that described in the section for traditional ~~real-time~~ PCR.

iii) *The real-time PCR reaction mixture contains:* TaqMan One-step real-time PCR SuperMix (Quanta, Biosciences), 0.3 µM of each primer, 0.1 µM of TaqMan probe, 5–50 ng of ~~*RNA*~~ *DNA*, and water in a reaction volume of 25 µl. For optimal results, the reaction mixture should be vortexed and mixed well.

iv) Amplification is performed with the master cycler Realplex 2.0 (Eppendorf). The cycling consists of initial denaturation at 95°C for 3 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds and annealing/extension at 60°C for 1 minute. After each cycle, the levels of fluorescence are measured.

v) At the end of the reaction, real time fluorescence measurements will be taken with a built in charge-coupled device (CCD) camera. A threshold will be set to be above the baseline that begins to detect the increase in signal associated with an exponential increase in PCR product. ~~Samples will be defined as negative if there is no Ct (threshold cycle) value is after 40 cycles.~~

vi) It is necessary to include a 'no template control' in each reaction run. This is to rule out the presence of fluorescence contaminants in the reaction mixture or in the heat block of the thermal cycler. A positive control should also be included, and this can be ~~an *in-vitro* transcribed RNA~~ plasmid DNA containing the target sequence, purified bacteria, or ~~RNA~~ DNA extracted from NHPB-infected hepatopancreas.

4.3.1.2.3.4. Sequencing

~~RT-~~PCR products may be cloned and sequenced or sequenced directly when necessary to confirm infection by NHPB or to identify false positives or nonspecific amplification (Aranguren *et al.,* 2010; Bustin *et al.,* 2009; Vincent & Lotz, 2005).

4.3.1.2.4. Agent purification

Methods for NHPB isolation and purification are available (Aranguren *et al.,* 2010; Vincent *et al.,* 2004; Vincent & Lotz, 2005)~~, but these are not recommended for routine diagnosis of NHP~~. The NHP bacterium is unculturable using traditional bacteriological methods, thus NHPB infection must be maintained through continual exposure of uninfected *L. vannamei* stock to a population undergoing an NHPB epidemic.

4.3.2 Serological methods

Not applicable because shrimp are invertebrate animals that do not produce specific antibodies that could be used to demonstrate infection by or prior exposure to NHPB.

5. Rating of tests against purpose of use

The methods currently available for targeted surveillance and diagnosis of NHPB 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
Bioassay	d	d	d	d	c	d
Direct LM	d	d	c	d	c	d
Histopathology	d	b	b	c	a	b
***In-situ* DNA probes**	a	a	a	a	a	a
Transmission EM	d	d	d	d	c	c
Antibody-based assays	d	d	c	c	b	b
Real-time PCR	a	a	a	a	a	a
~~**qPCR**~~	~~a~~	~~a~~	~~a~~	~~a~~	~~a~~	~~a~~
PCR	a	a	a	a	a	a
Sequencing	d	d	d	d	d	a

PLs = postlarvae; LM = light microscopy; EM = electron microscopy; PCR = polymerase chain reaction; ~~q~~PCR = ~~quantitative~~ PCR.

Annex 18 (contd)

6. Test(s) recommended for targeted surveillance to declare freedom from Necrotising Hepatopancreatitis

As indicated in Table 5.1, real-time PCR (Section 4.3.1.2.3.2) is the recommended method for targeted surveillance for reasons of availability, utility, and diagnostic specificity and sensitivity. When investigating acute mortality episodes as part of a targeted surveillance programme, demonstration of pathognomonic NHPB-induced lesions in the hepatopancreas by histology (with or without confirmation by ISH with NHPB-specific DNA probes) is a suitable method (Table 5.1).

7. Corroborative diagnostic criteria

7.1. Definition of suspect case

The presence of NHPB shall be suspected if at least one of the following criteria is met: ~~A suspect case is represented by:~~

Sudden high mortalities in late PL, juvenile or subadult *P. vannamei* or *P. stylirostris* in regions where NHPB is enzootic;

~~The sudden presence of numerous sea birds (gulls, cormorants, herons, terns, etc.) 'fishing' in one or more shrimp culture ponds;~~

Samples of cultured *P. vannamei* or *P. stylirostris* from ponds with feeding sea birds that present gross signs indicative of acute- or transition-phase NHP, such as a general atrophied hepatopancreas, reddish colouration, lethargy, soft shells, empty guts, and the presence of numerous irregular black spots on the cuticle;

Poor hatching success of eggs, and poor survival and culture performance of the larval and PL stages when broodstock are used from wild or farmed stocks where NHPB is enzootic.

7.2. Definition of confirmed case

Any combination of a molecular (PCR or ISH) test and a morphological (histology) test using at least two of the following three methods (with positive results):

- Histological demonstration of diagnostic acute-phase NHPB lesions in (especially) the atrophied hepatopancreas with moderate atrophy of the tubule mucosa, presence of bacteria~~l form~~ and infiltrating haemocytes involving one or more of the tubules (multifocal encapsulations).
- ISH positive histological signal to NHPB-type lesions.
- PCR positive results for NHPB.

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* *

NB: At the time of publication (2015) there was not yet
an OIE Reference Laboratory for Necrotising hepatopancreatitis
(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/).

Annex 19

CHAPTER 2.2.5.

TAURA SYNDROME

1. Scope

Taura syndrome (TS) is a viral disease of penaeid shrimp caused by infection with Taura syndrome virus (TSV) (Bonami *et al.,* 1997; Fauquet *et al.,* 2005; Lightner 1996a; Mari *et al.,* 1998).

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent, agent strains

The aetiological agent is TSV, as described by Bonami *et al.* (1997) and Mari *et al.* (1998; 2002). At least four genotypes (strains) have been documented based on the gene sequence encoding VP1 ~~(= CP2),~~ the largest and presumably dominant of the three major structural proteins of the virus. Based on VP1 ~~(= CP2)~~ sequence variations, these genotypic groups are: 1) the Americas group; 2) the South-East Asian group; 3) the Belize group; and 4) the Venezuelan group (Chang *et al.,* 2004; Erickson *et al.,* 2002; 2005; Nielsen *et al.,* 2005; Tang & Lightner, 2005; Wertheim *et al.,* 2009).

At least two distinct antigenic variants of TSV have been identified by their differential reactivity to monoclonal antibody MAb 1A1, produced to a reference isolate from the Americas (TSV USA-HI94 – GenBank AF277675) (Mari *et al.,* 2002; Poulos *et al.,* 1999): Type A represents those that react ~~to~~ with MAb 1A1 (in the enzyme-linked immunosorbent assay [ELISA], Western blots and ~~*in-situ* hybridisation [ISH]~~ immunohistochemistry (IHC) with infected tissues) and those that do not. The MAB 1A1 non-reactors were subdivided into Types B (TSV 98 Sinaloa, Mexico) and Type C (TSV 02 Belize), based on host species and virulence. All TSV isolates of the Americas and most, if not all, South-East Asian genotypes react with MAb 1A1. In marked contrast, none of the Belize genotype group reacts with MAb 1A1 (Erickson *et al.,* 2002; 2005), nor does a TSV isolate from the 2005 epizootic in Venezuelan shrimp farms.

TSV particles are 32 nm in diameter, non-enveloped icosahedrons and have a buoyant density of 1.338 g ml^{-1}. The genome of TSV consists of a linear, positive-sense single-stranded RNA 10,205 nucleotides in length, excluding the 3' poly-A tail, and it contains two large open reading frames (ORFs). ORF 1 contains the sequence motifs for nonstructural proteins, such as helicase, protease and RNA-dependent RNA polymerase. ORF 2 contains the sequences for TSV structural proteins, including the three major capsid proteins VP1, VP2 and VP3 (55, 40, and 24 kDa, respectively). The virus replicates in the cytoplasm of host cells (Bonami *et al.,* 1997; Mari *et al.,* 1998; 2002; Robles-Sikisaka *et al.,* 2001).

TSV has been assigned to the genus Aparavirus in the Family Dicistroviridae in the 9th report of the International Committee on Taxonomy of Viruses (ICTV; King *et al.,* 2012).

Other reported causes of TS: Taura syndrome in Ecuador was initially linked to fungicide contamination of shrimp farms, a contention that was supported by litigation for ~ 16 years after the disease was scientifically shown to have a viral aetiology (Bonami *et al.,* 1997; Hasson *et al.,* 1995; Lightner, 2005). Hence, several papers in the literature propose a toxic aetiology for TS (Intriago *et al.,* 1997; Jimenez, 1992; Jimenez *et al.,* 2000).

2.1.2. Survival outside the host

No information available.

2.1.3. Stability of the agent (effective inactivation methods)

No information available.

2.1.4. Life cycle

Not applicable.

2.2. Host factors

2.2.1. Susceptible host species

The principal host species for TSV are the Pacific white shrimp, *Penaeus vannamei*, and the Pacific blue shrimp, *P. stylirostris*. While the principal host species for TSV all belong to the penaeid subgenus *Litopenaeus*, other penaeid species can be infected with TSV by direct challenge, although disease signs do not develop. Documented natural and experimental hosts for TSV include: *P. setiferus, P. schmitti, P. monodon, P. chinensis, P. japonicus, P. aztecus, P. duorarum, P. indicus* and *Metapenaeus ensis* (Bondad-Reantaso *et al.,* 2001; Brock, 1997; Brock *et al.,* 1997; Chang *et al.,* 2004; Lightner, 1996a, 1996b; Overstreet *et al.,* 1997; Srisuvan *et al.,* 2005; Stentiford *et al.,* 2009; Wertheim *et al.,* 2009).

2.2.2. Susceptible stages of the host

TSV has been documented in all life stages (i.e. PL, juveniles and adults) of *P. vannamei* (the most economically significant of the two principal host species) except in eggs, zygotes and larvae (Lightner, 1996a).

2.2.3. Species or subpopulation predilection (probability of detection)

No data.

2.2.4. Target organs and infected tissue

TSV infects and has been shown to replicate (using ISH with specific DNA probes) principally in the cuticular epithelium (or hypodermis) of the general exoskeleton, foregut, hindgut, gills and appendages, and often in the connective tissues, the haematopoietic tissues, the lymphoid organ (LO), and antennal gland. The enteric organs (endoderm-derived hepatopancreas, midgut and midgut caeca mucosal epithelia) and smooth, cardiac, striated muscle, and the ventral nerve cord, its branches and its ganglia typically show no histological signs of infection by TSV and are usually negative for TSV by ISH (Bondad-Reantaso *et al.,* 2001; Hasson *et al.,* 1997; 1999a; 1999b; Jimenez *et al.,* 2000; Lightner, 1996a; Lightner & Redman 1998a; 1998b; Lightner *et al.,* 1995; Srisuvan *et al.,* 2005).

2.2.5. Persistent infection with lifelong carriers

Some members of populations of *P. vannamei* or *P. stylirostris* that survive TSV infections or epizootics may carry the virus for life (Hasson *et al.,* 1999a; 1999b) and, although not documented, are assumed to pass the virus to their progeny by vertical transmission.

2.2.6. Vectors

Sea birds: TSV has been demonstrated to remain infectious for up to 48 hours (after ingestion of TSV-infected shrimp carcasses) in the faeces passed by wild or captive sea gulls (*Larus atricilla*) and chickens (*Gallus domesticus,* used as a laboratory surrogate for all shrimp-eating birds) thus suggesting that the virus can retain infectivity when passed through the gastro-intestinal system of any bird species. These findings implicate birds as being an important mechanical vector for the transmission of the virus within affected farms or farming regions (Garza *et al.,* 1997; Vanpatten *et al.,* 2004).

Aquatic insects: the water boatman (*Trichocorixa reticulata* [*Corixidae*], an aquatic insect that feeds on shrimp carcasses in shrimp farm ponds), has also been shown to serve as a mechanical vector of TSV (Brock 1997; Lightner, 1995, 1996a, 1996b).

Frozen TSV-infected commodity products: TSV has been found in frozen commodity shrimp (*P. vannamei*) products in samples from markets in the USA that originated in Latin America and South-East Asia. Improper disposal of wastes (liquid and solid, i.e. peeled shells, heads, intestinal tracts, etc.) from value-added reprocessing of TSV-infected shrimp at coastal locations may provide a source of TSV that may contaminate wild or farmed stocks near the point of the waste stream discharge (Lightner, 1996b; Nunan *et al.,* 2004).

2.2.7. Known or suspected wild aquatic animal carriers

No data.

2.3. Disease pattern

TS is best known as a disease of nursery- or grow-out-phase *P. vannamei* that occurs within ~14–40 days of stocking PLs into grow-out ponds or tanks, hence, shrimp with TS are typically small juveniles of from ~0.05 g to <5 g. Larger shrimp may also be affected, especially if they are not exposed to the virus until they are larger juveniles or adults (Brock, 1997; Brock *et al.,* 1995; Lightner, 1996a, 1996b; Lotz, 1997).

2.3.1. Transmission mechanisms

Transmission of TSV can be by horizontal or vertical routes. Horizontal transmission by cannibalism or by contaminated water has been demonstrated (Brock, 1997; Hasson *et al.,* 1995; Lightner, 1996a, 1996b; White *et al.,* 2002). Vertical transmission from infected adult broodstock to their offspring is strongly suspected but has not been experimentally confirmed.

2.3.2. Prevalence

In regions where the virus is enzootic in farmed stocks, the prevalence of TSV has been found in various surveys to range from 0 to 100% (Brock, 1997; Jimenez *et al.,* 2000; Laramore, 1997).

2.3.3. Geographical distribution

TS is now widely distributed in the shrimp-farming regions of the Americas, South-East Asia and the Middle East (Bondad-Reantaso *et al.,* 2001; Brock, 1997; Chang *et al.,* 2004; Hasson *et al.,* 1999a; Lightner, 1996a, 1996b; Lightner *et al.,* 2012; Lotz *et al.,* 2005; Nielsen *et al.,* 2005; Tang & Lightner, 2005; Tu *et al.,* 1999; Wertheim *et al.,* 2009; Yu & Song, 2000).

The Americas: following its recognition in 1992 as a distinct disease of cultured *P. vannamei* in Ecuador (Brock *et al.,* 1995; Jimenez, 1992; Lightner *et al.,* 1995), TS spread rapidly throughout many of the shrimp-farming regions of the Americas through shipments of infected PL and broodstock (Brock, 1997; Brock *et al.,* 1997; Hasson *et al.,* 1999a; Lightner, 1996a, 1996b; Lightner *et al.,* 2012). Within the Americas, TS and/or TSV have been reported from virtually every penaeid shrimp-growing country in the Americas and Hawaii (Aguirre Guzman & Ascencio Valle, 2000; Brock, 1997; Lightner, 2011; Lightner *et al.,* 2012; Robles-Sikisaka *et al.,* 2001). TSV is enzootic in cultured penaeid shrimp stocks on the Pacific coast of the Americas from Peru to Mexico, and it has been occasionally found in some wild stocks of *P. vannamei* from the same region (Lightner & Redman, 1998a; Lightner *et al.,* 1995). TSV has also been reported in farmed penaeid stocks from the Atlantic, Caribbean, and Gulf of Mexico coasts of the Americas, but it has not been reported in wild stocks from the these regions (Hasson *et al.,* 1999a; Lightner, 1996a; 2005; 2011; Lightner *et al.,* 2012).

Asia and the Middle East: TSV was introduced into Chinese Taipei in 1999 with infected imported Pacific white shrimp, *P. vannamei*, from Central and South American sources (Tu *et al.,* 1999; Yu & Song, 2000). Since that original introduction, the virus has spread with movements of broodstock and PL to China (People's Rep. of), Thailand, Malaysia, and Indonesia where it has been the cause of major epizootics with high mortality rates in introduced unselected stocks of *P. vannamei* (Chang *et al.,* 2004; Lightner, 2011; Nielsen *et al.,* 2005; Tang & Lightner, 2005). Recently, TSV has also been associated with significant mortalities in *P. indicus* being farmed in Saudi Arabia (Wertheim *et al.,* 2009).

2.3.4. Mortality and morbidity

In on-farm epizootics of TS involving unselected (i.e. not selected for TSV resistance) stocks of *P. vannamei*, the principal host species for TSV, typical cumulative mortalities range from 40 to >90% in cultured populations of PL, juvenile, and subadult life stages. TSV-resistant lines of *P. vannamei* are available which show survival rates of up to 100% in laboratory challenge with all four TSV genotypes (Lightner *et al.,* 2009; Moss *et al.,* 2001).

2.3.5. Environmental factors

Outbreaks of TS are more frequent when salinities are below 30 ppt (Jimenez *et al.,* 2000).

Annex 19 (contd)

2.4. Control and prevention

2.4.1. Vaccination

No effective vaccines for TSV are available.

2.4.2. Chemotherapy

No scientifically confirmed reports of effective chemotherapy treatments.

2.4.3. Immunostimulation

No scientifically confirmed reports of effective immunostimulation treatments.

2.4.4. Resistance breeding

After TS emerged in Ecuador in 1992–1994, *P stylirostris* were found that possessed resistance to TSV (genotype 1, MAb 1A1 Type A). Following from this discovery and due to TSV reaching Mexico in 1994 where it caused crop failures of *P. vannamei,* selected lines of TSV-resistant *P. stylirostris* became the dominant shrimp farmed in western Mexico from 1995. However, in 1998–1999, a new 'strain' of TSV (Type B; Erickson *et al.,* 2002; Fegan & Clifford, 2001; Lightner, 1999; 2005; Zarin-Herzberg & Ascencio, 2001) emerged and caused massive epizootics in *P. stylirostris*. The emergence of this new 'strain' of TSV was soon followed in late 1999 by the introduction of white spot syndrome virus (WSSV) into shrimp farms in western Mexico, to which *P. stylirostris* had no resistance, effectively ending any interest in the culture of *P. stylirostris* in Mexico.

TSV-resistant domesticated stocks of *P. vannamei* and *P. stylirostris* have been developed. Some domesticated lines of TSV-resistant *P. vannamei* (that are also TSV-free) are in widespread use by the shrimp-farming industries of the Americas and South-East Asia (Clifford, 1998; Moss *et al.,* 2001; White *et al.,* 2002). After the appearance of TS in Central America, improved TS resistance was reported in wild caught *P. vannamei* PLs used to stock shrimp farms in the region (Laramore, 1997).

2.4.5. Restocking with resistant species

Selected lines of TS resistant *P. vannamei* have been developed and are commercially available (Clifford, 1998; Laramore, 1997; Moss *et al.,* 2001; White *et al.,* 2002).

2.4.6. Blocking agents

Resistance to TSV infection was reported by expression of the TSV coat protein antisense RNA in *P. vannamei* zygotes. Transgenic juveniles reared from zygotes protected in this manner showed improved resistance to TSV challenge by *per os* or intramuscular (IM) injection routes (Lu & Sun, 2005). Similar results have been produced by injection of short random double-stranded RNAi sequences into juvenile *P. vannamei* (Robalino *et al.,* 2004).

2.4.7. Disinfection of eggs and larvae

~~While~~ It is possible TSV might ~~is believed to~~ be transmitted vertically (transovarian transmission), despite ~~there have been~~ no published report documenting this route of transmission. Disinfection of eggs and larvae (Chen *et al.,* 1992) is good management practice and it is recommended for its potential to reduce TSV contamination of spawned eggs and larvae produced from them.

2.4.8. General husbandry practices

Some husbandry and disease control and management practices have been used ~~applied~~ successfully to reduce the risks TSV infections and disease occurring during farm grow-out. These include the application of polymerase chain reaction (PCR) prescreening of wild or pond-reared broodstock and/or their spawned eggs/nauplii and discarding those that test positive for the virus (Fegan & Clifford, 2001), fallowing and restocking of entire culture regions with TSV-free stocks (Dixon & Dorado, 1997), and the development of specific pathogen free (SPF) shrimp stocks of *P. vannamei* and *P. stylirostris* (Lightner, 1996b; 2005; Lotz *et al.,* 1995; Moss *et al.,* 2001; Pruder *et al.,* 1995; Wyban 1992; Wyban *et al.,* 2004). The adoption of the latter technology (SPF stocks) has proven to be among the most successful husbandry practice for the prevention and control of TS. Unfortunately, there is a misconception in the industry that SPF is a genetic trait rather than a condition of health status. The development of SPF *P. vannamei* that were free not only of TSV, but also of all the major known pathogens of penaeid shrimp, has resulted in the introduction of the species to Asia and to its surpassing *P. monodon* in 2005 as the dominant farmed shrimp species in Asia, as well as the Americas where the SPF stocks were developed (FAO, 2006; Lightner, 2005; Rosenberry, 2004).

3. Sampling

3.1. Selection of individual specimens

Suitable specimens for testing for infection by TSV include PL, juveniles and adults. While TSV may infect all life stages, infection severity, and hence virus load, may be below detection limits in spawned eggs and in the larval stages, so these life stages may not be suitable samples for TSV detection or certification of TS disease freedom.

3.2. Preservation of samples for submission

For routine histology or molecular assays, and guidance on preservation of samples for the intended test method see Chapter 2.2.0.

3.3. Pooling of samples

Samples taken for molecular tests may be combined as pooled samples representing no more than five specimens per pooled sample of juveniles, subadults and adults. However, for eggs, larvae and PL pooling of larger numbers (e.g. ~150 or more eggs or larvae or 50–150 PL depending on their size/age) may be necessary to obtain sufficient sample material (extracted nucleic acid) to run a diagnostic assay. See also Chapter 2.2.0.

3.4. Best organs and tissues

TSV infects tissues of ectodermal and mesodermal origin. The principal target tissue in the acute phase of TS is the cuticular epithelium. In chronic infections the LO is the principal target tissue.

Haemolymph or excised pleopods may be collected and used when non-lethal testing of valuable broodstock is necessary.

3.5. Samples/tissues that are not suitable

TSV is a systemic virus, and it does not replicate in enteric tissues (e.g. the hepatopancreas, the midgut, or its caeca). Hence, enteric tissues are inappropriate samples for detection of infection by TSV.

4. Diagnostic methods

4.1. Field diagnostic methods

4.1.1. Clinical signs

Only acute-phase TS disease can be presumptively diagnosed from clinical signs. See Section 4.2 for a description of gross clinical signs presented by shrimp with acute-phase TS disease.

4.1.2. Behavioural changes

Only shrimp with acute-phase TS disease present behavioural changes. Typically, severely affected shrimp apparently become hypoxic and move to the pond edges or pond surface where dissolved oxygen levels are higher. Such shrimp may attract seabirds in large numbers. In many TS disease outbreaks, it is the large numbers of seabirds attracted to the moribund shrimp that first indicate the presence of a serious disease outbreak (which is often either TS or WSD when sea birds are observed) to the farm manager.

4.2. Clinical methods

4.2.1. Gross pathology

TS disease has three distinct phases, acute, transition, and chronic, which are grossly distinguishable (Hasson *et al.,* 1999a; 1999b; Lightner, 1996a; 1996b; 2011; Lightner *et al.,* 1995). Gross signs presented by juvenile, subadult and adult shrimp in the transition phase of TS are unique and provide a presumptive diagnosis of the disease.

Acute phase: gross signs displayed by moribund *P. vannamei* with acute-phase TS include expansion of the red chromatophores giving the affected shrimp a general, overall pale reddish coloration and making the tail fan and pleopods distinctly red; hence 'red tail' disease was one of the names given by farmers when the disease first appeared in Ecuador (Lightner *et al.,* 1995). In such shrimp, close inspection of the cuticular epithelium in thin appendages (such as the edges of the uropods or pleopods) with a ×10 hand lens reveals signs of focal epithelial necrosis. Shrimp showing these gross signs of acute TS typically have soft shells, an empty gut and are often in the late D stages of the moult cycle. Acutely affected shrimp usually die during ecdysis. If the affected shrimp are larger than ~1 g, moribund shrimp may be visible to sea birds at the pond edges and surface. Thus, during the peak of severe epizootics, hundreds of sea birds (gulls, terns, herons, cormorants, etc.) may be observed feeding on affected moribund shrimp that accumulate at the surface of the affected pond surface and edges (Brock, 1997; Brock *et al.,* 1995; 1997; Garza *et al.,* 1997; Lightner, 1996a; 1996b; 2011; Lightner *et al.,* 1995; Vanpatten *et al.,* 2004).

Transition (*recovery*) *phase:* although only present for a few days during TS epizootics, the gross signs presented by shrimp in the transition phase can provide a tentative diagnosis of TSV infection. During the transition phase (which may be occurring while many shrimp in the affected populations are still in the acute phase and daily mortalities are high), fair to moderate numbers of shrimp in affected ponds show random, multifocal, irregularly shaped melanised cuticular lesions. These melanised spots are haemocyte accumulations indicating the sites resolving TS lesions in the cuticular epithelium. Such shrimp may or may not have soft cuticles and red-chromatophore expansion, and may be behaving and feeding normally (Brock, 1997; Hasson *et al.,* 1999b; Lightner, 1996a; 2011).

Chronic phase: after successfully moulting, shrimp in the transition phase move into the chronic phase of TS in which persistently infected shrimp show no obvious signs of disease (Brock, 1997; Hasson *et al.,* 1999b; Lightner, 1996a; 1996b; 2011; Lightner *et al.,* 1995). However, *P. vannamei* that are chronically infected with TSV may be less resistant to normal environmental stressors (i.e. sudden salinity reductions) than uninfected shrimp (Lotz *et al.,* 1995).

4.2.2. Clinical chemistry

Not applicable.

4.2.3. Microscopic pathology (for penaeid hosts)

TS disease in the acute and chronic phases can be diagnosed most reliably using histological methods (Hasson *et al.,* 1999b; Lightner, 1996a). Pathognomonic TSV-induced pathology is unique in acute-phase infections (Brock *et al.,* 1995; Lightner, 1996a; 2011). In chronic TSV infections, the only lesion typically presented by infected shrimp is the presence of an enlarged LO with multiple LO spheroids (LOS) (Hasson *et al.,* 1999b; Lightner 2011), which cannot be distinguished from LOS induced by chronic infections of other RNA viruses (Lightner, 1996a). When LOS are observed by routine histology and chronic TSV infection is suspected, a molecular test (ISH with TSV-specific probes, or reverse-transcription [RT] PCR [see Section 4.3.1.2.7]) is recommended for confirmation of TSV infection.

4.2.3.1. Acute phase of Taura syndrome

Diagnosis of TS in the acute phase of the disease is dependent on the histological demonstration (in haematoxylin and eosin [H&E] stained preparations) of multifocal areas of necrosis in the cuticular epithelium of the general body surface, appendages, gills, hindgut, and foregut (the oesophagus, anterior and posterior chambers of the stomach). Cells of the subcuticular connective tissues and adjacent striated muscle fibres basal to affected cuticular epithelium are occasionally affected. In some severe cases of acute-phase TS, the antennal gland tubule epithelium is also destroyed. Prominent in the multifocal cuticular lesions are conspicuous foci of affected cells that display an increased eosinophilia of the cytoplasm and pyknotic or karyorrhectic nuclei. Cytoplasmic remnants of necrotic cells are often extremely abundant in these TS acute-phase lesions and these are generally presented as spherical bodies (1–20 µm in diameter) that range in staining from eosinophilic to pale basophilic. These structures, along with pyknotic and karyorrhectic nuclei, give acute-phase TS lesions a characteristic 'peppered' or 'buckshot-riddled' appearance, which is considered to be pathognomonic for TS disease when there is no concurrent necrosis of the parenchymal cells of the LO tubules. The absence of necrosis of the LO in acute-phase TSV infections distinguishes TS disease from acute-phase yellowhead disease in which similar patterns of necrosis to those induced by TSV may occur in the cuticular epithelium and gills (Lightner, 1996a).

In TSV-infected tissues, pyknotic or karyorrhectic nuclei give a positive (for DNA) Feulgen reaction, which distinguishes them from the less basophilic to eosinophilic cytoplasmic inclusions that do not contain DNA. The absence of haemocytic infiltration or other signs of a significant host-inflammatory response distinguishes the acute phase of TS from the transitional phase of the disease (Bondad-Reantaso *et al.,* 2001; Brock, 1997; Brock *et al.,* 1995; 1997; Erickson *et al.,* 2002; 2005; Hasson *et al.,* 1995; 1999a; 1999b; Lightner, 1996a; Lightner *et al.,* 1995).

4.2.3.2. Transition (recovery) phase of Taura syndrome

In the transitional phase of TS, typical acute-phase cuticular lesions decline in abundance and severity and are replaced by conspicuous infiltration and accumulation of haemocytes at the sites of necrosis. The masses of haemocytes may become melanised giving rise to the irregular black spots that characterise the transition phase of the disease. In H&E sections, such lesions may show erosion of the cuticle, surface colonisation and invasion of the affected cuticle and exposed surface haemocytes by *Vibrio* spp. (Hasson *et al.,* 1999b; Lightner, 1996a; 2011). Sections of the LO during the transition phase of TS may appear normal with H&E staining. However, when sections of the LO are assayed for TSV by ISH with a specific cDNA probe (or by ISH with MAb 1A1 for TSV type A, genotype 1), large quantities of TSV are shown accumulating in the more peripheral parenchymal cells of the LO tubules (Hasson *et al.,* 1999b; Srisuvan *et al.,* 2005).

4.2.3.3. Chronic phase of Taura syndrome

Shrimp in the chronic phase of TS display no gross signs of infection, and histologically the only sign of infection is the presence of numerous prominent LOS, which may remain associated with the main body of the paired LO, or which may detach and become ectopic LOS bodies that lodge in constricted areas of the haemocoel (i.e. the heart, gills, in the subcuticular connective tissues, etc.). Such LOS are spherical accumulations of LO cells and haemocytes and may be distinguished from normal LO tissues by their spherical nature and the lack of the central vessel that is typical of normal LO tubules. When assayed by ISH with a cDNA probe for TSV (or with MAb 1A1 using ISH) some cells in the LOS give positive reactions to the virus, while no other target tissues react (Hasson *et al.,* 1999b; Lightner, 1996a; 1996b; 2011).

4.2.4. Wet mounts

Direct microscopy of simple unstained wet mounts from excised pieces of the gills, appendage tips, etc., examined by phase- or reduced-light microscopy may be used to demonstrate (and make a tentative diagnosis of acute-phase TS) focal lesions of acute-phase TS in cuticular epithelial cells. Preparations presenting TS acute-phase lesions will contain numerous spherical structures (see the histopathological methods in Section 4.2.3 above), which are pyknotic and karyorrhectic nuclei and cytoplasmic remnants of necrotic cells.

4.2.5. Smears

Not applicable.

4.2.6. Fixed sections

See Section 4.2.3.

4.2.7. Electron microscopy/cytopathology

Not currently applicable for diagnostic purposes.

4.3. Agent detection and identification methods

4.3.1. Direct detection methods

4.3.1.1. Microscopic methods

4.3.1.1.1. Wet mounts

See Section 4.2.4.

4.3.1.1.2. Smears

See Section 4.2.5.

4.3.1.1.3. Fixed sections

See Section 4.2.3.

4.3.1.2. Agent isolation and identification

4.3.1.2.1. Cell culture/artificial media

TSV has not been grown *in vitro*, as no crustacean cell lines exist (Lightner, 1996a; Pantoja *et al.,* 2004). Despite a publication that incorrectly reported that TSV infected human and monkey cell lines (Audelo del Valle *et al.,* 2003), two other laboratories repeated the study and both found that TSV does not infect or replicate in primate or human cell lines with known susceptibility to human picornaviruses (Luo *et al.,* 2004; Pantoja *et al.,* 2004).

4.3.1.2.2. Antibody-based antigen detection methods

An MAb for detection of TSV may be used to assay samples of haemolymph, tissue homogenates, or Davidson's AFA-fixed tissue sections from shrimp (Erickson *et al.,* 2002; 2005; Poulos *et al.,* 1999). TSV MAb 1A1 may be used to distinguish some variants or 'strains' of TSV from other strains (Erickson *et al.,* 2002; 2005).

4.3.1.2.3. Bioassay method

Confirmation of TSV infection may be accomplished by bioassay of TSV-suspect animals with SPF juvenile *P. vannamei* serving as the indicator of the virus (Brock *et al.,* 1997; Garza *et al.,* 1997; Hasson *et al.,* 1999b; 1995; Lightner, 1996a; Lotz, 1997; Overstreet *et al.,* 1997). Oral or injection protocols may be used. The oral method is relatively simple to perform and is accomplished by feeding chopped carcasses of suspect shrimp to SPF juvenile *P. vannamei* in small tanks (White *et al.,* 2002). The use of a negative control tank of indicator shrimp, which receive only SPF (TSV-free) tissue and normal shrimp feed is required. When the carcass feeding (*per os*) protocol is used to bioassay for TSV, TS-positive indicator shrimp (by gross signs and histopathology) are typically apparent within 3–4 days of initial exposure, and significant mortalities occur by 3–8 days after initial exposure. The negative control shrimp must remain negative (for at least 10–15 days) for gross or histological signs of TS disease and unusual mortalities (Hasson *et al.,* 1999b; Lightner, 1996a; White *et al.,* 2002).

With the injection bioassay protocol, a variety of sample types may be tested for TSV. Whole shrimp are used if they were collected during a TSV epizootic. Heads only should be used if shrimp display gross transition-phase lesions (multifocal melanised spots on the cuticle) or no clinical signs of infection (chronic phase) as the virus, if present, will be concentrated in the LO (Hasson *et al.,* 1999b; Lightner, 1996a). For non-lethal testing of broodstock, haemolymph samples may be taken and used to expose the indicator shrimp by IM injection (Lightner, 1996a).

To perform the IM (injection) bioassay for TSV:

Note that tissues and the resulting homogenate should be kept cool during the entire protocol by maintaining on ice.

i) Prepare a 1:2 or 1:3 ratio of TSV-suspect shrimp heads or whole shrimp with TN buffer (see Chapter 2.2.2, infectious hypodermal and haematopoietic necrosis [IHHN], for the composition of this buffer) or sterile 2% saline prepared with distilled water.

ii) Homogenise the mixture using a tissue grinder or blender. Do not permit the mixture to heat up by excessive homogenisation or grinding.

iii) Clarify the homogenate by centrifugation at 3000 ***g*** for 10 minutes. Decant and save the supernatant fluid. Discard the pellet.

iv) Centrifuge the supernatant fluid at 27,000 ***g*** for 20–30 minutes at 4°C. Decant and save the supernatant fluid. Discard the pellet.

v) Dilute the supernatant fluid from step iv to 1/10 to 1/100 with sterile 2% saline. This solution may now be used as the inoculum to inject indicator shrimp (or filter sterilised as described in step vi).

vi) Filter the diluted supernatant fluid from step v using a sterile syringe (size depends on the final volume of diluted supernatant) and a sterile 0.45 µm syringe filter. Multiple filters may have to be used as they clog easily. Filtrate should be collected in a sterile test tube or beaker. The solution can now be stored frozen (recommend –20°C for short-term [weeks] storage and –80°C for long-term [months to years] storage) or used immediately to inject indicator shrimp.

vii) Indicator shrimp should be from TSV-susceptible stocks of SPF *P. vannamei* (such as the 'Kona stock') (Moss *et al.,* 2001), which are commercially available from a number of sources in the Americas, and not from selected lines of known TSV-resistant stocks.

viii) Inject 0.01 ml per gram of body weight using a 1 ml tuberculin syringe. Indicator shrimp should be injected intramuscularly into the third tail segment. If the test shrimp begin to die within minutes post-injection, the inoculum contains excessive amounts of proteinaceous material and should be further diluted prior to injecting additional indicator shrimp. Sudden death occurring post-injection is referred to as 'protein shock', and is the result of systemic clotting of the shrimp's haemolymph in response to the inoculum (Lightner, 1996a; White *et al.,* 2002).

ix) Haemolymph samples may be diluted (1/10 or 1/20 in TN buffer), filter sterilised (if necessary), and injected into the indicator shrimp without further preparation.

x) If TSV was present in the inoculum, the indicator shrimp should begin to die within 24–48 hours post-injection. Lower doses of virus may take longer to establish a lethal infection and shrimp should be monitored for at least 10–15 days post-injection.

xi) The presence (or absence) of TSV in the indicator shrimp should be confirmed by histological analysis (and/or ISH by gene probe, if available) of Davidson's fixed moribund shrimp. If additional confirmation is needed beyond demonstration of pathognomonic TSV lesions, RT-PCR with sequencing of the resulting amplicon can be carried out.

4.3.1.2.4. Sentinel shrimp bioassay method

As a variation to the bioassay technique, a 'sentinel shrimp' system may be used. For example, TSV-sensitive stocks of small juvenile SPF *P. vannamei* may be held in net-pens in tanks, or in the same water system, with other shrimp of unknown TSV status to bioassay for the presence of infectious agents such as TSV.

4.3.1.2.5. Dot-blot immunoassay method

i) For the dot-blot immunoassay method, 1 µl of test antigen (purified virus, infected shrimp haemolymph or SPF shrimp haemolymph) is dotted on to the surface of MA-HA-N45 assay plates (Millipore, South San Francisco, California [CA], USA)[3].

ii) After air drying, the wells are blocked for 1 hour at room temperature with 200 µl of a buffer containing phosphate-buffered saline and 0.05% Tween 20 (PBST) mixed with 10% normal goat serum (Life Technologies, Gibco BRL) and 2% Hammersten casein (Amersham Life Sciences, Arlington Heights, Illinois, USA).

iii) The wells are washed three times with PBST and then reacted with 100 µl primary antibody (MAb or mouse polyclonal antibodies) for 30 minutes at room temperature.

iv) Alkaline-phosphatase-labelled goat anti-mouse IgG, γ chain specific, secondary antibody (Zymed, South San Francisco, CA) diluted 1/1000 in PBST plus 10% normal goat serum is used for detection (30 minutes at room temperature).

v) After washing three times with PBST, once with PBS and once with distilled water, the reactions are visualised by development for 15 minutes at room temperature with nitroblue tetrazolium and bromo-chloro-indoyl phosphate (Roche Diagnostics, Corp.) in Tris-NaCl (100 mM each) buffer containing 50 mM $MgCl_2$, pH 9.5.

vi) Reactions are stopped with distilled water.

[3] 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*.

vii) The reactions are graded using a scale from 0 to +4, with the highest intensity reaction being equivalent to the reaction generated using the MAb against the reference control consisting of semi-purified TSV. A negative reaction is one in which no coloured spot is visible in the well.

4.3.1.2.6. Other antibody-based methods

The TSV MAb 1A1 may be applicable to other antibody-based test formats (i.e. indirect fluorescent antibody [IFAT] or immunohistochemistry [IHC] tests with tissue smears, frozen sections, or deparaffinised fixed tissues). MAb 1A1 is applicable for use in an IHC format using Davidson's AFA-fixed tissue sections (Erickson *et al.,* 2002; 2005).

It is recommended that unexpected results from MAb-based tests for TSV should be interpreted in the context of clinical signs, case history, and in conjunction with other test results (e.g. RT-PCR test results, or findings from histology or ISH with a TSV-specific DNA probe – see appropriate sections in this chapter).

4.3.1.2.7. Molecular techniques

ISH and RT-PCR tests for TSV have been developed, and kits of RT-PCR methods for TSV are commercially available. The dot-blot method for TSV detection is not available.

4.3.1.2.7.1. DNA probes for ISH applications with non-radioactive cDNA probes

Non-radioactive, DIG-labelled cDNA probes for TSV may be produced in the laboratory. The ISH method provides greater diagnostic sensitivity than do more traditional methods for TSV detection and diagnosis that employ classic histological methods (Hasson *et al.,* 1999a; Lightner, 1996a; 1999; Lightner & Redman 1998b; Mari *et al.,* 1998). The ISH assay of routine histological sections of acute- and transition-phase lesions in the cuticular epithelium, other tissues, and of LOS in transition and chronic phase with a specific DIG-labelled cDNA probe to TSV, provides a definitive diagnosis of TSV infection (Hasson *et al.,* 1999a; 1999b; Lightner, 1996a; 1996b). Pathognomonic TSV-positive lesions display prominent blue to blue-black areas in the cytoplasm of affected cells when reacted with the cDNA probes. Not reacting to the probe are the prominent karyorrhectic nuclear fragments and pyknotic nuclei that contribute to the pathognomonic 'buckshot riddled' appearance of TS lesions (Lightner, 1996a; Mari *et al.,* 1998). (See Chapter 2.2.2 IHHN for details of the ISH method, and Chapter 2.2.0 Section B.5.3.ii for detailed information on the use of Davidson's AFA fixative.)

False-negative ISH results may occur with Davidson's fixed tissues if tissues are left in fixative for more than 24–48 hours. The low pH of Davidson's fixative causes acid hydrolysis of the TSV single-stranded RNA genome, resulting in false-negative probe results. This hydrolysis can be avoided through the use of neutral fixatives, including an 'RNA-friendly' fixative developed for shrimp, or by the proper use (avoiding fixation times over 24 hours) of Davidson's fixative (Hasson *et al.,* 1997; Lightner, 1996a; Lightner & Redman 1998).

4.3.1.2.7.2. Reverse-transcription (RT)-PCR method

Tissue samples (haemolymph, pleopods, whole small shrimp, etc.) may be assayed for TSV using RT-PCR. Primers designated as 9992F and 9195R, amplify a 231 base pair (bp) sequence of the TSV genome (Nunan *et al.,* 1998). The fragment amplified is from a conserved sequence located in the intergenic region and ORF 2 of TSV. Primer 9992F is located near the 3' end of intergenic region and 9195R is located on ORF 2 within VP2 (= CP1) (Mari *et al.,* 2002; Nunan *et al.,* 1998). A new pair of TSV primers (7171F and 7511R) has been developed and shown to have an improved sensitivity for TSV detection (Navarro *et al.,* 2009). These replacement primers are 9992F/9195R and they are located within ORF 2.

Primer	Product	Sequence	Temperature	G+C%
9992F	231 bp	5'-AAG-TAG-ACA-GCC-GCG-CTT-3'	69°C	55%
9195R		5'-TCA-ATG-AGA-GCT-TGG-TCC-3'	63°C	50%
7171F	341 bp	5'-CGA-CAG-TTG-GAC-ATC-TAG-TG-3'		50%
7511R		5'-GAG-CTT-CAG-ACT-GCA-ACT-TC-3'		50%

The RT-PCR method outlined below for TSV generally follows the method used in Nunan *et al.* (1998).

i) *Preparation of RNA template:* RNA can be extracted from fresh, frozen and ethanol-preserved tissues. Extraction of RNA should be performed using commercially available RNA tissue extraction kits, such as the High Pure RNA Tissue Kit (Roche, Penzberg, Germany) and following the manufacturer's procedures for production of quality RNA templates.

ii) The RT-PCR assay is carried out in solution, using 10 µl of total RNA extracted from haemolymph, frozen shrimp tissues, ethanol fixed tissue as the template (concentration of RNA = 1–100 ng ml^{-1}).

iii) The following controls should be included in every RT-PCR assay for TSV: a) known TSV-negative tissue sample; b) a known TSV-positive sample (tissue or purified virus); and c) a 'no-template' control.

iv) The GeneAmp® EZ rTth RNA PCR kit (Applied Bioscience, Forster City, CA) was ~~is~~ used for all amplification reactions described here. Alternative kits can be used and adjusted for use for this assay.

v) The optimised RT-PCR conditions (final concentrations in 50 µl total volume) for detection of TSV in shrimp tissue samples are: primers (~~0.46~~ 0.62 µM each), dNTPs (300 µM each), rTth DNA polymerase (2.5 U 50 $µl^{-1}$), manganese acetate (2.5 mM), in 5 × EZ buffer (25 mM Bicine, 57.5 mM potassium acetate, 40% [w/v] glycerol, pH 8.2).

vi) If the thermal cycler does not have a heated lid, then light mineral oil (50 µl) is overlaid on the top of the 50 µl reaction mixtures to prevent condensation or evaporation during thermal cycling.

vii) The RNA template and all the reagents are combined and reverse transcription is allowed to proceed at 60°C for 30 minutes, followed by 94°C for 2 minutes.

Note: The reaction conditions described here were optimised using an automatic Thermal Cycler GeneAmp 980 (Applied Biosystems). The conditions should be optimised for each thermal cycler using known positive controls.

viii) At the completion of reverse transcription, the samples are amplified for 40 cycles under the following conditions: denaturation at 94°C for 45 seconds, and then annealing/extension at 60°C for 45 seconds. A final extension step for 7 minutes at 60°C follows the last cycle and the process is terminated in a 4°C soak file.

ix) Following the termination of RT-PCR, the amplified cDNA solutions are drawn off from beneath the mineral oil and placed into clean 0.5 ml microfuge tubes.

x) A 10 µl sample of the amplified product can then be added to the well of a 2.0% agarose gel, stained with ethidium bromide (0.5 g ml^{-1}), and electrophoresed in 0.5 × TBE (Tris, boric acid, ethylene diamine tetra-acetic acid [EDTA]).

xi) A 1 kb DNA ladder (Invitrogen, Carlsbad, CA) is used as a marker.

xiii) Details of the composition of the reagents and buffers used here may be found in Chapter 2.2.2 IHHN.

4.3.1.2.7.3. Real-time PCR (qPCR) method for TSV

Quantitative RT-PCR methods have been developed for the detection of TSV. These methods have the advantages of speed, specificity and sensitivity. The sensitivity of qRT-PCR is ~100 copies of the target sequence from the TSV genome (Dahr *et al.,* 2002; Tang *et al.,* 2004).

Annex 19 (contd)

The real-time RT-PCR method using TaqMan chemistry described below for TSV generally follows the method used in Tang *et al.* (2004).

i) The PCR primers and TaqMan probe were selected from the ORF1 region of the TSV genomic sequence (GenBank AFAF277675) that encodes for nonstructural proteins. The primers and TaqMan probe were designed by the Primer Express software (Applied Biosystems). The upstream (TSV1004F) and downstream (TSV1075R) primer sequences are: 5'-TTG-GGC-ACC-AAA-CGA-CAT-T-3' and 5'-GGG-AGC-TTA-AAC-TGG-ACA-CAC-TGT-3'), respectively. The TaqMan probe, TSV-P1 (5'-CAG-CAC-TGA-CGC-ACA-ATA-TTC-GAG-CAT-C-3'), which corresponds to the region from nucleotide 1024 to 1051, is synthesised and labelled with fluorescent dyes 5-carboxyfluoroscein (FAM) on the 5' end and N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA) on the 3' end (Applied Biosystems, catalog no. 450025).

ii) *Preparation of RNA template:* the extraction and purification of RNA template from haemolymph, or shrimp tissue, is the same as that described in the section for traditional RT-PCR.

iii) It is necessary to include a 'no template control' in each reaction run. This is to rule out the presence of ~~fluorescence~~ contaminants in the reaction mixture or in the heat block of the thermal cycler. A positive control should also be included, and this can be an *in-vitro* transcribed RNA containing the target sequence, purified virions, or RNA extracted from TSV-infected tissue.

iv) The RT-PCR reaction mixture contains: TaqMan One-step RT-PCR Master Mix (Applied Biosystems, part no. 4309169), 0.3 µM of each primer, 0.1 µM of TaqMan probe, 5–50 ng of RNA, and water in a reaction volume of 25 µl. For optimal results, the reaction mixture should be vortexed and mixed well.

v) Amplification can be ~~is~~ performed with the GeneAmp 5700 Sequence Detection System (Applied osystems; ABI PRISM 7000, 7300, 7500, or newer models or equivalent thermocycler ~~and brands can also be used~~). The cycling consists of reverse transcription at 48°C for 30 minutes and initial denaturation at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds and annealing/extension at 60°C for 1 minute. The levels of fluorescence are measured at the end of each annealing/extension cycle.

vi) At the end of the reaction, real-time fluorescence measurements are analysed ~~will be taken with a built in charge-coupled device (CCD) camera~~. A threshold will be set to be above the baseline that begins to detect the increase in signal associated with an exponential increase in PCR product. Samples will be defined as negative if there is no Ct (threshold cycle) value ~~is~~ after 40 cycles ~~the Ct (threshold cycle) value is 40 cycles~~. Samples with a Ct value lower than 40 cycles are considered to be positive. ~~To confirm the real-time RT-PCR results, an aliquot of RT-PCR product can be subjected to electrophoresis on a 4% ethidium bromide agarose gel and exposed to UV light. A 72-bp DNA fragment can be visualised in the samples that are positive for TSV.~~

~~vi) It is necessary to include a 'no template control' in each reaction run. This is to rule out the presence of contaminants in the reaction mixture or in the heat block of the thermal cycler. A positive control should also be included, and this can be an *in-vitro* transcribed RNA containing the target sequence, purified virions, or RNA extracted from TSV-infected tissue.~~

4.3.1.2.7.4. Sequencing

RT-PCR products may be cloned and sequenced when necessary to confirm infection by TSV or to identify false positives or nonspecific amplification (Mari *et al.,* 2002; Nielsen *et al.,* 2005; Srisuvan *et al.,* 2005; Tang & Lightner, 2005; Wertheim *et al.,* 2009).

4.3.1.2.8. Agent purification

Methods for TSV isolation and purification are available (Bonami *et al.,* 1997; Hasson *et al.,* 1995; Mari *et al.,* 2002; Poulos *et al.,* 1999), but these are not recommended for routine diagnosis of TS.

4.3.2. Serological methods

Not applicable because shrimp are invertebrate animals which do not produce specific antibodies that could be used to demonstrate infection by or prior exposure to TSV.

5. Rating of tests against purpose of use

The methods currently available for surveillance, detection, and diagnosis of TSV 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 and/or not available 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. TSV surveillance, detection and diagnostic methods in penaeids

Method	Surveillance				Presumptive diagnosis	Confirmatory diagnosis
	Larvae	PLs	Juveniles	Adults		
Gross signs	d	d	c	c	b	c
Bioassay	d	d	d	d	c	b
Direct LM	d	d	c	d	c	d
Histopathology	d	b	b	c	a	a
Transmission EM	d	d	d	d	c	c
Antibody-based assays	d	d	c	c	b	b
DNA probes – *in situ*	d	c	b	b	a	a
RT-PCR, qRT-PCR	a	a	a	a	a	a
Sequence	d	d	d	d	d	a

PLs = postlarvae; LM = light microscopy; EM = electron microscopy;
RT-PCR = reverse-transcriptase polymerase chain reaction.

6. Test(s) recommended for targeted surveillance to declare freedom from Taura syndrome

As indicated in Table 5.1, RT-PCR (Section 4.3.1.2.7.2) is the recommended method for targeted surveillance for reasons of availability, utility, and diagnostic specificity and sensitivity.

When investigating acute mortality episodes as part of a targeted surveillance programme, demonstration of pathognomonic TSV-induced lesions in the cuticular epithelium by histology (with or without confirmation by ISH with TSV-specific DNA probes) is a suitable method (Table 5.1).

7. Corroborative diagnostic criteria

7.1. Definition of suspect case

A suspect case is represented by:

- Sudden high mortalities in late PL, juvenile or subadult *P. vannamei* or *P. stylirostris* in regions where TSV is enzootic;

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- The sudden presence of numerous sea birds (gulls, cormorants, herons, terns, etc.) 'fishing' in one or more shrimp culture ponds;
- Samples of cultured *P. vannamei* or *P. stylirostris* from ponds with feeding sea birds that present gross signs indicative of acute- or transition-phase TS, such as a general reddish colouration, lethargy, soft shells, empty guts, and the presence of numerous irregular black spots on the cuticle; or
- Demonstration of foci of necrosis in the cuticular epithelium using low magnification (i.e. a ×10 hand lens or by direct microscopic examination of wet mounts) to examine the edges of appendages such as uropods or pleopods, or the gills.

7.2. Definition of confirmed case

Any combination of a molecular (PCR or ISH) test and a morphological (histology) test using at least two of the following three methods (with positive results):

- Histological demonstration of diagnostic acute-phase TSV lesions in (especially) the cuticular epithelia of the foregut (oesophagus, anterior, or posterior chambers of the stomach) and/or in the gills, appendages, or general cuticle. Such TSV lesions are pathognomonic for TSV only when they occur without accompanying severe acute necrosis (with nuclear pyknosis and karyorrhexis) of the parenchymal cells of the lymphoid organ tubules (which may occur in acute-phase yellowhead virus infections).
- ISH-positive (with a TSV-specific cDNA probe) signal to TSV-type lesions in histological sections (i.e. cuticular acute-phase TS lesions) or to distinctive lymphoid organ spheroids (LOS) in the lymphoid organs of shrimp with chronic phase TS lesions.
- RT-PCR positive results for TSV.
- Sequencing of PCR product encompassing CP2 may be accomplished, as needed, to determine the TSV genotype (Tang & Lightner, 2005; Wertheim *et al.,* 2009).

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* *

NB: There is an OIE Reference Laboratory for Taura syndrome
(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 Taura syndrome

Annex 20

CHAPTER 2.2.8.

INFECTION WITH YELLOW HEAD VIRUS

1. Scope

For the purpose of this chapter, yellow head disease (YHD) is considered to be infection with yellow head virus genotype 1 (YHV1).

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent, agent strains

Yellow head virus genotype 1 (YHV1) is one of six known genotypes in the yellow head complex of viruses and is the only known agent of YHD. Gill-associated virus (GAV) is designated as genotype 2. GAV and four other known genotypes in the complex (genotypes 3–6) occur commonly in healthy *Penaeus monodon* in East Africa, Asia and Australia and are rarely or never associated with disease (Walker *et al.,* 2001, Wijegoonawardane *et al.,* 2008a). YHV and other genotypes in the yellow head complex are classified by the International Committee on Taxonomy of Viruses as a single species (Gill-associated virus) in the genus *Okavirus*, family *Roniviridae*, order *Nidovirales* (Cowley *et al.*, 2012). There is evidence of genetic recombination between genotypes (Wijegoonawardane *et al.*, 2009).

YHV forms enveloped, rod-shaped particles ~~(~~40–5060 nm × 150–180~~200~~ nm~~)~~ (Chantanachookin *et al.,* 1993; Wongteerasupaya *et al*., 1995). Envelopes are studded with prominent peplomers projecting approximately 11 nm from the surface. Nucleocapsids appear as rods (diameter 20–30 nm) and possess a helical symmetry with a periodicity of 5–7 nm. Virions comprise three structural proteins (nucleoprotein p20~~–24~~ and envelope glycoproteins gp64 and gp116) and a ~26 kb positive-sense single-stranded RNA genome.

2.1.2. Survival outside the host

YHV remains viable in aerated seawater for up to 72 hours (Flegel *et al.*, 1995b).

2.1.3. Stability of the agent (effective inactivation methods)

YHV can be inactivated by heating at 60°C for 15 minutes (Flegel *et al.*, 1995b). Little information is available on other inactivation methods but the virus appears to be susceptible to treatment with chlorine at 30 parts per million (0.03 mg ml^{-1}) (Flegel *et al.,* 1997).

2.1.4. Life cycle

High multiplicity YHV infections in cell culture have not been reported. Infection at a multiplicity of infection of 0.001 in primary cultures of lymphoid organ cells has indicated that maximum viral titres are obtained 4 days post-infection (Assavalapsakul *et al*., 2003). Clinical signs of YHD occur in *P. monodon* within 7–10 days of exposure. YHV replicates in the cytoplasm of infected cells in which long filamentous pre-nucleocapsids are abundant and virions bud into cytoplasmic vesicles in densely packed paracrystalline arrays for egress at the cytoplasmic membrane (Chantanachookin *et al.,* 1993).

Annex 20 (contd)

2.2. Host factors

2.2.1. Susceptible host species

YHD outbreaks have been reported ~~only~~ in the black tiger prawn (*P. monodon*) and the white Pacific shrimp (*P. vannamei*) (Chantanachookin *et al.,* 1993; Senapin *et al.,* 2010). The Pacific blue prawn (*P. stylirostris*), the daggerblade grass shrimp (*Palaemonetes pugio*), and the Jinga shrimp (*Metapenaeus affinis*) also fulfil the criteria required for listing a species susceptible to infection with YHV1 according to Article 1.5 of *Aquatic Animal Health Code*. Natural infections have also been detected in the kuruma prawn (*P. japonicus*), white banana prawn (*P. merguiensis*), Pacific blue prawn (*P. stylirostris*), white prawn (*P. setiferus*), red endeavour prawn (*Metapenaeus ensis*), mysid shrimp (*Palaemon styliferus*) and krill (*Acetes* sp.). Other species of penaeid and palemonid shrimp and prawns and krill that have been reported to be susceptible to experimental infection include: brown tiger prawn (*P. esculentus*), brown prawn (*P. aztecus*); pink prawn, hopper and brown-spotted prawn (*P. duorarum*), greentail prawn (*Metapenaeus bennettae*), Sunda river prawn (*Macrobrachium sintangense*), barred estuarine shrimp (*Palaemon serrifer*), the paste prawn (*Ascetes* sp.) and the daggerblade grass shrimp (*Palaemonetes pugio*) (Ma *et al.,* 2009). There are variations in the susceptibility of different species to disease. Laboratory trials have shown that YHV can cause high mortality in *P. monodon, P. vannamei, P. stylirostrus, P. aztecus, P. duorarum, M. sintangense, P. styliferus* and *P. serrifer* (Lightner *et al.*, 1998; Longyant *et al.*, 2005; 2006; Ma *et al.,* 2009). A survey of 16 crab species collected from the vicinity of shrimp farms in Thailand detected no evidence of either natural infection or experimental susceptibility (Longyant *et al.*, 2006). A critical review of susceptibility of crustaceans to yellow head disease and implications of inclusion in European legislation has been conducted (Stentiford *et al*., 2009). GAV has been detected in *P. monodon* and *P. esculentus* (Walker *et al.,* 2001). To date, infections by other genotypes in the YHV complex have been detected only in *P. monodon* (Wijegoonawardane *et al.*, 2008a). *Metapenaeus brevicornis* and *P. aztecus* also fulfil some of the criteria required for listing as susceptible but evidence was lacking to either confirm the identity of the pathogen under study as YHV1, to demonstrate a natural route of infection, or to definitively confirm an 'infected' status.

2.2.2. Susceptible stages of the host

Penaeus monodon are susceptible to YHV infection beyond PL15 (Khongpradit *et al.*, 1995). Experimental infections with GAV indicate that larger (~20 g) *P. japonicus* are less susceptible to disease than smaller (~6–13 g) shrimp of the same species (Spann *et al.*, 2000).

2.2.3. Species or subpopulation predilection (probability of detection)

~~Viruses in yellow head complex genotypes 2–6 are only known to occur commonly (prevalence up to 100%) in healthy *P. monodon*, which appears to be the natural host (Walker *et al.*, 2001; Wijegoonawardane *et al.*, 2008a; 2009). In contrast,~~ YHV (genotype 1) infections are usually detected only when disease is evident and whilst they do not occur commonly in healthy *P. monodon*, infections have been detected in healthy wild populations of *P. stylirostris* (Castro-Longoria *et al.,* 2008). During YHD outbreaks in aquaculture ponds, the YHV infection prevalence can be assumed to be high. Natural YHV infections have been detected in *P. japonicus, P. merguiensis, P. setiferus, M. ensis,* and *P. styliferus* (Cowley *et al.,* 2002; Flegel *et al.*, 1995a; 1995b), but there is little information available on the natural prevalence. Viruses in yellow head complex genotypes 2–6 are only known to occur commonly (prevalence up to 100%) in ~~healthy~~ *P. monodon*, which appears to be the natural host (Walker *et al.*, 2001; Wijegoonawardane *et al.*, 2008a; 2009).

2.2.4. Target organs and infected tissue

YHV targets tissues of ectodermal and mesodermal origin including lymphoid organ, haemocytes, haematopoietic tissue, gill lamellae and spongy connective tissue of the subcutis, gut, antennal gland, gonads, nerve tracts and ganglia (Chantanachookin *et al.,* 1993; Lightner, 1996).

2.2.5. Persistent infection with lifelong carriers

GAV persists as a chronic infection for at least 50 days in *P. esculentus* that survive experimental challenge (Spann *et al.*, 2003). The high prevalence of subclinical or chronic infection often found in healthy *P. monodon* infected with GAV (genotype 2) and genotypes 3–6 from postlarval stages onwardssuggests that these infections can perrsist for life (Walker *et al.*, 2001; Wijegoonawardane *et al.*, 2008a). There is also evidence that YHV (genotype 1) can persist in survivors of experimental infection (Longyant *et al.*, 2005; 2006).

2.2.6. Vectors

There are no known vectors of YHV.

2.2.7. Known or suspected wild aquatic animal carriers

Infection susceptibility and long-term persistence indicate the potential for a wide range of wild penaeid and palaemonid shrimp to act as carriers.

2.3. Disease pattern

2.3.1. Transmission mechanisms

YHV infection can be transmitted horizontally by injection, ingestion of infected tissue, immersion in sea water containing tissue extracts filtered to be free of bacteria, or by co-habitation of naive shrimp with infected shrimp (Flegel *et al.*, 1995b; Lightner, 1996). Infection of shrimp has also been established by injection of extracts of paste prawns (*Acetes* sp.) collected from infected ponds (Flegel *et al.*, 1995a). For GAV, vertical transmission of infection to progeny has been shown to occur from both male and female parents, possibly by surface contamination or infection of tissue surrounding fertilised eggs (Cowley *et al.,* 2002). The dynamics of how YHV infection spreads within aquaculture ponds have not been studied. However, the rapid accumulation of mortalities during disease outbreaks suggests that horizontal transmission occurs very effectively.

2.3.2. Prevalence

The infection prevalence of yellow head complex viruses in healthy *P. monodon* (as detected by nested polymerase chain reaction [PCR]) can be high (50–100%) in farmed and wild populations in Australia, Asia and East Africa as well as in *L. vannamei* farmed in Mexico (Castro-Longoria *et al.,* 2008; Cowley *et al*., 2004; Sanchez-Barajas *et al.*, 2009; Walker *et al.*, 2001; Wijegoonawardane *et al.*, 2008a). The prevalence of individual genotypes varies according to the geographical origin of the shrimp. ~~In contrast, except in situations of disease outbreaks in aquaculture ponds, the prevalence of YHV (genotype 1) is more commonly low (<1%) in healthy wild or farmed *P. monodon* (pers. comm.).~~ The use of detection methods less sensitive than nested PCR (e.g. histology, immunoblot, dot-blot, *in-situ* hybridisation), is likely in most cases to result in the real infection prevalence amongst populations of shrimp being underestimated.

2.3.3. Geographical distribution

YHD has been reported in Chinese Taipei, Indonesia, Malaysia, the Philippines, Sri Lanka, Thailand and Vietnam (Walker *et al.*, 2001). GAV and other genotypes in the yellow head complex have been detected in healthy *P. monodon* from Australia, Chinese Taipei, India, Indonesia, Malaysia, Mozambique, the Philippines, Thailand and Vietnam (Wijegoonawardane *et al.*, 2008a). YHV has also been detected in *P. vannamei* cultured in Mexico (Castro-Longoria *et al.,* 2008; Sanchez-Barajas *et al.*, 2009).

2.3.4. Mortality and morbidity

With *P. monodon* being farmed in ponds, disease caused by YHV (genotype 1) can cause up to 100% mortality within 3–5 days of the first appearance of clinical signs (Chantanachookin *et al.,* 1993). ~~GAV (genotype 2) has also been associated with morbidity and up to 80% mortality in ponds of *P. monodon* farmed in Australia.~~ Whilst mortalities can easily be induced by experimental exposure of *P. monodon* to YHV or GAV, bioassays have identified YHV to be far more virulent (~10^6-fold by lethal dose [LD_{50}] 50% end-point analysis) (Oanh *et al.,* 2011). Genotypes 3, 4, 5 and 6 have not yet been associated with disease (Wijegoonawardane *et al.*, 2008a).

2.3.5. Environmental factors

Elevated virus infection levels accompanied by disease can be precipitated by physiological stress induced by sudden changes in pH or dissolved oxygen levels, or other environmental factors (Flegel *et al*., 1997). The much higher virulence of YHV compared to GAV and other genotypes appears to ensure that the infection threshold required to cause disease is reached far more easily.

2.4. Control and prevention

2.4.1. Vaccination

No effective vaccination methods have been developed.

2.4.2. Chemotherapy

No effective commercial anti-viral product is yet available.

2.4.3. Immunostimulation

No scientifically confirmed reports.

2.4.4. Resistance breeding

Not reported.

2.4.5. Restocking with resistant species

All marine shrimp species farmed commercially appear to be susceptible to YHV.

2.4.6. Blocking agents

Injection of shrimp with double-stranded (ds) RNA homologous to ORF1a/1b gene regions of YHV or GAV (thus targeting the genome length viral RNA) can inhibit viral replication and prevent mortalities following experimental challenge. The antiviral action of the dsRNA appears to involve the RNA interference (RNAi) pathway (Tirasophon *et al.,* 2007).

2.4.7. Disinfection of eggs and larvae

Not reported.

2.4.8. General husbandry practices

Specific pathogen free (SPF) or PCR-negative seedstock and biosecure water and culture systems may be used to reduce the risk of disease.

3. Sampling

3.1. Selection of individual specimens

For diagnosis during a disease outbreak, moribund shrimp collected from pond edges are the preferred source of material for examination. Apparently normal shrimp should also be collected from the same ponds. For surveillance for evidence of infection in populations of apparently healthy shrimp, life stages from mysis onwards (mysis, postlarvae [PL], juveniles or adults) can provide tissue sources useful for testing.

3.2. Preservation of samples for submission

Moribund shrimp (or tissue from moribund shrimp) should be snap-frozen on-site in a dry ice/alcohol slurry and preserved frozen in dry ice, liquid nitrogen or in a –80°C freezer. Freezing at or above –20°C is unsuitable.

Tissue samples for PCR screening should be preserved in a minimum 3-fold excess of 90% analytical/reagent-grade (absolute) ethanol. The use of lower grade (laboratory or industrial grade) ethanol is not recommended. Commercial RNA preservatives (e.g. RNAlater) may also be used.

Tissue samples for histology should be preserved in Davidson's fixative. Formalin (10%) in seawater may be a useful alternative.

Tissues for electron microscopy should be sampled from live shrimp.

For guidance on sample preservation methods for the intended test methods, see Chapter 2.2.0.

3.3. Pooling of samples

For detecting YHV infection in large populations of shrimp, pooling of tissue samples is acceptable for screening or surveillance of batches of mysis to PL from a hatchery tank or batches of juvenile shrimp in a pond. For PCR analysis, pool size should be determined by tissue mass that can be processed without compromise in a single test. The total numbers of shrimp sampled, either as a single pool or as multiple smaller pools, are selected based on the infection prevalence expected and the required confidence limits of detection. Typically in populations comprising more than a 100,000 shrimp, if the prevalence of infection exceeds 5%, a total of 60 individuals tested in appropriate pool sizes will be required to detect YHV at a 95% confidence limit. However, definitive detection may be compromised if the YHV loads in the infected shrimp are very low or if tests less sensitive than two-step PCR or real-time PCR are employed. See also Chapter 2.2.0.

3.4. Best organs or tissues

In moribund shrimp suspected to be infected with YHV, lymphoid organ and gill are the most suitable sample tissues. For screening or surveillance of juvenile or adult shrimp that appear grossly normal, lymphoid organ is preferred. Gills or haemolymph can be used for non-sacrificial sampling.

3.5. Samples/tissues that are not suitable

Not determined.

4. Diagnostic methods

4.1. Field diagnostic methods

4.1.1. Clinical signs

Shrimp from late PL stages onwards can be infected experimentally with YHV. In cultured shrimp, infection can result in mass mortality occurring, usually in early to late juvenile stages. Moribund shrimp may exhibit a bleached overall appearance and a yellowish discoloration of the cephalothorax caused by the underlying yellow hepatopancreas, which may be exceptionally soft when compared with the brown hepatopancreas of a healthy shrimp. In many cases, the total loss of a pond crop occurs within a few days of the first appearance of shrimp showing gross signs of YHD (Chantanachookin *et al.,* 1993). Cessation of feeding, congregation of moribund shrimp at pond edges and a generally bleached appearance are always seen in YHD outbreaks. However, these disease features are not particularly distinctive for YHD, and in the absence of other more pathognomonic gross signs are not reliable even for preliminary diagnosis of YHD. Gross signs of GAV disease include swimming near the surface and at the pond edges, cessation of feeding, a reddening of body and appendages, and pink to yellow discoloration of the gills (Spann *et al.*, 1997). However, these signs can occur commonly in response to various stressors and thus are not considered pathognomonic for GAV disease. Shrimp chronically infected with YHV or GAV display normal appearance and behaviour.

4.1.2. Behavioural changes

Exceptionally high feeding activity followed by an abrupt cessation of feeding may occur within 2–4 days of the appearance of gross clinical signs of disease and mortality. Moribund shrimp may congregate at pond edges near the surface (Chantanachookin *et al.,* 1993).

4.2. Clinical methods

4.2.1. Gross pathology

See Section 4.1.

4.2.2. Clinical chemistry

None described.

4.2.3. Microscopic pathology

Fix the cephalothorax tissues of moribund shrimp suspected to be affected by YHD in Davidson's fixative, prepare tissue sections and stain with Meyer's haematoxylin and eosin (H&E) using standard histological procedures (Lightner, 1996). Examine tissues of ectodermal and mesodermal origin by light microscopy for the presence of moderate to large numbers of deeply basophilic, evenly stained, spherical, cytoplasmic inclusions approximately 2 µm in diameter or smaller (Chantanachookin *et al.,* 1993). Tissues of the lymphoid organ, stomach subcuticulum and gills are particularly informative.

4.2.4. Wet mounts

Fix whole shrimp or gill filaments overnight in Davidson's fixative (Lightner, 1996). After fixation, wash some gill filaments thoroughly with tap water to remove the fixative and stain with H&E (Lightner, 1996). After staining and dehydration, when the tissue is in xylene, place a gill filament on a microscope slide in a drop of xylene and, using a fine pair of needles (a stereo microscope is helpful), break off several secondary filaments. Replace the main filament in xylene where it can be stored indefinitely in a sealed vial as a permanent reference. Being careful not to let the xylene dry, tease apart the secondary filaments and remove any large fragments or particles that would thicken the mount unnecessarily. Add a drop of mounting fluid and a cover-slip and use light pressure to flatten the mount as much as possible. This procedure may also be used with thin layers of subcuticular tissue. Examine under a light microscope using a ×40 objective lens. For samples from YHD-affected shrimp, moderate to large numbers of deeply basophilic, evenly stained, spherical, cytoplasmic inclusions (approximately 2 µm in diameter or smaller) will be observed (Flegel *et al.,* 1997). Evidence of such pathology should be used to support results from haemolymph smears (see below) in making a presumptive diagnosis of YHD. As for the fixed tissues and gill filaments preserved in xylene, these whole-mount slides can be preserved as a permanent record.

If rapid results are required, the fixation step can be shortened to only 2 hours by replacing the acetic acid component of Davidson's fixative with a 50% dilution of concentrated HCl. For good fixation, this fixative should not be stored for more than a few days before use. After fixation, wash thoroughly to remove the fixative and check that the pH has returned to near neutral before staining. Do not fix for longer periods or above 25°C as this may result in excessive tissue damage that will make it difficult or impossible to identify specific pathology.

~~**4.2.5. Smears**~~

~~For moribund shrimp affected by YHD, haemolymph smears are not useful because haemocytes are usually depleted in the advanced stages of disease. In cases of suspected YHD where moribund shrimp have been sampled from a pond, haemolymph should be collected from grossly normal shrimp from the same pond. Draw the haemolymph into a syringe containing two volumes of either 25% formalin or Davidson's fixative modified by replacing the acetic acid component with either water or formalin. Mix thoroughly, ignore clots in the syringe, place a drop on a microscope slide, smear and then air-dry before staining with H&E or other standard blood smear stains. Dehydrate, add mounting fluid and a cover-slip. Examine under a light microscope using a ×40 objective lens. For YHD-affected shrimp, some smears will show moderate to high numbers of haemocytes with karyorrhectic or pyknotic nuclei. It is important that there is no evidence of concomitant bacterial infection in slides of haemocytes displaying such nuclei, as bacterial infections may cause similar changes in haemocytes. When making a presumptive diagnosis of YHD, the results from haemolymph smears should be considered in conjunction with the results from rapid-stained whole mounts (see above) or stained tissue sections.~~

4.2.5. Electron microscopy/cytopathology

For transmission electron microscopy (TEM), the most suitable tissues of shrimp suspected to be infected with YHV infection are lymphoid organ and gills. For screening or surveillance of grossly normal shrimp, the most suitable tissue is lymphoid organ.

Stun live shrimp by immersion in iced water until just immobilised or kill by injection of fixative. Quickly dissect and remove small portions of target tissue (no larger than a few mm in diameter) and fix in at least 10 volumes of 6% glutaraldehyde held at 4°C and buffered with sodium cacodylate ($Na[CH_3]_2AsO_2 \cdot 3H_2O$) solution (8.6 g Na cacodylate, 10 g NaCl, distilled water to make 100 ml, adjusted to pH 7 with 0.2 N HCl) or phosphate solution (0.6 g $NaH_2PO_4 \cdot H_2O$, 1.5 g Na_2HPO_4, 1 g NaCl, 0.5 g sucrose, distilled water to make 100 ml, adjusted to pH 7 with 0.2 N HCl). Fix for at least 24 h prior to processing. For long-term storage in fixative at 4°C, reduce glutaraldehyde to 0.5–1.0%. Processing involves post-fixation with 1% osmium tetroxide, dehydration, embedding, sectioning and staining with uranyl acetate and lead citrate according to standard TEM reagents and methods (Lightner, 1996).

In the cytoplasm of cells infected with YHV, both nucleocapsid precursors and complete enveloped virions are observed. Nucleocapsid precursors appear as long filaments approximately 15 nm in diameter that can vary markedly in length (80–450 nm) and that can sometimes be packed densely in paracrystalline arrays. Virions appear as rod-shaped, enveloped particles 40–50~~60~~ nm × 150–180~~200~~ nm with rounded ends and prominent projections (8–11 nm) extending from the surface. In the cell cytoplasm, virions are commonly seen to be localised or packed densely within intracellular vesicles. Virions may also be seen budding at the cytoplasmic membrane and in interstitial spaces. GAV virions and nucleocapsids are indistinguishable from YHV by TEM.

Lymphoid organ spheroids are commonly observed in healthy *P. monodon* chronically infected with YHV or GAV and lymphoid organ necrosis often accompanies disease (Spann *et al.*, 1997). However, spheroid formation and structural degeneration of lymphoid organ tissue also result from infection by other shrimp viruses (Lightner, 1996).

4.3. Agent detection and identification methods

4.3.1. Direct detection methods

4.3.1.1. Microscopic methods

4.3.1.1.1. Wet mounts

See Section 4.2.4.

4.3.1.1.2. Smears

See Section 4.2.5.

4.3.1.1.3. Fixed sections

See Section 4.2.3.

4.3.1.2. Agent isolation and identification

4.3.1.2.1. Cell culture/artificial media

Although primary shrimp cell culture methods are available, they are not recommended to isolate and identify YHV as a routine diagnostic method because of the high risk of them becoming contaminated with adventitious agents. No continuous cell lines suitable for YHV culture are yet available.

4.3.1.2.2. Antibody-based antigen detection methods

Reagents and protocols for detecting YHV proteins with antibodies have been published (Loh *et al.* 1998; Lu *et al.* 1994). Virions purified from haemolymph of experimentally infected shrimp have been used to produce antiserum in New Zealand white rabbits. From this antiserum, immunoglobulin (IgG) was purified using protein-G-linked columns and cross-reacting normal shrimp antigens were removed by adsorption to acetone-dried, ground shrimp muscle tissue and haemolymph. To detect YHV proteins by Western blotting, dilute 0.1 ml haemolymph collected from a live shrimp in an equal volume of citrate buffer and either run immediately or store at –80°C until used. Clarify 200 µl of the sample at 8000 ***g*** for 5 minutes and then pellet virions from the clarified supernatant by ultracentrifugation at 140,000 ***g*** for 5 minutes. Resuspend pellets in 100 µl 2 × loading buffer (2.5 ml 0.5 mM Tris/HCl pH 6.8, 4 ml 10% sodium dodecyl sulphate [SDS], 2 ml glycerol, 1 µl β-mercaptoethanol, 0.5 ml deionised distilled water) and heat at 95°C for 5 minutes. Load 10 µl sample onto a 5% SDS-polyacrylamide gel and electrophorese at 200 V. Blot the gel onto a 0.1 mm pore size nitrocellulose membrane in blotting buffer (3.03 g Tris-base, 14.4 g glycine, 200 ml methanol per litre) at 100 V for 1 hour. Rinse the membrane with phosphate buffered saline (PBS pH 7.4), block in 5% skim milk (in PBS) for 1 hour, and rinse with PBS for 5 minutes. Soak the membrane in a 1/1000 dilution of the anti-YHV antibody (IgG) for 1 hour, rinse three times with PBS for 5 minutes, and then soak for 1 hour in a 1/2500 dilution of goat anti-rabbit IgG-horseradish-peroxidase (HRP) conjugate. Rinse membrane three times with PBS for 5 minutes and then soak in HRP substrate 3,3',5,5'-tetramethylbenzidine , until blue-purple colour develops. Stop the reaction by soaking the membrane in distilled water. All incubations should be carried out at 25°C ± 2°C. Use a purified viral preparation as a positive control to identify positions of the YHV 116 kDa, 64 kDa and 20 kDa structural proteins. The Western blot YHV detection sensitivity is approximately 0.4 ng YHV protein (≈ 10^6 virions).

4.3.1.2.3. Molecular techniques

4.3.1.2.3.1 Reverse-transcription polymerase chain reaction (RT-PCR)

Three RT-PCR protocols are described. The first is a 1-step RT-PCR adapted from Wongteerasupaya *et al.* (1997) that can be used to detect YHV in shrimp affected by YHD. This protocol will detect YHV (highly virulent genotype first detected in Thailand in association with YHD) but not GAV or any of the other three genotypes currently recognised. The second is a more sensitive multiplex nested RT-PCR protocol adapted from Cowley *et al.* (2004). It can be used to differentiate YHV from GAV in diseased shrimp or for screening healthy carriers. This test will not detect all six known genotypes and genotype 3 may generate a PCR product indistinguishable in size from that generated with GAV (genotype 2). The test is available in a suitably modified form from a commercial source (YHV/GAV IQ2000, GeneReach Biotechnology Corp., Chinese Taipei). However, this kit is not currently listed as having completed the OIE's formal process for validating and certifying commercial tests (a list of certified test kits and manufacturers is available on the OIE website: http://www.oie.int/en/our-scientific-expertise/registration-of-diagnostic-kits/background-information/). The third is a sensitive multiplex RT-nested PCR protocol described by Wijegoonawardane *et al.* (2008b). This test can be used for screening healthy shrimp for any of the six genotypes of the yellow head complex of viruses (including YHV and GAV), but will not discriminate between genotypes. Assignment of genotype can be achieved by nucleotide sequence analysis of the RT-PCR product.

Sample preparation: For juvenile or adult shrimp, lymphoid organ, gill tissue or haemolymph may be used to prepare total RNA. Fresh tissue is preferred. Lymphoid organ and gill tissue preserved in 95% analytical-grade ethanol or RNAlater (various manufacturers), or stored frozen at –70°C are also suitable for total RNA preparation. Disrupt 10–20 mg lymphoid organ or gill tissue or 50 µl haemolymph in 500 µl TrizolTM4 reagent and extract total RNA according to the product manual. Resuspend RNA in 25 µl water treated with DEPC (diethyl-pyrocarbonate)-, heat at 55°C for 10 minutes, cool on ice and use immediately or store at –70°C until required. Ideally, a 1/200 dilution (i.e. 2.5 µl RNA in 500 µl DEPC-treated water) should be prepared, and UV absorbances at A260nm and A280 nm (a UV spectrophotometer is required) should be determined to quantify and check the quality of the RNA (ratio approximately 2:1). RNA yield will vary depending on the type and freshness of tissues, quality of the preservative used, and the length of time tissue has been preserved. However, RNA yields from fresh tissues would be expected to vary from 0.2 to 2.0 µg µl–1 and about half these amounts from alcohol-preserved tissues.

From a nursery tank or hatchery tank containing 100,000 PL or more, sample approximately 1000 PL from each of 5 different points. Pool the samples in a basin, gently swirl the water and then select samples of live PL that collect at the centre of the basin. Choose numbers of PL to be pooled and tested according to the assumed or infection prevalence. Homogenise tissue samples in an appropriate volume of Trizol™ reagent and extract RNA according to the product manual. Based on the standard Trizol™ extraction procedure, tissue masses equivalent to 25–30 × PL5, 15 × PL10 and 5 × PL15 are accommodated and produce high quality total RNA free of protein contamination.

For each set of RNA samples to be tested, DEPC-treated water and extracts known to contain YHV RNA and/or GAV RNA (as appropriate to the test) should be included as negative and positive controls, respectively.

Protocol 1: RT-PCR for specific detection of YHV in diseased shrimp

To synthesise cDNA, mix 2 µl RNA in 20 µl PCR buffer (10 mM Tris/HCl pH 8.3, 50 mM KCl) containing 2.5 U of M-MLV (Moloney murine leukaemia virus) reverse transcriptase, 1.0 U ribonuclease inhibitor, 0.75 µM antisense primer 144R, 1 mM each of dATP, dTTP, dCTP, and dGTP, and 5 mM $MgCl_2$, and incubate at 42°C for 15 minutes. Incubate the mixture at 100°C for 5 minutes to inactivate the reverse transcriptase and allow the mixture to cool to 5°C. Add PCR mixture (10 mM Tris/HCl pH 8.3, 50 mM KCl) containing 2.5 U *Taq* DNA polymerase, 2 mM $MgCl_2$ and 0.75 µM of sense primer 10F to give a final volume of 100 µl. Unless the instrument is fitted with a heated lid, overlay the tubes with 100 µl of mineral oil and conduct PCR amplification for 40 cycles at 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 30 seconds, and finishing at 72°C for 10 minutes. Alongside a suitable DNA ladder, apply a 20 µl aliquot of the PCR to a 2% agarose/TAE (Tris-acetate-EDTA [ethylene diamine tetra-acetic acid]) gel containing 0.5 µg ml^{-1} ethidium bromide and following electrophoresis, detect the 135 bp DNA band expected for YHV using a UV transilluminator.

[4] Reference to specific commercial products as examples does not imply their endorsement by the OIE. This applies to all commercial products referred to in this *Aquatic Manual*.

The sensitivity of the PCR is approximately 0.01 pg of purified YHV RNA (≈ 10^3 genomes).

PCR primer sequences:
10F: 5'-CCG-CTA-ATT-TCA-AAA-ACT-ACG-3'
144R: 5'-AAG-GTG-TTA-TGT-CGA-GGA-AGT-3'

Protocol 2: Nested RT-PCR for differential detection of YHV and GAV in healthy or diseased shrimp

For cDNA synthesis, 2 µl RNA (ideally 1.0 µg total RNA, if quantified),0.7 µl 50 pmol μl^{-1} primer GY5 and DEPC-treated water are added to 6 µl total, the mixture , incubated at 70°C for 10 minutes and chilled on ice. Add 2 µl Superscript II buffer × 5 (250 mM Tris/HCl pH 8.3, 375 mM KCl, 15 mM $MgCl_2$), 1 µl 100 mM DTT and 0.5 µl 10 mM dNTP stock mixture (i.e. 10 mM dATP, 10 mM dTTP, 10 mM dCTP, 10 mM dGTP) and mix gently. Preheat to 42°C for 2 minutes, add 0.5 µl 200 U μl^{-1} reverse transcriptase and incubate at 42°C for 1 hour. Heat the reaction at 70°C for 10 minutes, chill on ice and spin briefly in a microcentrifuge to collect the contents of the tube. For the first PCR step, prepare a 50 µl reaction mixture containing 1 × *Taq* buffer (10 mM Tris/HCl pH 8.3, 50 mM KCl, 0.1% Triton X-100), 1.5 mM $MgCl_2$, 35 pmol of each primer GY1 and GY4, 200 µM each of dATP, dTTP, dCTP and dGTP and 2.5 U *Taq* polymerase in a 0.5 ml thin-walled tube. Overlay the reaction mixture with 50 µl liquid paraffin, heat at 85°C for 2–3 minutes and then add 1 µl cDNA. Conduct PCR amplification using 35 cycles at 95°C for 30 seconds, 66°C for 30 seconds, and 72°C for 45 seconds, followed by final extension at 72°C for 7 minutes. For the second PCR step, prepare a 50 µl reaction mixture containing 2 µl of the first step PCR product, 1 × *Taq* buffer (above), 1.5 mM $MgCl_2$, 35 pmol of each primer GY2, Y3 and G6, 200 µM each of dATP, dTTP, dCTP and dGTP and 2.5 U *Taq* polymerase in a 0.5 ml thin-walled tube and overlay with liquid paraffin. Conduct PCR using amplification conditions as described above. Apply a 10 µl aliquot of the PCR to 2% agarose/TAE gels containing 0.5 µg ml^{-1} ethidium bromide alongside a suitable DNA ladder and detect using a UV transilluminator.

If the viral load is sufficiently high, a 794 bp DNA will be amplified from either GAV or YHV in the first PCR step. In the second PCR step, a 277 bp product indicates detection of YHV and a 406 bp product indicates detection of GAV. The presence of both 406 bp and 277 bp products indicates a dual infection with GAV and YHV. The detection sensitivity of the second-step PCR is ~1000-fold greater than the first-step PCR and GAV or YHV RNA can be detected to a limit of 10 fg lymphoid organ total RNA.

The sequences of RT-PCR primers generic for GAV and YHV (GY) or specific for GAV (G) or YHV (Y) are as follows:
GY1: 5'-GAC-ATC-ACT-CCA-GAC-AAC-ATC-TG-3'
GY2: 5'-CAT-CTG-TCC-AGA-AGG-CGT-CTA-TGA-3'
GY4: 5'-GTG-AAG-TCC-ATG-TGT-GTG-AGA-CG-3'
GY5: 5'-GAG-CTG-GAA-TTC-AGT-GAG-AGA-ACA-3'
Y3: 5'-ACG-CTC-TGT-GAC-AAG-CAT-GAA-GTT-3'
G6: 5'-GTA-GTA-GAG-ACG-AGT-GAC-ACC-TAT-3'

NB: Due to reported problems with primer specificity for some emerging strains, all PCR products generated using protocol 2 should be sequenced to confirm the virus genotype.

Protocol 3: Nested *RT*-PCR for detection of all currently known genotypes in the yellow head complex (including YHV and GAV)

For cDNA synthesis, mix 2 µl RNA (ideally 1.0 µg total RNA, if quantified), 50 ng random hexamer primers and 1.0 µl 10 mM dNTP and make up to a total volume of 14 µl in sterile DEPC-treated water, incubate at 65°C for 5 minutes and chill on ice. Add 4.0 µl Superscript III buffer × 5, 1.0 µl 100 mM DTT, 1.0 µl 40 U μl^{-1} RNaseOUT™ (Invitrogen) and 1.0 µl 200 U μl^{-1} reverse transcriptase and mix gently. Incubate at 25°C for 5 minutes and then at 42°C for 55 minutes, stop the reaction by heating at 70°C for 15 minutes, chill on ice and spin briefly in a microcentrifuge to collect the contents of the tube. For the first PCR step, add 1 µl cDNA to a total 25 µl reaction mixture containing 1 × *Taq* buffer (10 mM Tris/HCl, pH 9.0, 50 mM KCl, 0.1% Triton X-100), 1.5 µl 25 mM $MgCl_2$, 0.35 µl primer mix containing 25 pmol μl^{-1} of each primer pool (see below) YC-F1ab and YC-R1ab, 0.5 µl 10 mM dNTP mix and 0.25 µl 5 U μl^{-1} *Taq* DNA polymerase. Conduct PCR amplification using denaturation at 95°C for 1 minute followed by 35 cycles at 95°C for 30 seconds, 60°C for 30 seconds, 72°C for 40 seconds, followed by a final extension at 72°C for 7 minutes.

Annex 20 (contd)

For the second PCR step, use 1 µl of the first PCR product in the reaction mixture as prepared above but substituting primer pools YC-F2ab and YC-R2ab. Conduct PCR amplification using denaturation at 95°C for 1 minute followed by 35 cycles at 95°C for 30 seconds, 60°C for 30 seconds, 72°C for 30 seconds, followed by a final extension at 72°C for 7 minutes. Apply an 8 µl aliquot of the PCR to 2% agarose/TAE gels containing 0.5 µg ml^{-1} ethidium bromide alongside a suitable DNA ladder and detect using a UV transilluminator.

If the viral load is sufficiently high, a 358 bp DNA is amplified in the first PCR step. The second (nested) PCR step amplifies a 146 bp product. The detection of these products indicates detection of one of the six genotypes in the yellow head complex. Further assignment of genotype (if required) is possible by nucleotide sequence analysis of either PCR product followed by comparison with sequences of the known genotypes by multiple sequence alignment and phylogenetic analysis. The detection sensitivity limits of the first PCR step and nested PCR step are 2,500 and 2.5 RNA templates, respectively.

PCR primer sequences (each primer comprises a pool of equal quantities of two related oligonucleotide sequences):

YC-F1ab pool:	5'-ATC-GTC-GTC-AGC-TAC-CGC-AAT-ACT-GC-3'
	5'-ATC-GTC-GTC-AGY-TAY-CGT-AAC-ACC-GC-3'
YC-R1ab pool:	5'-TCT-TCR-CGT-GTG-AAC-ACY-TTC-TTR-GC-3'
	5'-TCT-GCG-TGG-GTG-AAC-ACC-TTC-TTG-GC-3'
YC-F2ab pool:	5'-CGC-TTC-CAA-TGT-ATC-TGY-ATG-CAC-CA-3'
	5'-CGC-TTY-CAR-TGT-ATC-TGC-ATG-CAC-CA-3'
YC-R2ab pool:	5'-RTC-DGT-GTA-CAT-GTT-TGA-GAG-TTT-GTT-3'
	5'-GTC-AGT-GTA-CAT-ATT-GGA-GAG-TTT-RTT-3'
Mixed base codes:	R(AG), Y(CT), M(AC), K(GT), S(GC), W(AT), H(ACT), B(GCT), V(AGC), D(AGT), N(AGCT).

4.3.1.2.3. *In-situ* hybridisation

The protocol of Tang *et al.* (2002) described is suitable for detecting YHV or GAV (Tang & Lightner, 1999). To preserve viral RNA accessibility, fix tissues sampled from live shrimp in neutral-buffered, modified Davidson's fixative without acetic acid (RF-fixative) (Hasson *et al.*, 1997). To achieve good tissue preservation whilst also preserving RNA accessibility, normal Davidson's fixative can be used as long as the fixation time is limited to 24 hours (maximum of 48 hours). Process the fixed tissue using standard histological methods and prepare 4 µm thick sections on Superfrost Plus slides (Fisher Scientific, Pennsylvania, USA). Prior to hybridisation, incubate sections at 65°C for 45 minutes, remove paraffin with Hemo-De (Fisher Scientific, Pennsylvania, USA), and rehydrate through a reducing ethanol concentration series to water. Digest sections with proteinase K (100 µg ml^{-1}, in 50 mM Tris/HCl pH 7.4, 10 mM NaCl, 1 mM EDTA) for 15 minutes at 37°C, followed by post-fixation in 0.4% formaldehyde for 5 minutes. Rinse in 2 × SSC (standard saline citrate), then pre-hybridise with 500 µl pre-hybridisation solution (4 × SSC, 50% formamide, 1 × Denhardt's, 0.25 mg ml^{-1} yeast RNA, 0.5 mg m^{-1} sheared salmon sperm DNA, 5% dextran sulphate) at 42°C for 30 minutes. For hybridisation, overlay the sections with 250 µl hybridisation solution containing a digoxigenin-labelled DNA probe (20–40 ng ml^{-1}) at 42°C overnight. The next day, wash the sections as follows: 2 × SSC once for 30 minutes at room temperature; 1 × SSC twice for 5 minutes at 37°C; 0.5 × SSC twice for 5 minutes at 37°C. Incubate the sections with sheep anti-digoxigenin-alkaline phosphatase conjugate (Roche) at 37°C for 30 minutes. Wash with 0.1 M Tris/HCl pH 7.5, 0.15 M NaCl twice for 10 minutes at room temperature and rinse with 0.1 M Tris/HCl pH 9.5, 0.1 M NaCl. Incubate with nitroblue tetrazolium and 5-bromo-4-chloro-3-indoyl phosphate in the dark for 1–2 h for colour development. Counterstain with Bismarck Brown Y (0.5%), dehydrate through a series of ethanol and Hemo-De, add Permount (Fisher Scientific, Pennsylvania, USA) and cover with a cover-slip. YHV-infected cells give a blue to purple-black colour against the brown counter stain. Include positive controls of YHV-infected tissue and negative controls of uninfected shrimp tissue. The digoxigenin-labelled DNA probe can be prepared by PCR labelling using the following primers:

YHV1051F:	5'-ACA-TCT-GTC-CAG-AAG-GCG-TC-3'
YHV1051R:	5'-GGG-GGT-GTA-GAG-GGA-GAG-AG-3'

4.3.1.2.3 Agent purification

A YHV purification method based on density gradient ultracentrifugation is described (Wongteersupaya *et al.* 1995). Approximately 250 healthy juvenile *P. monodon* shrimp (approximately 10 g) should ideally be used as a source of virus for purification. After acclimatising for several days in 1500 litre tanks (approximately 80 shrimp/tank) at a salinity of 3.5 parts per thousand (mg ml^{-1}), inoculate each shrimp intramuscularly with 100 µl of a 1/100 gill extract suspension prepared from YHV-infected shrimp. At 2 days post-infection, harvest moribund shrimp showing typical signs of YHD. Use a syringe to draw haemolymph from the sinuses at the base of the walking legs and mix carefully on ice with the same volume of lobster haemolymph medium (LHM) (486 mM NaCl, 15 mM $CaCl_2$, 10 mM KCl, 5 mM $MgCl_2$, 0.5 mM Na_2HPO_4 8.1 mM $MgSO_4$, 36 mM $NaHCO_3$, 0.05% dextrose in Minimal Eagle's Medium, adjusted pH 7.6 with 1 N NaOH). Centrifuge the mixture at 480 ***g*** for 30 minutes at 4°C to remove cellular debris. Ultracentrifuge the supernatant at 100,000 ***g*** for 1 hour at 4°C. Discard the supernatant and gently resuspend the pellet overnight at 4°C in 1 ml LHM. Layer this suspension over a continuous gradient of 20–40% Urografin and ultracentrifuge at 100,000 ***g*** for 1 hour at 4°C. After centrifugation, collect the viral band by using a Pasteur pipette and dilute with NTE buffer (0.02 M EDTA, 0.2 M NaCl, 0.2 M Tris/HCl [pH 7.4]) to a final volume of 12 ml. Ultracentrifuge the suspension at 100,000 ***g*** for 1 hour at4°C and resuspend the pellet (purified virus) in 100 µl TE buffer (10 mM Tris/HCl, 1 mM EDTA [pH 7.4]) and store in 20 µl aliquots at –80°C until required.

4.3.1.2.4 Bioassay

The bioassay procedure is based on that described by Spann *et al.* (1997), but similar procedures have been described by several other authors (Lu *et al.,* 1994). The bioassay should be conducted in susceptible shrimp (see Section 2.2 above) ideally that have been certified as SPF and have been obtained from a biosecure breeding facility. Alternatively, susceptible wild or farmed shrimp to be used for bioassay should be screened by nested RT-PCR using RNA extracted from haemolymph to confirm the absence of pre-existing chronic infections with YHV, GAV or related viruses. Throughout the procedure, shrimp should be maintained under optimal conditions for survival of the species in laboratory tank systems.

Collect moribund shrimp from a YHD-affected ponds or shrimp suspected of being carriers of infection and maintain at 4°C or on ice. Remove and discard the tail and appendages. If necessary, the whole shrimp or the retained cephalothorax may be snap-frozen and stored at –80°C or in liquid nitrogen until required. Thaw stored samples rapidly in a 37°C water bath within two snap-seal plastic bags and then maintain at 4°C or on ice during all procedures. Remove the carapace and calciferous mouth-parts. Suspend the remaining tissues in six volumes of TN buffer (0.02 M Tris/HCl, pH 7.4, 0.4 M NaCl) and homogenise in a tissue grinder to form a smooth suspension. Clarify the homogenate at 1300 ***g*** for 20 minutes at 4°C. Remove the supernatant fluid below the lipid layer and pass through a 0.45 µm filter. Maintain the filtrate at 4°C for immediate use or snap-freeze and store in aliquots at –80°C or in liquid nitrogen. Thaw the filtrate rapidly at 37°C and maintain on ice prior to use.

Inject at least 12 juvenile (1–5 g) shrimp of a known susceptible species (*P. monodon*, *P. esculentus*, *P. japonicus*, *P. merguiensis*, *P. vannamei*, *P. stylirostris*), with 5 µl of filtrate per gram body weight into the second abdominal segment using a 26-gauge needle. Inject two equivalent groups of at least 12 shrimp with TN buffer and a filtered tissue extract prepared from uninfected shrimp. One additional group of at least 12 shrimp should be injected last with a known and calibrated positive control inoculum from shrimp infected with YHV or GAV (as required). Maintain each group of shrimp in a separate covered tank with a separate water supply for the duration of the bioassay. Ensure no inadvertent transfer of water between tanks by good laboratory practice. Observe the shrimp and record mortalities for at least 21 days or until the test and positive control groups reach 100% mortality. Collect at least one moribund shrimp from each of the four groups for examination by histology, TEM, *in situ* nucleic acid hybridisation, and PCR or Western-blot analysis to confirm the presence of YHV or GAV (as required) in the sample (refer to the Sections above for test procedures).

NOTE: shrimp to be tested that are suspected of being carriers of low level chronic infections may produce an inoculum containing a very low dose of virus. In bioassay, such an inoculum may not necessarily cause mortalities, gross signs of disease or histology characteristic of a lethal infection. In this event, molecular tests (PCR or ISH) or TEM must be applied to the bioassay shrimp.

Annex 20 (contd)

4.3.2. Serological methods

Not applicable.

5. Rating of tests against purpose of use

The methods currently available for targeted surveillance and diagnosis of YHD 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	d	d	d	d	a	d
Histopathology	d	d	c	c	a	d
Transmission EM	d	d	c	c	d	b
Antibody-based assays	d	d	c	c	a	b
DNA probes – *in situ*	d	d	c	c	b	a
PCR	a	a	a	a	a	a
Sequence	a	a	a	a	d	a

PLs = postlarvae; LM = light microscopy; EM = electron microscopy; PCR = polymerase chain reaction.

6. Test(s) recommended for targeted surveillance to declare freedom from infection with yellow head virus

Nested RT-PCR (Section 4.3.1.2.3.1; Protocol 3) followed by confirmatory sequencing of the amplified PCR product is the prescribed method for declaring freedom. Two-step PCR negative results are required. The very rare case when a two-step PCR positive result cannot be confirmed by sequencing is also considered to be a negative result. ~~As genetic recombination between genotypes can occur, the detection of any genotype is considered to be evidence of the presence of YHD.~~

7. Corroborative diagnostic criteria

7.1. Definition of suspect case

A suspect case of ~~YHD~~ YHV genotype 1 is defined as a disease outbreak in marine shrimp with rapidly accumulating mortalities (up to 100%) in the early to late juvenile stages, which may be preceded by cessation of feeding and congregation of shrimp at pond edges. Moribund shrimp may exhibit a bleached overall appearance and a yellowish discoloration of the cephalothorax caused by the underlying yellow hepatopancreas. Histological examination of fixed lymphoid organ tissues should reveal moderate to large numbers of deeply basophilic, evenly stained, spherical, cytoplasmic inclusions (approximately 2 µm in diameter or smaller).

7.2. Definition of confirmed case

YHD may be confirmed by the detection of high levels of disseminated infection in tissues of ectodermal and mesodermal origin by *in situ* hybridisation in conjunction with the detection of amplified products of the prescribed size using discriminatory RT-PCR assays and sequencing, as described in Section 4.3 of this chapter. As low-level chronic infections with yellow head complex viruses are common in some regions, detection of the presence of virus is not, in itself, evidence of aetiology.

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NB: There is an OIE Reference Laboratory for Yellow head 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 Yellow head disease

Annex 21

CHAPTER 2.4.7.

INFECTION WITH *PERKINSUS OLSENI*

1. Scope

For the purpose of this chapter, infection with *Perkinsus olseni* is considered to be infection with *P. olseni*. *Perkinsus atlanticus* is considered to be a synonym.

2. Disease information

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2.2. Host factors

2.2.1. Susceptible host species

Perkinsus olseni has an extremely wide host range. Known hosts include the clams *Anadara trapezia*, *Austrovenus stutchburyi*, *Ruditapes decussatus*, *R. philippinarum, Tridacna maxima, T. crocea, Protothaca jedoensis* and *Pitar rostrata* (Goggin & Lester, 1995; Villalba *et al.,* 2004; Cremonte *et al.,* 2005; Park *et al.,* 2006; Sheppard & Phillips, 2008); oysters ~~*Crassostrea gigas*~~, *Crassostrea* ~~*C.*~~ *ariakensis*, and *C. sikamea* (Villalba *et al.,* 2004); pearl oysters *Pinctada margaritifera, P. martensii*, and *P. fucata* (Goggin & Lester, 1995; Sanil *et al.,* 2010); abalone *Haliotis rubra, H. laevigata, H. scalaris,* and *H. cyclobates* (Goggin & Lester, 1995). Other bivalve and gastropod species might be susceptible to this parasite, especially in the known geographical range. Members of the families Arcidae, Malleidae, Isognomonidae, Chamidae and Veneridae are particularly susceptible, and their selective sampling may reveal the presence of *P. olseni* when only light infections occur in other families in the same habitat.

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NB: There is an OIE Reference Laboratory for Infection with *Perkinsus olseni*
(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 *Perkinsus olseni*

Annex 22

CHAPTER 1.1.

NOTIFICATION OF DISEASES, AND PROVISION OF EPIDEMIOLOGICAL INFORMATION

Article 1.1.1.

For the purposes of the *Aquatic Code* and in terms of Articles 5, 9 and 10 of the OIE Organic Statutes, every Member Country of the OIE shall recognise the right of the *Headquarters* to communicate directly with the *Competent Authority* of its *territory* or *territories*.

All *notifications* and all information sent by the OIE to the *Competent Authority* shall be regarded as having been sent to the country concerned and all *notifications* and all information sent to the OIE by the *Competent Authority* shall be regarded as having been sent by the country concerned.

Article 1.1.2.

1) Member Countries shall make available to other Member Countries, through the OIE, whatever information is necessary to minimise the spread of important *diseases* of *aquatic animals* and their *pathogenic agents* and to assist in achieving better world-wide control of these *diseases*.

2) To achieve this, Member Countries shall comply with the *notification* requirements specified in Articles 1.1.3. and 1.1.4.

3) To assist in the clear and concise exchange of information, reports shall conform as closely as possible to the current OIE *disease* reporting format.

4) The detection of the *pathogenic agent* of a *listed disease* in an *aquatic animal* should be reported, even in the absence of clinical signs. Recognising that scientific knowledge concerning the relationship between *pathogenic agents* and clinical *disease* is constantly developing and that the presence of an infectious agent does not necessarily imply the presence of clinical *disease*, Member Countries shall ensure through their reports that they comply with the spirit and intention of point 1 above.

5) In addition to notifying findings in accordance with Article 1.1.3. and 1.1.4., Member Countries shall also provide information on the measures taken to prevent the spread of *diseases*. Information shall include possible *quarantine* measures and restrictions on the movement of *aquatic animals*, *aquatic animal products*, *biological products* and other miscellaneous objects which could by their nature be responsible for transmission of *disease*. In the case of *diseases* transmitted by vectors, the measures taken against such vectors shall also be specified.

Article 1.1.3.

The *Competent Authority* shall, under the responsibility of the Delegate, send to the *Headquarters* of the OIE:

1) in accordance with relevant provisions in the *disease*-specific chapters, *notification*, through the World Animal Health Information System (WAHIS) or by fax or e-mail within 24 hours of any of the following events:

   a) first occurrence of a *listed disease* in a country, a *zone* or a *compartment*;

   b) re-occurrence of a *listed disease* in a country, a *zone* or a *compartment* following the final report that declared the *outbreak* ended;

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c) first occurrence of a new strain of a *pathogenic agent* of a *listed disease* in a country, a *zone* or a *compartment*;

d) a sudden and unexpected change in the distribution or increase in incidence or virulence of, or morbidity or mortality caused by the *pathogenic agent* of a *listed disease*, present within a country, a *zone* or a *compartment*;

e) occurrence of a *listed disease* in a new host species.

In deciding whether findings justify immediate *notification* (within 24 hours), Member Countries must ensure that they comply with the obligations of Chapters 5.1. and 5.2. (especially Article 5.1.1.), to report developments that may have implications for *international trade*;

2) weekly reports subsequent to a *notification* under point 1 above, to provide further information on the evolution of the event which justified the *notification*. These reports should continue until the *disease* has been eradicated or the situation has become sufficiently stable so that six-monthly reporting under point 3 will satisfy the obligation of the Member Country to the OIE; for each event notified, a final report on the event should be submitted;

3) six-monthly reports on the absence or presence and evolution of *listed diseases* and information of epidemiological significance to other Member Countries;

4) annual reports concerning any other information of significance to other Member Countries.

Article 1.1.4.

Competent Authorities shall, under the responsibility of the Delegate, send to the *Headquarters*:

1) a *notification* through WAHIS or by fax or e-mail, when an *emerging disease* has been detected in a country, a *zone* or a *compartment*;

2) periodic reports subsequent to a *notification* of an *emerging disease*~~, as described under point 1~~. These should continue ~~until~~:

a) for the time necessary to have reasonable certainty that:

~~b~~i) the *disease* has been eradicated; or

~~c~~ii) the situation has becomes ~~sufficiently~~ stable; or

b) until sufficient scientific information is available to determine whether it meets the criteria for listing.

Article 1.1.5.

1) The *Competent Authority* of a country in which an *infected zone* or *compartment* was located shall inform the *Headquarters* when this *zone* or *compartment* is free from the *disease*.

2) An *infected zone* or *compartment* for a particular *disease* shall be considered as such until freedom from the *disease* has been demonstrated in accordance with recommendations in Chapter 1.4. and the relevant recommendations described in the *disease*-specific chapters in Sections 8 to 11. ~~a period exceeding the *infective period* specified in the *Aquatic Code* has elapsed after the last reported *case* and when full prophylactic and appropriate *aquatic animal* health measures have been applied to prevent possible reappearance or spread of the *disease*. These measures will be found in detail in various *disease*-specific chapters of the *Aquatic Code*.~~

3) A Member Country may be considered to regain freedom from a specific *disease* when all relevant conditions given in the *Aquatic Code* have been fulfilled.

4) The *Competent Authority* of a Member Country which sets up one or several *free zones* or *free compartments* shall inform the *Headquarters*, giving necessary details, including the criteria on which the free status is based, the requirements for maintaining the status and indicating clearly the location of the *zones* or *compartments* on a map of the territory of the Member Country.

Article 1.1.6.

1) Although Member Countries are only required to notify *listed diseases*, and *emerging diseases*, they are encouraged to inform the OIE of other important *aquatic animal* health events.

2) The *Headquarters* shall communicate by e-mail or World Animal Health Information Database (WAHID) to *Competent Authorities* all *notifications* received as provided in Articles 1.1.2. to 1.1.5. and other relevant information.

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Annex 23

CHAPTER 1.2.

CRITERIA FOR ~~LISTING AQUATIC~~ ~~ANIMAL~~ THE INCLUSION OF DISEASES IN THE OIE LIST

Article 1.2.1.

Introduction

This chapter describes the criteria for the inclusion of *aquatic animal diseases* in the OIE list ~~listing *diseases* in Chapter 1.3.~~

The objective of listing is to support Member Countries~~'~~ by providing information needed to take appropriate action ~~efforts~~ to prevent the transboundary spread of important *diseases* of *aquatic animals*. This is achieved by ~~through~~ transparent, timely and consistent ~~reporting~~ *notification*.

For the *diseases* listed in accordance with Article 1.2.2., the corresponding *disease*-specific chapters ~~in the *Aquatic Code*~~ assist Member Countries in the harmonisation of *disease* detection, prevention and control and provide standards for safe *international trade* in *aquatic animals* and their products.

The requirements for *notification* of *listed diseases* are detailed in Chapter 1.1.

Principles for selection of diagnostic tests are described in Chapter 1.1.2 of the *Aquatic Manual*.

Article 1.2.2.

The c~~C~~riteria for the inclusion of a ~~listing an aquatic animal~~ *disease* in the OIE list are as follows:

~~*Diseases* proposed for listing should meet the relevant criteria as set out in A. Consequences, B. Spread and C. Diagnosis. Therefore, to be listed, a *disease* should have the following characteristics: 1 or 2 or 3; and 4 or 5; and 6; and 7; and 8. Such proposals should be accompanied by a *case definition* for the *disease* under consideration.~~

~~No.~~		~~Criteria for listing~~	~~Explanatory notes~~
~~A. Consequences~~			
~~1.~~OR	b.	The *disease* has been shown to cause a significant ~~production losses at a national or multinational (zonal or regional) level~~ impact on the health of *aquatic animals* at the level of country or a *zone* taking into account the occurrence and severity of the clinical signs, including direct production losses and mortality.	~~There is a general pattern that the disease will lead to losses in susceptible species, and that morbidity or mortality are related primarily to the infectious agent and not management or environmental factors. (Morbidity includes, for example, loss of production due to spawning failure.) The direct economic impact of the disease is linked to its morbidity, mortality and effect on product quality.~~

Annex 23 (contd)

~~2.~~OR	c.~~Or~~	The *disease* has been shown to, or scientific evidence indicates that it ~~is likely to~~ would, cause a significant impact on the health of ~~morbidity or mortality in~~ *wild aquatic animal* populations taking into account the occurrence and severity of the clinical signs, including direct production losses and mortality, and ecological threats.	~~Wild aquatic animal populations can be populations that are commercially harvested (wild fisheries) and hence are an economic asset. However, the asset could be ecological or environmental in nature, for example, if the population consists of an endangered species of aquatic animal or an aquatic animal potentially endangered by the disease.~~
AND			
~~3.~~4.	a.~~Or~~	~~The agent is of public health concern.~~ Natural transmission to humans has been proven, and human infection is associated with severe consequences.	
~~And B. Spread~~			
~~4.~~	-	~~Infectious aetiology of the disease is proven.~~	-
~~5.~~	~~Or~~	~~An infectious agent is strongly associated with the disease, but the aetiology is not yet known.~~	~~Infectious diseases of unknown aetiology can have equally high-risk implications as those diseases where the infectious aetiology is proven. Whilst disease occurrence data are gathered, research should be conducted to elucidate the aetiology of the disease and the results be made available within a reasonable period of time.~~
~~No.~~		**~~Criteria for listing~~**	**~~Explanatory notes~~**
~~And B. Spread~~			
~~6.~~1.	~~And~~	~~Likelihood of i~~International spread~~,~~ of the agent ~~including~~ (via live *aquatic animals*~~,~~or their products, or fomites) has been proven.	~~International trade in aquatic animal species susceptible to the disease exists or is likely to develop and, under international trading practices, the entry and establishment of the disease is likely.~~
AND			
~~7.~~2.	~~And~~	At least one ~~Several~~ *country*~~ies~~ ~~or countries with zones~~ has demonstrated freedom or impending freedom from the *disease* in populations of susceptible *aquatic animals*, ~~may be declared free of the disease~~ based on the ~~general surveillance~~ provisions ~~principles outlined in~~ of Chapters 1.4. and 1.5.	~~Free countries/zones could still be protected. Listing of diseases that are ubiquitous or extremely widespread would render notification unfeasible. However, individual countries that run a control programme on such a disease can propose its listing provided they have undertaken a scientific evaluation to support their request. Examples may be the protection of broodstock from widespread diseases, or the protection of the last remaining free zones from a widespread disease.~~

~~**And C. Diagnosis**~~			
AND			
~~8.~~3.		~~A repeatable and robust~~ Reliable means of detection~~/~~ and diagnosis exist~~s~~ and a precise *case* definition is available to clearly identify *cases* and allow them to be distinguished from other *diseases*.	~~A diagnostic test should be widely available and preferably has undergone a formal standardisation and validation process using routine field samples (See *Aquatic Manual*.) or a robust case definition is available to clearly identify cases and allow them to be distinguished from other pathologies.~~

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Annex 24

CHAPTER 9.2.

INFECTION WITH YELLOW HEAD VIRUS GENOTYPE 1

Article 9.2.1.

For the purposes of the *Aquatic Code*, infection with yellow head virus (YHD) means *infection* with yellow head virus genotype 1 (YHV). YHV is classified as a species in the genus *Okavirus*, family *Roniviridae* and order *Nidovirales*.

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

Article 9.2.2.

Scope

The recommendations in this chapter apply to: *Penaeus monodon*, *Penaeus vannamei*, *Penaeus stylirostris*, *Palaemonetes pugio* and *Metapenaeus affinis* ~~giant tiger prawn (*Penaeus monodon*), brown tiger prawn (*P. esculentus*) and Kuruma prawn (*P. japonicus*)~~. These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

[…]

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Annex 25

CHAPTER 2.2.X.

ACUTE HEPATOPANCREATIC NECROSIS DISEASE

1. Scope

For the purpose of this chapter, acute hepatopancreatic necrosis disease (AHPND), also known as early mortality syndrome (EMS), is considered to be infection with unique strains of *Vibrio parahaemolyticus*, namely AHPND-causing *V. parahaemolyticus* (VP_{AHPND}).

The disease has two distinct phases:

i) An acute phase characterised by acute progressive, massive degeneration of the hepatopancreas (HP) tubules from medial to dorsal, with significant rounding and sloughing of HP tubule epithelial cells into the HP tubules, HP collecting ducts and posterior stomach in the absence of bacterial cells (FAO, 2013; NACA, 2012; 2014; Nunan *et al.,* 2014; Soto-Rodriguez *et al.,* 2015; Tran *et al.*, 2013a; 2013b; 2014a; 2014b).

ii) The terminal stage is characterised by marked intra-tubular haemocytic inflammation and development of massive secondary bacterial infections that occur in association with the necrotic and sloughed HP tubule cells (FAO, 2013; NACA, 2012; 2014; Nunan *et al.,* 2014; Soto-Rodriguez *et al.,* 2015; Tran *et al.,* 2013a; 2013b; 2014a; 2014b).

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent, agent isolates

AHPND has a bacterial aetiology (Tran, 2013a; 2013b; Zhang *et al.,* 2012). It is caused by specific virulent strains of *Vibrio parahaemolyticus*, namely VP_{AHPND}, which contains one or more extrachromosomal plasmids, including a unique, previously unreported, large, plasmid with a size of ~70 kbp (Gomez-Gil *et al.,* 2014; Gomez-Jimenez *et al*., 2014; Kondo *et al.,* 2014; Yang *et al.,* 2014). This plasmid has been designated pVPA3-1, and its size may vary slightly. Removal (or "curing") of pVA1 abolishes the AHPND-causing ability of the virulent strain of *V. parahaemolyticus.* A pVA1-cured strain fails to induce the massive sloughing of cells in the hepatopancreatic tubules that is a primary histopathological characteristic of disease (Lee *et al.,* 2015).

VP_{AHPND} expresses a deadly plasmid-encoded toxin (Pir^{vp}), which is homologous to the Pir (*Photorhabdus* insect-related) binary toxin. The toxin is formed from two subunits, $PirA^{vp}$ and $PirB^{vp}$, but unlike other Pir binary toxins, *V. parahaemolyticus* PirB ($PirB^{vp}$, a 50.1 kDa protein) alone is capable of inducing AHPND histopathology in the hepatopancreatic tubules, while $PirA^{vp}$ (a 12.7 kDa protein) causes only minor histological changes (Han *et al.,* 2015; Lee *et al.,* 2015; Sirikharin *et al.,* 2015).

Within a population of AHPND-causing bacteria, natural deletion of the Pir^{vp} region may occur in a few individuals (Tinwongger *et al.,* 2014). This deletion is due to the instability caused by the repeat sequences/transposase that flank the pir toxin operon, and although different strains exhibit different levels of stability, when the deletion occurs, it means that a virulent strain of *V. parahaemolyticus* will lose its ability to induce AHPND. However, if the pir toxin sequence is used as a target for detection, then a colony that has this deletion will produce a negative result even though the colony was derived from an isolate of virulent bacteria.

The plasmid pVA1 also carries a cluster of genes related to conjugative transfer, which means that this plasmid is potentially able to transfer to other bacteria. So far, however, there have been no published reports that any bacteria other than *V. parahaemolyticus* carry pVA1. The pVA1 plasmid also carries the *pnd*A gene, which is associated with a post-segregational killing (psk) system. For a bacterium that

harbours a plasmid with the psk system (PSK^+), only progeny that inherit the PSK^+ plasmid will be viable. Progeny that do not inherit the PSK^+ plasmid will die because the stable *pnd*A mRNA will be translated to PndA toxin that will kill the bacterium. The presence of a psk system on a plasmid thus ensures that the plasmid is inherited during bacterial replication. The pVA1 plasmid will therefore be passed on to subsequent generations of VP_{AHPND} producing $PirA^{vp}$ and $PirB^{vp}$. Hence, when $PirA^{vp}$ is present there is little or no histopathology. When $PirB^{vp}$ is present, its larger size of 50.1 kDa is adequate to produce an enzyme that denudes the hepatopancreatic tubules (Lee *et al.,* 2015).

2.1.2. Survival outside the host (i.e. in the natural environment)

Not known.

2.1.3. Stability of the agent

Not known.

2.1.4. Life cycle

Not applicable.

2.2. Host factors

2.2.1. Susceptible host species (common and Latin names)

Penaeus vannamei (white leg or Pacific white shrimp); *P. monodon* (black tiger prawn) and *P. chinensis* (fleshy prawn).

2.2.2. Susceptible stages of the host

In the acute phase, this disease is characterised by a massive acute progressive degeneration of the HP tubules from medial to dorsal, with significant rounding and sloughing of HP tubule epithelial cells into the HP tubules, HP collecting ducts and posterior stomach in the absence of bacterial cells (FAO, 2013; Nunan *et al.,* 2014; Soto-Rodriguez *et al.,* 2015; Tran *et al.,* 2013a; 2013b; 2014a; 2014b).

In the terminal phase of ANDHP, is characterised by marked intra-tubular haemocytic inflammation and development of massive secondary bacterial infections that occur in association with the necrotic and sloughed HP tubule cells (FAO, 2013; Leaño & Mohan, 2013; NACA, 2012; 2014; Nunan *et al.,* 2014; Soto-Rodriguez *et al.,* 2015; Tran *et al.,* 2013a; 2013b; 2014a; 2014b).

2.2.3. Species or sub-population predilection (probability of detection)

Not applicable.

2.2.4. Target organs and infected tissue

Gut-associated tissues and organs.

2.2.5. Persistent infection with lifelong carriers

See Section 2.1.4 Life cycle.

2.2.6. Vectors

None are known.

2.2.7. Known or suspected wild aquatic animal carriers

None are known (except in South-East Asia, some molluscs and certain polychaetes).

2.3. Disease pattern

2.3.1. Transmission mechanisms

Mortalities are expected within 30 days of stocking shrimp ponds with postlarvae (PL) or juveniles (from 15 mg to ~1 g in weight) (Nunan *et al.,* 2014; Leaño & Mohan, 2013; Soto-Rodriguez *et al.,* 2015; Tran *et al.,* 2013b).

With laboratory infections, mortality can be induced within 12 hours of exposure to strains of VP_{AHPND} by the *per os* route if the coated feed contains 10^8 CFU (colony-forming units) per gram of inoculum (Nunan *et al.,* 2014; Soto-Rodriguez *et al.,* 2015; Tran *et al.,* 2013b).

Alternatively mortalities can be induced is with a bath challenge, provided that the challenge bath begins with 10^8 CFU per gram of inoculum (Nunan *et al.,* 2014; Soto-Rodriguez *et al.,* 2015; Tran *et al.,* 2013b).

2.3.2. Prevalence

Nunan *et al.,* 2014, Soto-Rodriguez *et al.,* 2015, and Tran *et al.,* 2013b found a near 100% prevalence in pond-reared stocks in South-East Asia and in Mexico after 2013.

2.3.3. Geographical distribution

The disease has been introduced into south-east China (People's Rep. of), Vietnam, Malaysia, Thailand and Mexico. In other countries in the East and South-East Asia regions, and neighbouring Mexico, farms may have been exposed to the toxin-producing strains of *V. parahaemolyticus* (Nunan *et al.,* 2014; Soto-Rodriguez *et al.,* 2015; Tran *et al.,* 2013b; Zhang *et al.*, 2012).

2.3.4. Mortality and morbidity

Joshi *et al.,* 2014a; 2014b; Nunan *et al.,* 2014; Soto-Rodriguez *et al.,* 2015; and Tran *et al.,* 2013b found a near 100% mortality and morbidity prevalence in pond-reared stocks in South-East Asia and in Mexico.

2.3.5. Environmental factors (e.g. temperature, salinity, season, etc.)

Water sources with low salinity (below ~5 to ~10 ppt) seem to reduce the prevalence of the disease. Although AHPND can be found year round in South-East Asia, the hot and dry season from April to July seems to be the peak. Bad feed management, algal bloom or crash are also factors that may lead to AHPND in endemic areas.

2.4. Control and prevention

2.4.1. Vaccination

Not applicable.

2.4.2. Chemotherapy

Not useful.

2.4.3. Immunostimulation

Not useful.

2.4.4. Resistance breeding

An AHPND line with some resistance to the disease has been developed in Mexico and in Ecuador. This was accomplished through mass selection over 10 years for growth and survival, rather than for SPF (specific-pathogen free) stock development (Lightner, unpublished data).

2.4.5. Restocking with resistant species

None available.

2.4.6. Blocking agents

None available.

2.4.7. Disinfection of eggs and larvae

None known.

2.4.8. General husbandry practices

None known.

3. Sampling

3.1. Selection of individual specimens

Samples of moribund shrimp or shrimp that show clinical signs (see Section 4.1.1) should be selected for AHPND detection. It is assumed that adults (broodstock) can carry toxin-bearing strains (especially PirB^{vp}) of *V. parahaemolyticus* (Han *et al.,* 2015; Lee *et al*., 2015; Nunan *et al.,* 2014; Soto-Rodriguez *et al.,* 2015; Tran *et al.,* 2013b). Therefore, broodstock without clinical signs may also be selected for testing, but only for testing for the presence of PirB^{vp} toxin.

3.2. Preservation of samples for submission

Carefully selected shrimp samples can be submitted to a variety of laboratories for diagnosis of AHPND. The samples can be submitted in 90% ethanol for polymerase chain reaction (PCR) detection, or preserved in Davison's AFA fixative for routine histopathology (Joshi *et al.,* 2014a; 2014b; Leaño & Mohan, 2013; Lee *et al.,* 2015; Nunan *et al.,* 2104; Sirikharin *et al.,* 2014; Soto-Rodriguez *et al.,* 2015; Tran *et al.,* 2013b).

3.3. Pooling of samples

For molecular testing, samples of shrimp cephalothoracies can be selected (pooled when less than 0.5 g).

Samples, especially PL or specimens up to 0.5 g can be pooled. Larger shrimp should not be pooled and should be processed individually (Lightner, unpublished data).

3.4. Best organs or tissues

Gut-associated tissues and organs, such as hepatopancreas, stomach, the midgut, the hindgut, and faeces of selected shrimp for samples. Valuable broodstock may be worth saving, and from these only faeces should be collected.

3.5. Samples/tissues that are not appropriate (i.e. when it is never possible to detect)

Samples other than gut-associated tissues and organs are not appropriate (FAO, 2013; NACA, 2012; 2014; Nunan *et al.,* 2014; Soto-Rodriguez *et al.,* 2015; Tran *et al.,* 2013b).

4. Diagnostic methods

4.1. Field diagnostic methods

4.1.1. Gross signs

The clinical signs could be used for presumptive diagnosis, which can be further confirmed by histopathology observed at the animal level include a pale to white HP due to pigment loss in the connective tissue capsule, significant atrophy of the HP, soft shells and guts with discontinuous contents or no contents, black spots or streaks sometimes visible within the HP, soft HP which does not squash easily between the thumb and forefinger, and the onset of clinical signs and mortality starting as early as 10 days post-stocking (NACA, 2012; 2014).

4.1.2. Behavioural changes

Not applicable.

4.2. Clinical methods

4.2.1. Clinical chemistry

None are known.

4.2.2. Microscopic pathology

An acute phase characterised by an acute, massive progressive degeneration of the HP tubules from medial to dorsal, with significant rounding and sloughing of HP tubule epithelial cells into the HP tubules, HP collecting ducts and posterior stomach in the absence of bacterial cells (FAO, 2013; Nunan *et al.,* 2014; Soto-Rodriguez *et al.,* 2015; Tran *et al.,* 2013a; 2013b; 2014a; 2014b).

The terminal phaseis characterised by marked intra-tubular haemocytic inflammation and development of massive secondary bacterial infections that occur in association with the necrotic and sloughed HP tubule cells (FAO, 2013; Leaño & Mohan, 2013; NACA, 2012; 2014; Nunan *et al.,* 2014; Soto-Rodriguez *et al.,* 2015; Tran *et al.,* 2013a; 2013b; 2014a; 2014b).

4.2.3. Wet mounts

Not applicable.

4.2.4. Smears

Not applicable.

4.2.5. Fixed sections (for ISH):

In-situ hybridisation is a useful technique provided it is done properly. The result of an ISH test will be apparent as a Bismarck Brown stained material will remain. This will be used to distinguish ANHPD tissues from those tissues which are not affected.

4.2.6. Electron microscopy/cytopathology

None reported to date (February 2015).

4.3. Agent detection and identification methods

4.3.1. Direct detection methods

4.3.1.1. Microscopic methods

See Section 2.2.2.

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

On marine or blood agar, the strains of VP_{AHPND} are capable of swarming (Han *et al.,* 2015). Hence, it is possible to isolate $PirB^{vp}$ toxin-producing forms of *V. parahaemolyticus* on standard media used for isolation of bacteria from diseased shrimp or other samples, especially because $PirB^{vp}$ produces a more potent toxin than $PirA^{vp}$ (Lee *et al.,* 2015; Soto-Rodriguez *et al.,* 2015). The identity of the *V. parahaemolyticus* may be confirmed by use of a PCR method to detect lecithin dependent haemolysin gene (Taniguchi *et al.,* 1985) and their probable ability to cause AHPND by PCR methods described in section 4.3.1.2.3. This must be followed by bioassay to confirm ability to cause AHPND.

4.3.1.2.1. Cell culture/artificial media

No methods are available.

4.3.1.2.2. Antibody-based antigen detection methods

None is available to date (February 2015).

4.3.1.2.3. Molecular techniques

4.3.1.2.3.1. PCR protocols for detection of AHPND causing bacteria from cultures or infected shrimp

Although the AP1 and AP2 methods are not recommended for detection of VP_{AHPND}, they are included here because they may find utility in studying the environmental prevalence and distribution of bacterial isolates carrying pVA1 plasmid variants that lack the $PirA^{vp}$ and $PirB^{vp}$ genes, would not cause AHPND and would give negative results with the methods recommended here for VP_{AHPND} detection.

Methods for detection of isolates of VP_{AHPND} by PCR have been developed. The most successful methods target the unique genes for AHPND toxin $Pir A^{vp}$ (12.7 kDa) and/or $PirB^{vp}$ (50.1 kDa) that together cause sloughing of shrimp hepatopancreatic cells. These toxin genes have been found as episomal elements in all AHPND isolates so far sequenced (Gomez-Gil *et al.,* 2014; Kondo *et al.,* 2014; Yang *et al.,* 2014) and GenBank Accession number KM067908. Two earlier, preliminary PCR detection methods (AP1 and AP2) that targeted the plasmid carrying the toxin genes (Yang *et al.,* 2014) were announced on the internet at the website of the Network of Aquaculture Centres in Asia-Pacific (NACA) in December 2013 (Flegel & Lo, 2014) but were later abandoned because of 3% false positive results from non-AHPND bacteria that carried the plasmid without the toxin genes.

To overcome the problem of false positive results, methods have been developed that target the AHPND toxin genes. The first such method (AP3) was announced in June 2014 and targeted the 12.7 kDa *pir*A^{vp} gene (Sirikharin *et al.,* 2014). It was validated for 100% positive and negative predictive value by testing with 104 AHPND-causing and non-pathogenic bacteria (including other *Vibrio* and non-*Vibrio* species) that had previously been confirmed by bioassay (Kwai *et al.,* 2014; Sirikharin *et al.,* 2015). A subsequent publication using 9 AHPND-causing and 11 non-pathogenic isolates of *V. parahaemolyticus* from Mexico (Soto-Rodriguez *et al.,* 2015) reported that the AP3 method gave the highest positive (90%) and negative (100%) predictive values of five PCR methods tested, including one commercial method.

The AP3 method and four other more recently published methods that target the AHPND *pir*A^{vp} gene (the $Pir^{vp}A$ method and the VpPirA-284) and *pir*B^{vp} ($Pir^{vp}B$ method and the VpPirB-392) are one-step PCR methods of relatively low sensitivity when used for detection of AHPND-causing bacteria at carrier levels or in environmental samples such as sediments and biofilms. For such samples, a preliminary enrichment step is recommended since experience has shown that these PCR methods are not sensitive enough to detect low numbers of bacterial cells at carrier levels and that adaptation to a nested PCR protocol was not successful due to the occurrence of non-specific amplicons.

An additional two-tube nested PCR method called AP4 has been devised and found to give 100% positive predictive value for AHPND-causing bacteria using the same 104 bacterial isolates used to validate AP3 above (announcement at www.enaca.org and manuscript in preparation). This method does not give rise to non-specific amplicons and has a minimum sensitivity for 1 fg of DNA extracted from AHPND-causing bacteria, allowing it to be used directly with tissue and environmental samples without an enrichment step.

4.3.1.2.3.1.1 Enrichment of samples prior to DNA extraction

Preliminary enrichment cultures for detection of AHPND-causing bacteria at carrier levels or in environmental samples may be carried out in any suitable medium (e.g. tryptic–soy broth) or alkaline peptone water containing 2.5% NaCl supplement) incubated for 4 hours at around 30°C with shaking. After this, let any debris settle, remove the cloudy supernatant for centrifugation to pellet the bacteria it contains and discard the supernatant solution. Extract DNA from the bacterial pellet.

4.3.1.2.3.1.2 Agent purification

The causative agent of AHPND may be isolated in pure culture from diseased shrimp, carrier shrimp or environmental samples using standard microbiological media used for isolation of *Vibrio* species from such sources (Lightner, 1996; Tran *et al*., 2013a; 2013b). Isolation of pure cultures must be followed by PCR analysis and/or bioassays to confirm the ability to cause AHPND.

AP1 (AHPND Primer set 1)

AP1F: 5'-CCT-TGG-GTG-TGC-TTA-GAG-GAT-G-3'

AP1R: 5'-GCA-AAC-TAT-CGC-GCA-GAA-CAC-C-3'

AP1 Amplicon sequence 700 bp (Lee *et al.,* 2015).

AP2 (AHPND Primer set 2)

AP2F: 5'-TCA-CCC-GAA-TGC-TCG-CTT-GTG-G-3'

AP2R: 5'-CGT-CGC-TAC-TGT-CTA-GCT-GAA-G-3'

AP2 Amplicon sequence 700 bp (Lee *et al.,* 2015).

4.3.1.2.3.1.3 DNA extraction

A general DNA extraction method may be used to extract DNA from the stomach or hepatopancreatic tissue of a putatively infected shrimp, from cultures of purified bacterial isolates or from bacterial pellets from enrichment cultures (see above). The amount of template DNA in a 25 µl PCR reaction volume should be in the range of 0.01–1 ng of DNA when extracted from bacterial isolates (i.e. directly from a purified culture) and in the range of 10–100 ng of total DNA when extracted from shrimp tissues or from a bacterial pellet from an enrichment culture.

4.3.1.2.3.1.4 PCR primers for one-step PCR detection of AHPND bacteria

Five one-step PCR methods called AP3, Pir^{vp}A, Pir^{vp}B, VpPirA and VpPirB have been developed (see above) for detection of VP_{AHPND}. The AP3, Pir^{vp}*A* and VpPirA methods target the *pir*A^{vp} gene while the Pir^{vp}*B* and VpPirB methods target the *pir*^{vp}*B* gene. These primers are listed in Table 4.1 together with the size of their expected amplicons.

Table 4.1. *PCR primers for one-step PCR detection of* VP_{AHPND}

Method name	Primers	Target gene	Expected amplicon size	Reference
AP3	AP3-F: 5'-ATG-AGT-AAC-AAT-ATA-AAA-CAT-GAA-AC-3' AP3-R: 5'-GTG-GTA-ATA-GAT-TGT-ACA-GAA-3'	*pir*A^{vp}	333	Sirikharin *et al*., 2014 Sirikharin *et al.,* 2015
Pir^{vp}A	*Pir*^{vp}*A* F: 5'-ATG-AGT-AAC-AAT-ATA-AAA-CAT-G-3' *Pir*^{vp}*A* R: 5'-TTA-GTG-GTA-ATA-GAT-TGT-ACA-G-3'	*pir*A^{vp}	336	Lee *et al.,* 2015
Pir^{vp}*B*	*PirvpB* F: 5'-GAG-CCA-GAT-ATT-GAA-AAC-ATT-TGG-3' *PirvpB* R: 5'-CCA-CGC-AGC-GAG-TTC-TGT-AAT-GTA-3'	*pirB*^{vp}	438	Lee *et al.,* 2015,
VpPirA-284	VpPirA-284F: 5'-TGA-CTA-TTC-TCA-CGA-TTG-GAC-TG-3' VpPirA-284R: 5'-CAC-GAC-TAG-CGC-CAT-TGT-TA-3'	*pir*A^{vp}	284	KM067908 Han *et al.,* 2015
VpPirB-392	VpPirB-392F: 5'-TGA-TGA-AGT-GAT-GGG-TGC-TC-3' VpPirB-392R: 5'-TGT-AAG-CGC-CGT-TTA-ACT-CA-3'	*pirB*^{vp}	392	KM067908 Han *et al.,* 2015

Note that the primer sequences and amplicons for the two methods that target the pir*A*^{vp} *gene differ slightly.*

4.3.1.2.3.1.5 PCR primers for nested PCR for detection of AHPND bacteria

A two-tube, nested PCR method called AP4 has been devised and found to give 100% positive predictive value for AHPND-causing bacteria using the same 104 bacterial isolates used to validate AP3 above (www.enaca.org) (Sritunyalucksana *et al.,* 2015). Nonspecific amplicons do not arise from this method and it has a minimum sensitivity for 1 fg of DNA

extracted from AHPND-causing bacteria, allowing it to be used directly with tissue and environmental samples that may have low levels of AHPND bacteria. The target sequence consists of a chimeric DNA fragment comprising the full *pir*A^{vp} gene sequence plus the 12 bp linker plus the full succeeding *pir*B^{vp} gene sequence for a total of 1269 bp. The first-step and second-step PCR primers are listed in Table 4.2 below. The primers were designed from the China (People's Rep. of) isolate of AHPND bacteria (Yang *et al.,* 2014). The expected amplicons are 1269 bp for the outer primers AP4-F1 and AP4-R1 and 230 bp for the inner primers AP4-F2 and AP4-R2. At high concentrations of target DNA, additional bands for amplicons may occur as the product of residual primer AP4-F1 working with AP4-R2 (357 bp) or AP4-F2 with AP4-R1 (1142 bp) in the nested step.

Table 4.2. *Primers for the AP4, two-step PCR method for detection of AHPND-causing bacteria*

Method name	Primers	Expected amplicon size	Reference
AP4 Step 1	AP4-F1: 5'-ATG-AGT-AAC-AAT-ATA-AAA-CAT-GAA-AC-3' AP4-R1: 5'-ACG-ATT-TCG-ACG-TTC-CCC-AA-3'	1269	Sirikharin *et al.,* 2015
AP4 Step 2	AP4-F2: 5'-TTG-AGA-ATA-CGG-GAC-GTG-GG-3' AP4-R2: 5'- GTT-AGT-CAT-GTG-AGC-ACC-TTC-3'	230	

Note that the AP4-F1 primer sequence is equal to that of AP3-F.

4.3.1.2.3.1.6 Protocol for the AP3, 1-step PCR method

This protocol follows the method described by Sirikharin *et al.* 2014. The PCR reaction mixture consists of 10x PCR mix (Invitrogen[5]) 2.5 µl, 50 mM $MgCl_2$, 0.7 µl, 10 mM dNTPs, 0.4 µl, 10 µM AP3-F1, 0.5 µl, 10 µM AP3-R1, 0.5 µl, 0.3 µl of Taq DNA pol (5 units μl^{-1}, Invitrogen) and 100 ng of template DNA in a total volume of 25 µl made up with distilled water. The PCR protocol is 94°C for 5 minutes followed by 30 cycles of 94°C for 30 seconds, 53°C for 30 seconds and 72°C for 40 seconds with a final extension step at 72°C for 5 minutes and hold at 4°C.

4.3.1.2.3.1.7 Protocol for the VpPirA-284 1-step PCR method

Perform PCR with PuReTaq ready-to-go PCR beads (GE Healthcare). This consists of a 3-minute step at 94°C to denature DNA prior to the primers binding and activation of the Taq DNA polymerase, followed by 35 cycles of 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds, and a final extension at 72°C for 7 minutes.

Prepare a 25 µl PCR reaction with a PuReTaq ready-to-go PCR bead. Each reaction contains 0.2 µM of each primer, 10 mM Tris/HCl (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$, 2.5 U of Taq DNA polymerase, and 1 µl of extracted DNA.

4.3.1.2.3.1.8 Protocol for the Pir^{vp}B 1-step PCR method

PCR consists of an initial preheating stage of 5 minutes at 94°C, followed by 25–30 cycles of 30 seconds denaturation at 94°C, 30 seconds annealing at 60°C and 60 seconds extension at 72°C, and a final 10 minutes extension at 72°C. The amplified PCR products are analysed in 2% agarose gels, stained with ethidium bromide, and visualised under ultraviolet transillumination. AHPND positive samples give a positive band at 336 bp and 400 bp with the PirA^{vp} and PirB^{vp} primer sets, respectively. No band is produced by non-AHPND samples (Lee *et al.,* 2015).

4.3.1.2.3.1.9 Protocol for the VpPirB-392 1-step PCR method

The protocol for this method is the same as that for the VpPirA-284 method above

[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*.

4.3.1.2.3.1.10 Protocol for the AP4 nested PCR method

This protocol follows the method described by Sritnyalucksana *et al.,* 2015. The first PCR reaction mixture consists of 10× PCR mix (Invitrogen) 2.5 µl, 50 mM $MgCl_2$, 1.5 µl, 10 mM dNTPs, 0.5 µl, 10 µM AP4-F1, 0.5 µl, 10 µM AP4-R1, 0.5 µl, 0.3 µl of Taq DNA pol (5 units μl^{-1}, Invitrogen) and 100 ng of template DNA in a total volume of 25 µl made up with distilled water. The PCR protocol is 94°C for 2 minutes followed by 30 cycles of 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 90 seconds with a final extension step at 72°C for 2 minutes and hold at 4°C.

The nested PCR reaction mixture consists of 10× PCR mix (Invitrogen) 2.5 µl, 50 mM $MgCl_2$, 1.5 µl, 10 mM dNTPs, 0.5 µl, 10 µM AP4-F2, 0.375 µl, 10 µM AP4-R2, 0.375 µl, 0.3 µl of Taq DNA pol (5 units μl^{-1}, Invitrogen) and 2 µl of the first PCR reaction in a total volume of 25 µl. The nested PCR protocol is 94°C for 2 minutes followed by 25 cycles of 94°C for 20 seconds, 55°C for 20 seconds and 72°C for 20 seconds and hold at 4°C.

4.3.1.2.3.1.11 Controls for all PCR methods

The following controls should be included in all AHPND PCR assays: a) DNA template extracted from a known negative sample, such as specific-pathogen-free shrimp tissues; b) DNA template from a known positive sample, including AHPND-affected shrimp tissue, or DNA from a VP_{AHPND} bacteria culture, or plasmid DNA that contains the target region of the specific set of primers; c) a none-template control, i.e. adding nuclease-free water as the template.

4.3.1.2.3.1.12 Analysis of PCR products by agarose gel electrophoresis

After PCR, load 5–10 µl of the PCR reaction mix onto a 1.5% agarose gel (containing 0.5 µg ml^{-1} ethidium bromide). Look for the expected amplicons appropriate for the PCR method used (Tables 4.1 and 4.2).

4.3.2. Serological methods

Not applicable.

5. Rating of tests against purpose of use

As an example, the methods currently available for targeted surveillance and diagnosis of AHPND 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	PL	Juveniles	Adults		
Gross signs	d	d	b	c	b	a
Bioassay	d	d	b	d	b	b
Direct LM	d	d	d	c	c	c
Histopathology	d	c	a	c	a	a
Transmission EM	d	d	d	d	d	a
PCR	d	b	a	a	a	a
Sequence	d	d	a	a	a	a

PL = postlarvae; LM = light microscopy; EM = electron microscopy; PCR = polymerase chain reaction.

Annex 25 (contd)

6. Test(s) recommended for targeted surveillance to declare freedom from AHPND

Two years of freedom from AHPND is adequate to declare freedom from the acute hepatopancreatic necrosis disease (VP_{AHPND}) (Leaño & Mohan, 2013; NACA, 2012; 2014; Nunan *et al.,* 2014; Soto-Rodriguez *et al.,* 2015; Tran *et al.,* 2013b).

7. Corroborative diagnostic criteria

7.1. Definition of suspect case

AHPND shall be suspected if at least one of the following criteria is met:

Histopathology indicative of AHPND

or

Detection of VP_{AHPND}

or

Mortality associated with clinical signs of AHPND.

7.2. Definition of confirmed case

AHPND is considered to be confirmed if the following criteria are met:

Detection of VP_{AHPND}

and

Histopathology indicative of AHPND

or

Mortality associated with clinical sings of AHPND

or

Positive results by bioassay.

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Annex 26

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

Original: English

January 2015

REPORT OF THE MEETING OF THE OIE AD HOC GROUP ON NOTIFICATION OF ANIMAL DISEASES AND PATHOGENIC AGENTS

Paris, 6–8 January 2015

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The OIE *ad hoc* Group on Notification of Animal Diseases and Pathogenic Agents met at the OIE Headquarters from 6 to 8 January 2015.

The members of the Group and other participants are listed in Appendix I. The meeting was chaired by Dr Toni Tana and Dr Allan Sheridan acted as rapporteur.

Dr Alex Thiermann, Advisor of the Director General and President of the Terrestrial Animal Health Code Commission, welcomed the participants on behalf of the Director General, Dr Bernard Vallat, and thanked them for having accepted the OIE's invitation. He reminded the participants of the importance of this Group in the OIE's work, gathering together many experts coming from different regions. He reiterated that this Group has a unique and critical mandate. The aim is to review disease listing criteria bearing in mind all diseases, not just specific ones considered important at particular times. He also reminded the Group that a listed disease was not more important than other diseases, but the criteria define it as a disease, based on its epidemiologic characteristics, that requires rapid dissemination of information to facilitate control efforts by the veterinary services. The Group was also asked to consider providing more clarity and discipline on how to report on emerging diseases and when reporting ceases to be necessary. The conclusions of this Group should also help the OIE to find a way to encourage Member Countries to improve the level and quality of disease notification and events reporting.

Dr Paula Cáceres, Head of World Animal Health Information and Analysis Department, presented the objectives of the meeting: to examine and evaluate the disease listing criteria in both the *Terrestrial* and *Aquatic Codes* for inclusion of diseases, infections and infestations in the OIE Lists. The Group was requested to assess the need for further definition of disease notification obligations of Member Countries for emerging diseases. The Group was also asked to assist the OIE considering deleting the reporting of non OIE-listed diseases in the annual report and, based on the amended Chapter 1.1 adopted by the OIE World Assembly of May 2014, replacing it by the information on emerging diseases declared endemic. Dr Cáceres also spoke with the Group on day two and clarified the description of the role and responsibilities of expert members that was presented by Dr Thiermann. The aim of an expert member of an *ad hoc* Group appointed by the OIE is to work to further the OIE's mission, bringing their unique regional experiences and awareness to the task at hand without advocating a position at odds with that of the OIE.

The Group reviewed and agreed to its terms of reference; they are listed in Appendix II.

Annex 26 (contd)

The Group endorsed the proposed agenda presented in Appendix III.

Dr Vallat, the Director General, joined the meeting on day two to support the activities of the Group. He reminded the Group that the OIE organises such Groups periodically to review existing standards and improve them on the basis on new scientific information. He highlighted that one of the main missions of the OIE is to promote the transparency of the animal health situation worldwide using appropriate standards and reporting mechanisms. He explained that the system for disease notification was built and modernised over years, with increasing requirements for data collection and reporting. He also stated that the OIE's capacity building work with Member Countries through the network of focal points aims to facilitate disease reporting and improve the quality of information. He emphasised the importance of recent changes in the definition and obligations of emerging diseases due to increasing numbers and importance of these diseases.

He reminded the Group that one of the main objectives should be to simplify the criteria for the listing of diseases for the benefit of Members. Dr Vallat emphasised that the OIE continued to work on modernising the WAHIS reporting system aiming to improve the mapping system, official status displaying, data mining and analysis of the information collected, as well as to include the information on genotype collected from the network of reference laboratories and the information related to antimicrobial resistance. He added also that three departments, namely the Scientific and Technical Department, the International Trade Department and the World Animal Health Information and Analysis Department, worked on the harmonisation of definitions between the *Terrestrial* and *Aquatic Codes* and WAHIS guidelines.

The desire is to have this report discussed by three Specialist Commissions concerned during their February 2015 meetings. Observers from the three Commissions whose work is directly linked to this *ad hoc* Group are listed in Appendix I as other participants who attended this meeting.

The Group appointed a Chair and rapporteur and briefly discussed their role and responsibilities. The Group agreed that it was critically important to remember that the criteria for listing of diseases could not be looked at in isolation from each other or other elements of Chapters 1.1 and 1.2 in each of the OIE *Terrestrial Animal Health Code* (*Terrestrial Code*) and *Aquatic Animal Health Code* (*Aquatic Code*). In addition the Group noted that the purpose of listing and reporting on diseases was subordinate to the OIE's overall mandate for animal health.

Following the meeting the proposed changes to the chapters were compiled and are presented in this report as the following Appendices:

Terrestrial Code, Chapter 1.1	Appendix IV
Aquatic Code, Chapter 1.1	Appendix V
Terrestrial Code, Chapter 1.2	Appendix VI
Aquatic Code, Chapter 1.2	Appendix VII (showing proposed changes)
Aquatic Code, Chapter 1.2	Appendix VIII (changes proposed)

Chapter 1.3 of the *Aquatic Code* is equivalent to Article 1.2.3. of the *Terrestrial Code*. It consists of the *Aquatic Code*'s listed diseases.

1. Examine and evaluate the disease listing criteria in the *Terrestrial* and *Aquatic Codes*

Chapter 1.2

The Group discussed the need to clearly define for Members the purposes for listing diseases contained in Chapter 1.2. of the *Terrestrial Code* and Chapter 1.3 of the *Aquatic Code* .

Annex 26 (contd)

The Group reviewed each article in the chapters related to the listing criteria for including terrestrial and aquatic animal diseases. The Group noted that there were some differences between the Chapters in the two Codes; however, and as per earlier requests by some Member Countries, the Group looked for ways to better harmonise the criteria for listing of terrestrial and aquatic diseases where it was feasible.

The Group noted that the titles of the two chapters and the first line of the introduction were different but conveyed the same information. For that reason the Group requested the Code Commissions to assess whether these aspects of the Chapters could be harmonised.

The Group also discussed the need for specificity when an *ad hoc* Group is appointed to consider whether a disease, infection or infestation is proposed for listing under these criteria. The Group advised the Code Commission representative that the criteria can be applied to either a disease or to a specified strain of a disease. The Group agreed it was essential that *ad hoc* groups are provided with clarity as to whether they are to consider a disease or a specified strain of a disease in their terms of reference.

Terrestrial Code: Article 1.2.1

The Group agreed that the aim of listing was to facilitate notification by and to the Member Countries to allow them to take appropriate and (where possible) co-ordinated action to prevent the spread of diseases as far as possible through control exercised by the veterinary authorities over animals and animal products. The Group agreed that this should be clear in the first paragraph of this article and recommended also insertion of the term 'timely' to highlight the importance of providing information soon enough so other Members can take effective action.

The Group agreed that the role of the *Terrestrial Code* in providing standards for disease control and safe trade in animals and animal products was important and should be mentioned. This had already been done in the *Aquatic Code* and insertion of that form of wording in the second paragraph of the introduction was recommended by the Group.

The Group agreed that details on the mechanisms for notification are provided in Chapter 1.1 and there was no benefit gained by restating them.

The Group further agreed that it would assist Members to have reference in this section to the principles for selection of an appropriate diagnostic test. This was discussed when considering point C. 8 of Article 1.2.2. of the *Aquatic Code*.

Aquatic Code: Article 1.2.1

The same rationale for amending the article in the *Terrestrial Code* was seen as appropriate in this instance.

Terrestrial Code: Article 1.2.2

Point 1. The Group was advised that two aspects of this article elicited Member comments – 'international spread' and 'proven'. After discussion the Group agreed that the term 'international spread' was clear and in need of no further elaboration. The Group also agreed that the term 'proven' had a clear scientific meaning in the context of the *Terrestrial Code* and that it was not to be considered as a legal term.

Point 2. The Group was advised by the Code Commission and agreed that the term 'demonstrated freedom' would incorporate historical disease freedom as per the provisions of Article 1.4.6. of the *Terrestrial Code*. The Code Commission further advised and the Group agreed that 'impending freedom' would be applicable to countries with control programmes with eradication as the end point in an advanced stage. In addition the Group was advised by the Code Commission and agreed that the 'negligible risk' categorisation of certain countries in respect of BSE would be equivalent to 'freedom' for this Article. The Group recommended to simplify the wording related to surveillance provisions in the *Terrestrial Code*, as has been done in the *Aquatic Code*.

Annex 26 (contd)

Point 3. a. The Group agreed to maintain this criterion as written.

Point 3.b. The Group was advised of comments by Members indicating a lack of consistent understanding of the terms 'significant morbidity and mortality'. Some members wished for quantification of incidence and some requested a specific definition for the term 'morbidity' as it applies to the *Terrestrial Code*. The Group discussed whether a definition of the term or further elaboration of its meaning within the article was the best way to improve clarity. After extensive discussion and review of a draft definition the Group agreed that simplification of the higher level statement and specification of the criteria that should be used within the article would be more useful. The use of the term 'significant impact on health' is now proposed to be accompanied by a mechanism by which it can be evaluated.

The Group also discussed the significance of positive serological results in relation to the listing criteria. A positive serological result, or titre, in an animal is evidence of prior exposure to an agent (micro-organism, protein, etc.) leading to an immunological response. However, exposure to an infectious agent may, or may not, result in illness in an individual. The Group agreed that positive serological results in the absence of clinical signs are not considered to be signs of disease or morbidity and are not to be considered as evidence of 'a significant impact on health'.

During these discussions the Group also considered whether additional criteria were necessary. One suggestion was to allow for relisting of a disease that has been delisted but for which control measures remain in place in a number of countries. The Group discussed this proposal and did not support the suggestion. Countries are allowed to implement animal health-based measures under WTO rules for non-listed diseases if they provide a risk assessment and the measures are the least-trade-restrictive that are necessary to protect that country's status. Delisting decisions are agreed at General Session when a disease does not meet the listing criteria. However, delisted diseases could be proposed for relisting if their behaviour changed in such a way that they subsequently met the listing criteria, so the Group did not see any benefit to include this proposal.

The Code Commission advised that use of the term '*zone*' in this article would include *containment zones* established to control disease.

Point 3.c. The Group aligned the wording of this point with that of point 3.b. In addition the Group agreed to change the term 'wild animal populations' to '*wildlife*'. '*Wildlife*' is defined in the *Terrestrial Code* and includes other wildlife categories of economic value previously excluded, in particular *captive wild animals*. The Group further considered this aspect in relation to the equivalent requirement in the *Aquatic Code* (point A. 2.of Article 1.2.2) and agreed that addition of the term 'ecological threats' was of significant value here as well.

Point 4. The Group agreed to maintain this criterion as written.

Re-ordering of the Article 1.2.2: The Group considered it easier for Members to apply the criteria if the only 'or' options were at the end of the section. Reordering to suit the suggestion was performed by moving the previous point 4 of Article 1.2.2. to point 3 of Article 1.2.2.

Aquatic Code: Article 1.2.2

The Group reviewed the reason for having explanatory notes in light of recent proposed changes to the *Aquatic Code*, and agreed to incorporate relevant information into the assessment criteria**.** In addition it was noted that removal of the explanatory notes means that the table format is no longer required and the Group recommends to the Code Commission alignment of the format of this Article with the corresponding Article 1.2.2. of the *Terrestrial Code*.

The Group further agreed that the second paragraph of Article 1.2.2. that describes in detail how to apply the criteria was unnecessary and that alignment of the first sentence of the article with that in the equivalent section of the Terrestrial Code was appropriate.

Annex 26 (contd)

No. A. 1. This article corresponds to point 3.b of Article 1.2.2. of the *Terrestrial Code*. The Group agreed that the new article in the *Terrestrial Code* was broader and should be proposed for use in this article of the *Aquatic Code*. The Group also agreed that the explanatory note referring to morbidity was liable to create confusion and was no longer required.

No. A. 2. This article corresponds to point 3. c. of Article 1.2.2. of the *Terrestrial Code*. The Group aligned the wording of this clause with that in point A.1 of Article 1.2.2. The Group decided following review of the explanatory notes that consideration of ecological aspects of the disease impact was of significant value given the broad mandate of the OIE. As the term 'ecological' also incorporates consideration of environmental factors the Group did not feel it was appropriate to add 'environmental' as was previously in the explanatory note. The explanatory note was no longer seen as necessary given these changes to the article.

No. A. 3. This article corresponds to point 3. a of Article 1.2.2. of the *Terrestrial Code*. The Group noted that this article did not incorporate the concept of severity of consequences, which the Group agreed was important. The Group reviewed use of the corresponding article in the *Terrestrial Code* and agreed to recommend that the same wording be used in the *Aquatic Code*.

No. B. 4. This article corresponds to no article in the *Terrestrial Code*. The Group agreed this article was no longer necessary as the glossary definition of 'disease' in the *Aquatic Code* specifies an infectious aetiology.

No. B. 5. This article corresponds to no article in the *Terrestrial Code*. The Group agreed this Article was not appropriate as a disease with a suspected infectious aetiology would be reported as an emerging disease (as defined in the glossary to the *Aquatic Code*).

No. B. 6. This article corresponds to point 1 of Article 1.2.2. of the *Terrestrial Code*. The Group noted when discussing this point that, in light of removal by the Code Commission of the article on emerging diseases from this chapter, the current wording was no longer appropriate. The Group agreed that use of the same wording as in the *Terrestrial Code* would be appropriate and that the information in the guidance note was not needed.

No. B. 7. This article corresponds to point 2 of Article 1.2.2. of the *Terrestrial Code*. The use of the term '*zone*' in this context was explained as covering bodies of water within a country as well as those that may be shared by a number of countries. The Group agreed that the competent authority of at least one country would need to propose 'freedom' as the term 'several countries' is undefined and if a single country is free then this status is worth protecting. The Group also discussed that the minimum requirement in the *Terrestrial Code* was the important feature. If one country could be free, others could take action to gain that same status and may be encouraged to do so. For these reasons the Group agreed to harmonise the text with that used in point 2 of Article 1.2.2. of the *Terrestrial Code* and remove the explanatory text.

No. C. 8. This article corresponds to point 4 of Article 1.2.2.. of the *Terrestrial Code*. The Group discussed the terms 'repeatable and robust' in relation to diagnostic testing. The single term ''reliable' is often used in relation to test performance and the Group agreed that this term could be used here together with some information on the criteria that can be applied when selecting a test for use. The Group reviewed the explanatory notes for this article and agreed that the appropriate chapter of the *Aquatic Manual*, Chapter 1.1.2, should provide that information for application by Members. It was further agreed that this would be best placed in the Introduction, under Article 1.2.1. Following these changes it was seen that the wording of the equivalent article in the *Terrestrial Code*, that includes specification of the need for case definition in the explanatory note, would be appropriate for the *Aquatic Code* as well. The explanatory note was then removed.

Re-ordering of points of the Article 1.2.2 of the *Aquatic Code*: For the same reason that reordering of these points was performed in the *Terrestrial Code*, and for harmonisation, reordering of the revised Article 1.2.2 was performed, as shown in Appendix VII (in tracked changes) and Appendix VIII (as a clean version with all suggested changes to that chapter accepted).

Annex 26 (contd)

Terrestrial Code: Article 1.2.3

While no changes to the text were made, the Group agreed that it was worthwhile asking the Code Commission to consider splitting Article 1.2.3. of the *Terrestrial Code*, which includes all diseases currently listed in the *Terrestrial Code*, into a separate chapter. This has been done in the *Aquatic Code*, where Chapter 1.3 is the disease list. The Group considered there could be advantages in that approach as a change suggested to the *Terrestrial Code*'s disease list would then purely affect the list and not open the criteria for review without reason.

2. Assessment of the need for further definition of disease notification obligations of Member Countries for emerging diseases

The Group sought clarification of what the OIE was seeking by raising this agenda item. Dr Caceres presented the current situation related to the *notification* of *emerging diseases* and a flow chart describing the World Animal Health Information System (WAHIS). The Group was informed that, once an *emerging disease* has been declared as endemic or stable, a country is no longer required to provide the OIE with further information concerning the disease. Is there a need to change point 1.1.4 in the *Terrestrial Code* to facilitate on-going reporting of information on these diseases?

The Group discussed the provisions of Article 1.1.4 of the *Terrestrial Code* regarding *notification* of *emerging diseases*. The Group agreed that the phrase in point 2 of Article 1.1.4. 'as described under point 1' was unnecessary and poorly referenced. The Group proposed it be deleted.

A suggestion was made that point 2 of Article 1.1.4. might benefit from having a time period specified during which countries would be required to continue submitting reports. That would be cut short if listing was proposed or the disease became sufficiently stable. The Group discussed this point in relation to whether a net benefit would be gained from the additional reporting that may occur. The Group agreed that the existing criteria for reporting ensure that the situation on a country is well described for other Members. In addition, the existing criteria allow for a return to reporting of the disease under appropriate circumstances so there is no net benefit by mandating a time period. The discussion did include however that there needs to be reliability of reporting up to the time a disease was sufficiently stable, or eradicated. The Group agreed to incorporate the phrase 'sufficient time to have reasonable certainty that' in the first sentence of point 2 of Article 1.1.4. for that reason and amend the punctuation of sub points a., b. and c. to clarify that reporting should continue until either the disease has been eradicated or becomes sufficiently stable within the country, or until it has been assessed for listing. The Group agreed that determining whether a disease was emerging and whether it had met either of the first two of those criteria for ceasing reporting was the responsibility of the Member country's Delegate.

The Group considered whether the WAHIS/WAHID system facilitates reporting in line with the *Terrestrial Code* requirements of 1.1.3 and 1.1.6. After discussion it was agreed the existing system does not support countries to supply the data mandated in point 4 of Article 1.1.3. In addition, the level of detail requested in WAHIS is not reflected in the mandatory reporting requirements of Article 1.1.3.

The Group recommends that the OIE consider appointing an *ad hoc* Group to refine WAHIS and Article 1.1.3. of the *Terrestrial Code* in order to define clearly for Members the level of compulsory data that is expected in reports. The Group agreed that this *ad hoc* Group should also consider changes to the WAHIS system to facilitate the agreed reporting in an appropriate manner. This should include facilitation of reporting of non-listed diseases (including those previously delisted) under Article 1.1.6 that Members consider would be of use to other Members and should also be consistent with Member's obligations under Article 1.1.4.

Annex 26 (contd)

3. In case of significant proposed changes in criteria, analyse and comment on the results of recent *ad hoc* Groups on some emerging diseases (e.g. PED, MERS, Schmallenberg)

The Group analysed the newly proposed criteria for the listing of diseases and agreed that the changes it recommended were to clarify already existing criteria. As there were no major modifications proposed the Group was not requested, under its Terms of Reference, to review the reports on *emerging diseases* such as PED, MERS and Schmallenberg.

4. Analyse new emerging diseases such as Ebola and the consequences for disease information reporting

Dr Marija Popovic, chargée de mission at the World Animal Health Information and Analysis Department, gave a brief background and on the evolution on the voluntary reporting of non OIE listed wildlife diseases using the spread sheet questionnaire and lately by the *WAHIS –Wild* platform. She stated that Ebola virus disease is classified under the disease "*infection with filovirus"* and that this include Marburg virus. The reporting was developed to provide Member Countries with an early warning system as the diseases involved had an impact on livestock health, human health, wildlife conservation, biodiversity and environmental integrity.

Dr Popovic advised that no quantitative data has been received so far from any Member Country on Ebola. The OIE followed up unofficial information in the last quarter of 2014 regarding domestic pigs affected by Ebola disease but the respective Member Country advised the information was incorrect.

The Group critically assessed the consequences of reporting on new *emerging diseases* by Members. Regarding Ebola, as specified by the Terms of Reference, the Group agreed it was not competent to assess Ebola disease (*infection with filovirus*). The Group then considered whether listing would be of value to Members if Ebola could be seen to meet the amended listing criteria.

The Group acknowledged that Ebola is an important disease due to its potential impact on human health. However, it needs to be considered in the framework of Article 1.2.1 with the purpose of listing in mind. If listing and consequent mandatory reporting of information on a given disease facilitates the taking of appropriate action by Members to prevent transboundary spread of that disease then listing is consistent with the mission of the OIE. The Group agreed that, for Ebola, it was difficult to see how listing would achieve that outcome. The Group discussed that this situation illustrated how important it was, not to apply the listing criteria to a disease without considering the broader context of the OIE's mission in terms of official notification, particularly as captured in the revised Article 1.2.1. Members with Ebola can nevertheless provide reports as it could be considered an emerging disease or voluntary reporting may be considered under Article 1.1.6 as an important animal health event. The Group agreed in relation to Ebola that Members should be encouraged to report.

5. Consider the deletion of reporting non-OIE-listed diseases in the annual report (Article 1.1.3 point 4) and consider replacing it by the information on emerging diseases declared endemic (Article 1.1.4)

The Group reviewed point 4 of Article 1.1.3. of the *Terrestrial Code* regarding the *notification* of diseases, *infections* and *infestations*, and provision of epidemiological information particularly related to the annual report.

Dr Cáceres briefly informed the Group about the current content of the annual report.

The Group discussed the content of the annual report on the non OIE-Listed diseases. The Group agreed that the information requested in the annual report on non-listed diseases is not supported by point 4 of Article 1.1.3. The discussion included consideration of how useful this information is for Members on the basis of advice from the OIE advisers present that the reports are rarely if ever interrogated by Members. The Group agreed that it was no longer appropriate to include these diseases in WAHIS and suggested that the WAHIS form be changed to reflect this.

Annex 26 (contd)

The Group agreed that the WAHIS system for gathering disease information should be flexible, facilitating the input of information on the reporting form that Members consider may be of use for other Members, and reiterated that this was not the case at present. The Group agreed that allowing provision of free text in an 'other comments' box could also be considered to encourage the Member Countries to provide information as per Article 1.1.6. The Group considered a suggestion that OIE should encourage Members to provide data on emerging diseases and that this be done by providing a specific area on the OIE website where emerging diseases are listed. While not covered by the Terms of Reference, the Group discussed this point and considered that its usefulness could be assessed by the suggested *ad hoc* Group for the WAHIS system if appointed by OIE.

The Group agreed that the official data collection should be focused on the OIE-Listed and *emerging diseases*. Previous recommendations by the group on appointing an *ad hoc* Group to review the WAHIS and WAHID systems were reiterated.

6. Other business

The Group discussed the definitions of *emerging disease* in the *Terrestrial* and *Aquatic Codes*. The Group agreed that, while it would be better for Members if the definitions were consistent, it did not have sufficient background information to consider whether there were necessary reasons for the differences in the definitions. The Group suggested both Code Commissions to work together to assess whether and how it would be possible to more closely harmonise the definitions of 'emerging disease' in both Codes.

7. Finalisation and adoption of the draft report

The Group finalised and adopted the draft report

…/Appendices

Annex 26 (contd)

Appendix I

MEETING OF THE

***AD HOC* GROUP ON NOTIFICATION OF ANIMAL DISEASES AND PATHOGENIC AGENTS**

Paris, 6–8 January 2015

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Annex 26 (contd)

Appendix II

TERMS OF REFERENCE
OF
THE *AD HOC* GROUP ON NOTIFICATION OF ANIMAL DISEASES AND PATHOGENIC AGENTS

Paris, 6–8 January 2015

The *ad hoc* Group is kindly requested to:

1) On the basis of Chapter 1.2 of the OIE *Terrestrial Animal Health Code* and *Aquatic Animal Health Code*, assist the OIE in addressing the following points:

   a) Examine and evaluate the disease listing criteria for inclusion of diseases, infections and infestations in the OIE List.

   b) Assess the need for further definition of disease notification obligations of Member Countries for emerging diseases and modification of the reporting obligations when an emerging disease becomes endemic.

   c) In case of significant proposed changes in criteria, analyse and comment on the results of recent *ad hoc* Groups on some emerging diseases (e.g. PED, MERS, Schmallenberg).

   d) Analyse new emerging diseases such as Ebola and the consequences for disease information reporting.

2) On the basis of the adopted amended Chapter 1.1 during the OIE World Assembly of May 2014, assist the OIE in addressing the following point:

   a) Consider the deletion of reporting non-OIE-listed diseases in the annual report (Article 1.1.3 point 4) and consider replacing it by the information on emerging diseases declared endemic (Article 1.1.4).

3) Any other business

Annex 26 (contd)

Appendix III

MEETING OF THE

***AD HOC* GROUP ON NOTIFICATION OF ANIMAL DISEASES AND PATHOGENIC AGENTS**

Paris, 6–8 January 2015

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Agenda

1. Opening

2. Appointment of chairperson and rapporteur

3. Terms of reference for the *ad hoc* Group meeting

   3.1. Examine and evaluate the disease listing criteria for inclusion of diseases, infections and infestations in the OIE List.

   3.2. Assess the need for further definition of disease notification obligations of Member Countries for emerging diseases and modification of the reporting obligations when an emerging disease becomes endemic.

   3.3. In case of significant proposed changes in criteria, analyse and comment on the results of recent *ad hoc* Groups on some emerging diseases (e.g. PED, MERS, Schmallenberg).

   3.4. Analyse new emerging diseases such as Ebola and the consequences for disease information reporting.

   3.5. Consider the deletion of reporting non-OIE-listed diseases in the annual report (Article 1.1.3 point 4) and consider replacing it by the information on emerging diseases declared endemic (Article 1.1.4).

4. Any other business

5. Finalisation and adoption of the draft report

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Annex 26 (contd)

Appendix IV

CHAPTER 1.1.

NOTIFICATION OF DISEASES, INFECTIONS AND INFESTATIONS, AND PROVISION OF EPIDEMIOLOGICAL INFORMATION

Article 1.1.1.

For the purposes of the *Terrestrial Code* and in terms of Articles 5, 9 and 10 of the OIE Organic Statutes, Member Countries shall recognise the right of the *Headquarters* to communicate directly with the *Veterinary Authority* of its territory or territories.

All *notifications* and all information sent by the OIE to the *Veterinary Authority* shall be regarded as having been sent to the country concerned and all *notifications* and all information sent to the OIE by the *Veterinary Authority* shall be regarded as having been sent by the country concerned.

Article 1.1.2.

1) Member Countries shall make available to other Member Countries, through the OIE, whatever information is necessary to minimise the spread of important animal *diseases*, and their aetiological agents, and to assist in achieving better worldwide control of these *diseases*.

2) To achieve this, Member Countries shall comply with the *notification* requirements specified in Articles 1.1.3. and 1.1.4.

3) To assist in the clear and concise exchange of information, reports shall conform as closely as possible to the official OIE *disease* reporting format.

4) The detection of the aetiological agent of a *listed disease* in an *animal* should be reported, even in the absence of clinical signs. Recognising that scientific knowledge concerning the relationship between *diseases* and their aetiological agents is constantly developing and that the presence of an aetiological agent does not necessarily imply the presence of a *disease*, Member Countries shall ensure, through their reports, that they comply with the spirit and intention of point 1 above.

5) In addition to notifying new findings in accordance with Articles 1.1.3. and 1.1.4., Member Countries shall also provide information on the measures taken to prevent the spread of *diseases*, *infections* and *infestations*. Information shall include quarantine measures and restrictions on the movement of *animals*, animal products, biological products and other miscellaneous objects which could by their nature be responsible for their transmission. In the case of *diseases* transmitted by *vectors*, the measures taken against such *vectors* shall also be specified.

Article 1.1.3.

Veterinary Authorities shall, under the responsibility of the Delegate, send to the *Headquarters*:

1) in accordance with relevant provisions in the *disease*-specific chapters, *notification* through the World Animal Health Information System (WAHIS) or by fax or e-mail, within 24 hours, of any of the following events:

   a) first occurrence of a *listed disease*, *infection* or *infestation* in a country, a *zone* or a *compartment*;

   b) re-occurrence of a *listed disease*, *infection* or *infestation* in a country, a *zone* or a *compartment* following the final report that declared the *outbreak* ended;

   c) first occurrence of a new strain of a pathogen of a *listed disease*, *infection* or *infestation* in a country, a *zone* or a *compartment*;

Annex 26 (contd)

Appendix IV (contd)

d) a sudden and unexpected change in the distribution or increase in incidence or virulence of, or morbidity or mortality caused by, the aetiological agent of a *listed disease*, *infection* or *infestation* present within a country, a *zone* or a *compartment*;

e) occurrence of a *listed disease*, *infection* or *infestation* in an unusual host species;

2) weekly reports subsequent to a *notification* under point 1 above, to provide further information on the evolution of the event which justified the *notification*. These reports should continue until the *disease*, *infection* or *infestation* has been eradicated or the situation has become sufficiently stable so that six-monthly reporting under point 3 will satisfy the obligation of the Member Country; for each event notified, a final report on the event should be submitted;

3) six-monthly reports on the absence or presence, and evolution of *listed diseases*, *infections* or *infestations* and information of epidemiological significance to other Member Countries;

4) annual reports concerning any other information of significance to other Member Countries.

Article 1.1.4.

Veterinary Authorities shall, under the responsibility of the Delegate, send to the *Headquarters*:

1) a *notification* through WAHIS or by fax or e-mail, when an *emerging disease* has been detected in a country, a *zone* or a *compartment*;

2) periodic reports subsequent to a *notification* of an *emerging disease*~~, as described under point 1~~. These should continue ~~until~~ for sufficient time to have reasonable certainty that:

a) the *disease*, *infection* or *infestation* has been eradicated; or

b) the situation becomes sufficiently stable;

or until

c) sufficient scientific information is available to determine whether it meets the criteria for listing.

Article 1.1.5.

1) The *Veterinary Authority* of a country in which an *infected zone* was located shall inform the *Headquarters* when this zone is free from the *disease*, *infection* or *infestation*.

2) An *infected zone* for a particular *disease*, *infection* or *infestation* shall be considered as such until a period exceeding the *infective period* specified in the *Terrestrial Code* has elapsed after the last reported *case*, and when full prophylactic and appropriate animal health measures have been applied to prevent possible reappearance or spread of the *disease*, *infection* or *infestation*. These measures will be found in detail in the various chapters of Volume II of the *Terrestrial Code*.

3) A Member Country may be considered to regain freedom from a specific *disease*, *infection* or *infestation* when all relevant conditions given in the *Terrestrial Code* have been fulfilled.

4) The *Veterinary Authority* of a Member Country which sets up one or several *free zones* shall inform the *Headquarters* giving necessary details, including the criteria on which the free status is based, the requirements for maintaining the status and indicating clearly the location of the *zones* on a map of the territory of the Member Country.

Annex 26 (contd)

Appendix IV (contd)

Article 1.1.6.

1) Although Member Countries are only required to notify *listed diseases*, *infections* and *infestation* and *emerging diseases*, they are encouraged to inform the OIE of other important animal health events.

2) The *Headquarters* shall communicate by e-mail or World Animal Health Information Database (WAHID) to *Veterinary Authorities* all *notifications* received as provided in Articles 1.1.2. to 1.1.5. and other relevant information.

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Annex 26 (contd)

Appendix V

CHAPTER 1.1.

NOTIFICATION OF DISEASES, AND PROVISION OF EPIDEMIOLOGICAL INFORMATION

Article 1.1.1.

For the purposes of the *Aquatic Code* and in terms of Articles 5, 9 and 10 of the OIE Organic Statutes, every Member Country of the OIE shall recognise the right of the *Headquarters* to communicate directly with the *Competent Authority* of its *territory* or *territories*.

All *notifications* and all information sent by the OIE to the *Competent Authority* shall be regarded as having been sent to the country concerned and all *notifications* and all information sent to the OIE by the *Competent Authority* shall be regarded as having been sent by the country concerned.

Article 1.1.2.

1) Member Countries shall make available to other Member Countries, through the OIE, whatever information is necessary to minimise the spread of important *diseases* of *aquatic animals* and their *pathogenic agents* and to assist in achieving better world-wide control of these *diseases*.

2) To achieve this, Member Countries shall comply with the *notification* requirements specified in Articles 1.1.3. and 1.1.4.

3) To assist in the clear and concise exchange of information, reports shall conform as closely as possible to the current OIE *disease* reporting format.

4) The detection of the *pathogenic agent* of a *listed disease* in an *aquatic animal* should be reported, even in the absence of clinical signs. Recognising that scientific knowledge concerning the relationship between *pathogenic agents* and clinical *disease* is constantly developing and that the presence of an infectious agent does not necessarily imply the presence of clinical *disease*, Member Countries shall ensure through their reports that they comply with the spirit and intention of point 1 above.

5) In addition to notifying findings in accordance with Article 1.1.3. and 1.1.4., Member Countries shall also provide information on the measures taken to prevent the spread of *diseases*. Information shall include possible *quarantine* measures and restrictions on the movement of *aquatic animals*, *aquatic animal products*, *biological products* and other miscellaneous objects which could by their nature be responsible for transmission of *disease*. In the case of *diseases* transmitted by vectors, the measures taken against such vectors shall also be specified.

Article 1.1.3.

The *Competent Authority* shall, under the responsibility of the Delegate, send to the *Headquarters* of the OIE:

1) in accordance with relevant provisions in the *disease*-specific chapters, *notification*, through the World Animal Health Information System (WAHIS) or by fax or e-mail within 24 hours of any of the following events:

   a) first occurrence of a *listed disease* in a country, a *zone* or a *compartment*;

   b) re-occurrence of a *listed disease* in a country, a *zone* or a *compartment* following the final report that declared the *outbreak* ended;

   c) first occurrence of a new strain of a *pathogenic agent* of a *listed disease* in a country, a *zone* or a *compartment*;

Annex 26 (contd)

Appendix V (contd)

d) a sudden and unexpected change in the distribution or increase in incidence or virulence of, or morbidity or mortality caused by the *pathogenic agent* of a *listed disease*, present within a country, a *zone* or a *compartment*;

e) occurrence of a *listed disease* in a new host species.

In deciding whether findings justify immediate *notification* (within 24 hours), Member Countries must ensure that they comply with the obligations of Chapters 5.1. and 5.2. (especially Article 5.1.1.), to report developments that may have implications for *international trade*;

2) weekly reports subsequent to a *notification* under point 1 above, to provide further information on the evolution of the event which justified the *notification*. These reports should continue until the *disease* has been eradicated or the situation has become sufficiently stable so that six-monthly reporting under point 3 will satisfy the obligation of the Member Country to the OIE; for each event notified, a final report on the event should be submitted;

3) six-monthly reports on the absence or presence and evolution of *listed diseases* and information of epidemiological significance to other Member Countries;

4) annual reports concerning any other information of significance to other Member Countries.

Article 1.1.4.

Competent Authorities shall, under the responsibility of the Delegate, send to the *Headquarters*:

1) a *notification* through WAHIS or by fax or e-mail, when an *emerging disease* has been detected in a country, a *zone* or a *compartment*;

2) periodic reports subsequent to a *notification* of an *emerging disease*, ~~as described under point 1~~. These should continue ~~until~~ for sufficient time to have reasonable certainty that:

a) the *disease* has been eradicated; or

b) the situation becomes sufficiently stable;

or until

c) sufficient scientific information is available to determine whether it meets the criteria for listing.

Article 1.1.5.

1) The *Competent Authority* of a country in which an *infected zone* or *compartment* was located shall inform the *Headquarters* when this *zone* or *compartment* is free from the *disease*.

2) An *infected zone* or *compartment* for a particular *disease* shall be considered as such until a period exceeding the *infective period* specified in the *Aquatic Code* has elapsed after the last reported *case* and when full prophylactic and appropriate *aquatic animal* health measures have been applied to prevent possible reappearance or spread of the *disease*. These measures will be found in detail in various *disease*-specific chapters of the *Aquatic Code*.

3) A Member Country may be considered to regain freedom from a specific *disease* when all relevant conditions given in the *Aquatic Code* have been fulfilled.

4) The *Competent Authority* of a Member Country which sets up one or several *free zones* or *free compartments* shall inform the *Headquarters*, giving necessary details, including the criteria on which the free status is based, the requirements for maintaining the status and indicating clearly the location of the *zones* or *compartments* on a map of the territory of the Member Country.

Annex 26 (contd)

Appendix V (contd)

Article 1.1.6.

1) Although Member Countries are only required to notify *listed diseases*, and *emerging diseases*, they are encouraged to inform the OIE of other important *aquatic animal* health events.

2) The *Headquarters* shall communicate by e-mail or World Animal Health Information Database (WAHID) to *Competent Authorities* all *notifications* received as provided in Articles 1.1.2. to 1.1.5. and other relevant information.

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Annex 26 (contd)

Appendix VI

CHAPTER 1.2.

CRITERIA FOR THE INCLUSION OF DISEASES, INFECTIONS AND INFESTATIONS IN THE OIE LIST

Article 1.2.1.

Introduction

~~The aim of t~~This chapter ~~is to~~ describe~~s~~ the criteria for the inclusion of *diseases*, *infections* and *infestations* on the OIE list.

The objective of listing is to support Member Countries~~'~~ by providing information needed to take appropriate action ~~efforts~~ to prevent the transboundary spread of important animal *diseases*, including *zoonoses*~~,~~. This is achieved by ~~through~~ transparent, timely and consistent *notification* ~~reporting~~.

Each *listed disease* normally has a corresponding chapter that ~~to~~ assists Member Countries in the harmonisation of *disease* detection, prevention and control~~.~~ and provides standards for safe *international trade* in *animals* and their products.

Requirements for *notification* are detailed in Chapter 1.1. ~~and *notifications* are to be made through WAHIS or, if not possible, by fax or e-mail as described in Article 1.1.3.~~

Principles for selection of diagnostic tests are described in Chapter 1.1.5 of the *Terrestrial Manual*.

Article 1.2.2.

The criteria for the inclusion of a *disease, infection* or *infestation* in the OIE list are as follows:

1) International spread of the agent (via live *animals* or their products, *vectors* or fomites) has been proven.

AND

2) At least one country has demonstrated freedom or impending freedom from the *disease*, *infection* or *infestation* in populations of susceptible *animals*, based on the ~~animal health surveillance~~ provisions of ~~the *Terrestrial Code*, in particular those contained in~~ Chapter 1.4.

AND

3) ~~A~~ Reliable means of detection and diagnosis exist~~s~~ and a precise *case* definition is available to clearly identify *cases* and allow them to be distinguished from other *diseases*, *infections* and *infestations*.

AND

~~3.~~4)

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

OR

b) The *disease* has been shown to cause a significant impact on the health of ~~morbidity or mortality in~~ domestic *animals* at the level of a country or a *zone* taking into account the occurrence and severity of the clinical signs, including direct production losses and mortality.

Annex 26 (contd)

Appendix VI (contd)

OR

c) The *disease* has been shown to, or scientific evidence indicates that it would, cause a significant impact on the health of ~~morbidity or mortality in *wild*~~ *wildlife* ~~*animal*~~ ~~populations~~ taking into account the occurrence and severity of the clinical signs, including direct production losses and mortality, and ecological threats.

~~AND~~

~~4. A reliable means of detection and diagnosis exists and a precise *case* definition is available to clearly identify *cases* and allow them to be distinguished from other *diseases*, *infections* and *infestations*.~~

Article 1.2.3.

The following *diseases, infections* and *infestations* are included in the OIE list.

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

1) The following are included within the category of multiple species *diseases*, *infections* and *infestations*:

- Anthrax
- Bluetongue
- Brucellosis (*Brucella abortus*)
- Brucellosis (*Brucella melitensis*)
- Brucellosis (*Brucella suis*)
- Crimean Congo haemorrhagic fever
- Epizootic haemorrhagic disease
- Equine encephalomyelitis (Eastern)
- Foot and mouth disease
- Heartwater
- Infection with Aujeszky's disease virus
- Infection with *Echinococcus granulosus*
- Infection with *Echinococcus multilocularis*
- Infection with rabies virus
- Infection with Rift Valley fever virus
- Infection with rinderpest virus
- Infection with *Trichinella* spp.
- Japanese encephalitis
- New World screwworm (*Cochliomyia hominivorax*)
- Old World screwworm (*Chrysomya bezziana*)
- Paratuberculosis
- Q fever
- Surra (*Trypanosoma evansi*)
- Tularemia
- West Nile fever.

2) The following are included within the category of cattle *diseases* and *infections*:
   - Bovine anaplasmosis
   - Bovine babesiosis
   - Bovine genital campylobacteriosis
   - Bovine spongiform encephalopathy
   - Bovine tuberculosis
   - Bovine viral diarrhoea
   - Enzootic bovine leukosis
   - Haemorrhagic septicaemia
   - Infectious bovine rhinotracheitis/infectious pustular vulvovaginitis
   - Infection with *Mycoplasma mycoides* subsp. *mycoides* SC (Contagious bovine pleuropneumonia)
   - Lumpy skin disease
   - Theileriosis
   - Trichomonosis
   - Trypanosomosis (tsetse-transmitted).
3) The following are included within the category of sheep and goat *diseases* and *infections*:
   - Caprine arthritis/encephalitis
   - Contagious agalactia
   - Contagious caprine pleuropneumonia
   - Infection with *Chlamydophila abortus* (Enzootic abortion of ewes, ovine chlamydiosis)
   - Infection with peste des petits ruminants virus
   - Maedi–visna
   - Nairobi sheep disease
   - Ovine epididymitis (*Brucella ovis*)
   - Salmonellosis (*S.* abortus ovis)
   - Scrapie
   - Sheep pox and goat pox.
4) The following are included within the category of equine *diseases* and *infections*:
   - Contagious equine metritis
   - Dourine
   - Equine encephalomyelitis (Western)
   - Equine infectious anaemia
   - Equine influenza
   - Equine piroplasmosis
   - Glanders
   - Infection with African horse sickness virus
   - Infection with equid herpesvirus-1 (EHV-1)
   - Infection with equine arteritis virus
   - Venezuelan equine encephalomyelitis.

Annex 26 (contd)

Appendix VI (contd)

5) The following are included within the category of swine *diseases* and *infections*:
   - African swine fever
   - Infection with classical swine fever virus
   - Nipah virus encephalitis
   - Porcine cysticercosis
   - Porcine reproductive and respiratory syndrome
   - Transmissible gastroenteritis.
6) The following are included within the category of avian *diseases* and *infections*:
   - Avian chlamydiosis
   - Avian infectious bronchitis
   - Avian infectious laryngotracheitis
   - Avian mycoplasmosis (*Mycoplasma gallisepticum*)
   - Avian mycoplasmosis (*Mycoplasma synoviae*)
   - Duck virus hepatitis
   - Fowl typhoid
   - Infection with avian influenza viruses
   - Infection with influenza A viruses of high pathogenicity in birds other than poultry including wild birds
   - Infection with Newcastle disease virus
   - Infectious bursal disease (Gumboro disease)
   - Pullorum disease
   - Turkey rhinotracheitis.
7) The following are included within the category of lagomorph *diseases* and *infections*:
   - Myxomatosis
   - Rabbit haemorrhagic disease.
8) The following are included within the category of bee *diseases*, *infections* and *infestations*:
   - Infection of honey bees with *Melissococcus plutonius* (European foulbrood)
   - Infection of honey bees with *Paenibacillus larvae* (American foulbrood)
   - Infestation of honey bees with *Acarapis woodi*
   - Infestation of honey bees with *Tropilaelaps* spp.
   - Infestation of honey bees with *Varroa* spp. (Varroosis)
   - Infestation with *Aethina tumida* (Small hive beetle).
9) The following are included within the category of other *diseases* and *infections*:
   - Camelpox
   - Leishmaniosis.

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Annex 26 (contd)

Appendix VII

AQUATIC CODE

CHAPTER 1.2.

CRITERIA FOR ~~LISTING AQUATIC~~ ~~ANIMAL~~ THE INCLUSION OF DISEASES IN THE OIE LIST

Article 1.2.1.

Introduction

This chapter describes the criteria for listing *diseases* in Chapter 1.3.

The objective of listing is to support Member Countries~~:~~ by providing information needed to take appropriate action ~~efforts~~ to prevent the transboundary spread of important *diseases* of *aquatic animals*. This is achieved by ~~through~~ transparent, timely and consistent ~~reporting~~ *notification*.

For the *diseases* listed in accordance with Article 1.2.2., the corresponding *disease*-specific chapters ~~in the *Aquatic Code*~~ assist Member Countries in the harmonisation of *disease* detection, prevention and control and provide standards for safe *international trade* in *aquatic animals* and their products.

The requirements for *notification* of *listed diseases* are detailed in Chapter 1.1.

Principles for selection of diagnostic tests are described in Chapter 1.1.2 of the *Aquatic Manual*.

Article 1.2.2.

The c~~C~~riteria for the inclusion of a ~~listing an aquatic animal~~ *disease* in the OIE list are as follows:

~~*Diseases* proposed for listing should meet the relevant criteria as set out in A. Consequences, B. Spread and C. Diagnosis. Therefore, to be listed, a *disease* should have the following characteristics: 1 or 2 or 3; and 4 or 5; and 6; and 7; and 8. Such proposals should be accompanied by a *case definition* for the *disease* under consideration.~~

~~No.~~		~~Criteria for listing~~	~~Explanatory notes~~
		~~A. Consequences~~	
~~1.~~OR	b.	The *disease* has been shown to cause a significant ~~production losses at a national or multinational (zonal or regional) level~~ impact on the health of *aquatic animals* at the level of country or a *zone* taking into account the occurrence and severity of the clinical signs, including direct production losses and mortality.	~~There is a general pattern that the disease will lead to losses in susceptible species, and that morbidity or mortality are related primarily to the infectious agent and not management or environmental factors. (Morbidity includes, for example, loss of production due to spawning failure.) The direct economic impact of the disease is linked to its morbidity, mortality and effect on product quality.~~
~~2.~~OR	c.~~Or~~	The *disease* has been shown to, or scientific evidence indicates that it ~~is likely to~~ would, cause a significant impact on the health of ~~morbidity or mortality in~~ *wild aquatic animal* populations taking into account the occurrence and severity of the clinical signs, including direct production losses and mortality, and ecological threats.	~~Wild aquatic animal populations can be populations that are commercially harvested (wild fisheries) and hence are an economic asset. However, the asset could be ecological or environmental in nature, for example, if the population consists of an endangered species of aquatic animal or an aquatic animal potentially endangered by the disease.~~

Annex 26 (contd)

Appendix VII (contd)

AND			
3.4.	a.Or	The agent is of public health concern. Natural transmission to humans has been proven, and human infection is associated with severe consequences.	
And B. Spread			
4.	-	Infectious aetiology of the disease is proven.	-
5.	Or	An infectious agent is strongly associated with the disease, but the aetiology is not yet known.	Infectious diseases of unknown aetiology can have equally high-risk implications as those diseases where the infectious aetiology is proven. Whilst disease occurrence data are gathered, research should be conducted to elucidate the aetiology of the disease and the results be made available within a reasonable period of time.

No.		**Criteria for listing**	**Explanatory notes**
And B. Spread			
6.1.	And	Likelihood of iInternational spread, of the agent including (via live *aquatic animals*, their products or fomites) has been proven.	International trade in aquatic animal species susceptible to the disease exists or is likely to develop and, under international trading practices, the entry and establishment of the disease is likely.
AND			
7.2.	And	At least one Several *country*ies or countries with zones has demonstrated freedom or impending freedom from the *disease* in populations of susceptible *aquatic animals*, may be declared free of the disease based on the general surveillance provisions principles outlined in of Chapters 1.4. and 1.5.	Free countries/zones could still be protected. Listing of diseases that are ubiquitous or extremely widespread would render notification unfeasible. However, individual countries that run a control programme on such a disease can propose its listing provided they have undertaken a scientific evaluation to support their request. Examples may be the protection of broodstock from widespread diseases, or the protection of the last remaining free zones from a widespread disease.
And C. Diagnosis			
AND			
8.3.		A repeatable and robust Reliable means of detection/ and diagnosis exists and a precise *case* definition is available to clearly identify *cases* and allow them to be distinguished from other *diseases*.	A diagnostic test should be widely available and preferably has undergone a formal standardisation and validation process using routine field samples (See *Aquatic Manual*.) or a robust case definition is available to clearly identify cases and allow them to be distinguished from other pathologies.

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Annex 26 (contd)

Appendix VIII

AQUATIC CODE

CHAPTER 1.2.

CRITERIA FOR THE INCLUSION OF DISEASES IN THE OIE LIST

Article 1.2.1.

Introduction

This chapter describes the criteria for listing *diseases* in Chapter 1.3.

The objective of listing is to support Member Countries by providing information needed to take appropriate action to prevent the transboundary spread of important *diseases* of *aquatic animals.* This is achieved by transparent, timely and consistent *notification*.

For the *diseases* listed in accordance with Article 1.2.2., the corresponding *disease*-specific chapters assist Member Countries in the harmonisation of *disease* detection, prevention and control and provide standards for safe *international trade* in *aquatic animals* and their products.

The requirements for *notification* of *listed diseases* are detailed in Chapter 1.1.

Principles for selection of diagnostic tests are described in Chapter 1.1.2 of the *Aquatic Manual*.

Article 1.2.2.

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

1) International spread, of the agent (via live aquatic animals, their products or fomites) has been proven.

AND

2) At least one country has demonstrated freedom or impending freedom from the disease in populations of susceptible aquatic animals, based on the general surveillance provisions of Chapters 1.4. and 1.5.

AND

3) Reliable means of detection and diagnosis exist and a precise case definition is available to clearly identify cases and allow them to be distinguished from other diseases.

AND

4)

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

OR

b) The disease has been shown to cause a significant impact on the health of aquatic animals at the level of country or a zone taking into account the occurrence and severity of the clinical signs, including direct production losses and mortality.

Annex 26 (contd)

Appendix VIII (contd)

OR

c) The disease has been shown to, or scientific evidence indicates that it would, cause a significant impact on the health of wild aquatic animal populations taking into account the occurrence and severity of the clinical signs, including direct production losses and mortality, and ecological threats.

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

Annex 27

Original: English
February 2015

REPORT OF THE FIRST MEETING OF OIE AD HOC GROUP ON SUSCEPTIBILITY OF CRUSTACEAN SPECIES TO INFECTION WITH OIE LISTED DISEASES

Paris, 10–12 February 2015

The OIE *ad hoc* Group on Susceptibility of crustacean species to infection with OIE listed diseases (the *ad hoc* Group) met at OIE Headquarters on 10–12 Februray 2015. Dr Grant Stentiford chaired the meeting.

1. Welcome and introduction

The members of the *ad hoc* Group and other participants at the meeting are listed at Annex I. The adopted agenda is provided as Annex II.

On behalf of Dr Bernard Vallat, Director General of the OIE, the Head of the International Trade Department, Dr Derek Belton, welcomed all members and thanked them for their agreement to work with the OIE on this important topic.

2. Objectives of the meeting

Chapter 1.5. 'Criteria for listing species as susceptible to infection with a specific pathogen' was introduced into the 2014 edition of the OIE *Aquatic Animal Health Code (Aquatic Code)*. The purpose of this chapter is to provide criteria for determining which host species should be listed as susceptible in each disease-specific chapter of the *Aquatic Code.* These criteria are to be applied progressively to each disease-specific chapter in the *Aquatic Code*. Furthermore, for species where there is some evidence of susceptibility but insufficient evidence to demonstrate susceptibility through the approach described in Article 1.5.3., information will be included in the relevant disease-specific chapter of the *Manual of Diagnostic Tests for Aquatic Animals (Aquatic Manual)*.

Assessment of the scientific evidence will be undertaken by the *ad hoc* Group and the assessments will be provided to Member Countries for comment prior to any change in the list of susceptible species in Article X.X.2. of the crustacean disease-specific chapters of the *Aquatic Code* and *Manual.*

The objectives of the meeting were therefore as follows:

1) To consider the general elements of the three-stage approach for determining susceptibility of given host taxa to infection with the listed crustacean pathogens
2) To determine the specific evidence required to fulfil stages 2 (identification of the pathogen) and 3 (determination of infection) for yellow head virus (YHV) genotype 1 (hereafter YHV1)
3) To apply specific criteria defined in 2 to the available peer-reviewed and grey literature pertaining to YHV1 for determining susceptibility according to *Aquatic Code* chapter 1.5.
4) To classify evidence pertaining to susceptibility of specific host taxa to YHV1 to either Group 1 (*Aquatic Code*-listed), Group 2 (*Aquatic Manual*-listed) or Group 3 (other, e.g. mechanical vector)
5) To report on the outcome of the analysis prior to the meeting of the Aquatic Animal Health Standards Commission in March 2015.

3. Terms of Reference

The Terms of Reference were adopted and the final version is shown in Annex III.

Annex 27 (contd)

4. Discussion of working documents and other relevant documents

Prior to the meeting, the *ad hoc* Group was provided with the European Food Safety Authority (EFSA) opinion relating to susceptibility of aquatic animals to pathogens listed in EC Directive 2006/88 (EFSA, 2008). Furthermore, a peer-reviewed paper arising from this work and focussed on application of the criteria outlined by EFSA to susceptibility of hosts to the crustacean diseases white spot disease (WSD), Taura syndrome (TS) and yellowhead disease (YHD), was also provided (Stentiford *et al.*, 2009).

The *ad hoc* Group agreed that the three-stage approach as outlined in Article 1.5.3. would be adequate (given appropriate supporting data) to define a host as 'susceptible' to 'infection' with YHV1.

The Group wished to raise the following specific points related to Articles within Chapter 1.5.:

Article 1.5.4. In reference to crustacean diseases, the *ad hoc* Group agreed that while injection of an infectious agent can be considered as an experimental invasive route, it does not mimic a natural route of infection. This contrasts the previous review of susceptibility of crustacean hosts to pathogens by EFSA (EFSA, 2008) in which injection was considered to potentially mimic a natural route of infection in crustaceans.

Article 1.5.4. In reference to consideration of 'environmental factors' associated with route of transmission/natural pathways, the Group considered this a relevant inclusion when considering the evidence reported in specific studies (e.g. did the temperature at which a given challenge study was conducted mimic expected natural conditions for the host/pathogen?).

Article 1.5.5. In reference to use of section 7 of the appropriate chapter of the *Aquatic Manual* to provide a means to 'identify' the pathogen under consideration, the Group considered it vital that section 7 be modified to include appropriate molecular diagnostic (PCR) and phylogenetic elements. In most cases this should take the form of PCR followed by sequencing of the amplicon, whilst in some cases pathogen taxon-specific PCR assays may be sufficient. If this information is not consistently provided in section 7, it is difficult to confirm the taxonomy of the pathogen under consideration against the reference listed pathogen.

Article 1.5.6. In reference to criteria A-D, the Group discussed and agreed a set of descriptive elements for each criterion, related to YHV1. These descriptions take account of previous work by EFSA (2008) and Stentiford *et al.* (2009) for susceptibility to YHV1. The descriptions to be applied to assess susceptibility to YHV1 in accordance with Chapter 1.5. of the *Aquatic Code* are thus:

A: Replication	B: Viability / Infectivity	C: Pathology / Clinical signs	D: Location
Presence of characteristic inclusion bodies and positive labelling of inclusion bodies by ISH or IFAT Presence of virions in inclusion bodies by TEM Demonstration of increasing copy number over time with RT-qPCR Serial passage from individual to SPF individual of the same species*	Single passage bioassay to a SPF (target pathogen) of any susceptible host species and confirmation of pathogen identification**	Characteristic inclusion bodies, with pyknosis and karyorrhectic nuclei in target tissues and haemocytic infiltration and/or clinical signs***	Haemocytes, heart, peripheral nerves, eye, lymphoid organ and sinuses, connectiv tissue****

Key:*To demonstrate replication by this approach requires evidence for multiple passages in confirmed target pathogen-free hosts of the same species as being assessed. **To demonstrate viability or infectivity of the target pathogen within the host being assessed, single passage in any known susceptible SPF host is required. ***Clinical signs typical of YHV1 may provide evidence for fulfilment of this category when evidence from histopathology is not available. However, clinical signs according to the *Manual* chapter may not present equally in all host taxa and may not be specific for infection with YHV1. ****Lymphoid organ not present in most non-penaeid host taxa.

Article 1.5.5. In reference to specific identification of YHV1, the Group noted that as currently written, there are several inconsistencies between the *Aquatic Code* Chapter 9.2. (Infection with Yellow Head Virus) and the *Aquatic Manual* Chapter 2.2.8. (Yellow Head Disease). Since only infection with Yellow Head Virus (genotype 1) of the Yellowhead complex of viruses (i.e. that including GAV, etc.) is listed by the OIE, it was recommended that the *Code* and *Manual* chapters should be modified to clarify this point. To this end, and for the purposes of defining susceptibility of hosts to the listed pathogen (YHV1), publications were scrutinised to provide specific evidence that the pathogen under study was in fact YHV1 and not (potentially) other viruses within the complex. Where this definition could not be made, poor evidence for fulfilling stage 2 of the approach (see Articles 1.5.3. and 1.5.5.) was noted. In such cases, and where other studies could not be identified to fulfil this evidence gap, a host taxon would not be proposed for listing in the *Aquatic Code* (Group 1).

Article 1.5.7. In reference to outcomes of the assessment for each host taxon, those hosts for which identification of the agent is confirmed (section 1.5.5.) and where evidence to fulfil either criteria A, or at least two of B, C, D of Article 1.5.6. (see table above) is provided, hosts may be classified as Group 1 (for inclusion in the *Code*). This outcome is strengthened somewhat where infections are natural rather than experimental (Article 1.5.4.). In cases where evidence to fulfil some of those criteria listed in Article 1.5.6. (see table above) are not achieved, and/or where identification of the pathogen is not sufficient to confirm YHV1 (Article 1.5.5.), or where infection was due to invasive experimental procedures only, hosts may be classified as Group 2 (for inclusion in the *Manual*).

In all other cases, where evidence for 'infection' cannot be demonstrated conclusively (e.g. where only PCR evidence for presence of the pathogen was provided), hosts were classified to a third group, Group 3. Group 3 hosts may potentially include those taxa to be listed in section 2.2.6. of the *Manual* chapter under the heading of 'Vector'.

In reviewing susceptibility of aquatic hosts to YHV1 the *ad hoc* Group considered the taxonomic range over which susceptible hosts exist. Such an approach may be used to better inform on risk related to the importation of potentially susceptible hosts. Evidence for susceptibility to YHV1 is available from only two families (*Penaeidae* and *Palaemonidae*) within the Decapod suborder (Dendrobranchiata). Susceptibility was tested (though not demonstrated) in several other families (including Bracyhura, the crabs) of the suborder Pleocyemata. An understanding of taxonomic spread in host range is a new concept in addressing susceptibility and will undoubtedly highlight the variation in virulence strategies for the pathogens (including those of fish and molluscs) listed the *Aquatic Code* and *Manual*. Where specific evidence does not exist for susceptibility of a given host taxon to YHV1 (or other listed crustacean diseases), a risk analysis based upon taxonomic range should be conducted.

5. Outcome of the analysis

The *ad hoc* Group recommended to retain the listing of *Penaeus monodon* as a species susceptible to YHV1 infection. The assessment undertaken by the *ad hoc* Group against the criteria is provided below:

STAGE 1

Transmission has been obtained naturally (Wijegoonawardane *et al.*, 2008 and Boonyaratpalin *et al.*, 1993) in accordance with Article 1.5.4. The *ad hoc* Group considered that this criterion was met.

AND STAGE 2

The identity of the pathogenic agent has been confirmed (Wijegoonawardane *et al.*, 2008) in accordance with Article 1.5.5. The *ad hoc* Group considered that this criterion was met.

AND STAGE 3

There is evidence of infection with the pathogenic agent in the suspect host species (Wijegoonawardane *et al.*, 2008; Boonyaratpalin *et al.*, 1993; Longyant *et al.*, 2006) in accordance with criteria A to D in Article 1.5.6. The *ad hoc* Group considered that this criterion was met.

Annex 27 (contd)

In addition:

The *ad hoc* Group recommended to list ***Penaeus vannamei*** as a species susceptible to YHV1 infection. The assessment undertaken by the *ad hoc* Group against the criteria is provided below.

STAGE 1

Transmission has been obtained naturally (Songsuk *et al.*, 2011), and by experimental procedures (Lightner *et al.*, 1998) that mimic natural pathways for *infection* in accordance with Article 1.5.4. The *ad hoc* Group considered that this criterion was met.

AND STAGE 2

The identity of the *pathogenic agent* has been confirmed (Songsuk *et al.*, 2011) in accordance with Article 1.5.5. The *ad hoc* Group considered that this criterion was met.

AND STAGE 3

There is evidence of *infection* with the *pathogenic agent* in the suspect host species (Lightner *et al.*, 1998; Songsuk *et al*., 2011) in accordance with criteria A to D in Article 1.5.6. The *ad hoc* Group considered that this criterion was met.

The *ad hoc* Group recommended to list ***Penaeus stylirostris*** as a species susceptible to YHV1 infection. The assessment undertaken by the *ad hoc* Group against the criteria is provided below.

STAGE 1

Transmission has been obtained naturally (Castro-Longoria *et al.*, 2008), in accordance with Article 1.5.4. The *ad hoc* Group considered that this criterion was met.

AND STAGE 2

The identity of the *pathogenic agent* has been confirmed (Castro-Longoria *et al.*, 2008) in accordance with Article 1.5.5. The *ad hoc* Group considered that this criterion was met.

AND STAGE 3

There is evidence of *infection* with the *pathogenic agent* in the suspect host species (Lu *et al.*, 1994; Castro-Longoria *et al.*, 2008) in accordance with criteria A to D in Article 1.5.6. The *ad hoc* Group considered that this criterion was met.

The *ad hoc* Group recommended to list ***Metapenaeus affinis*** as a species susceptible to YHV1 infection. The assessment undertaken by the *ad hoc* Group against the criteria is provided below.

STAGE 1

Transmission has been obtained by experimental procedures that mimic natural pathways for *infection* (Longyant *et al.*, 2006) in accordance with Article 1.5.4. The *ad hoc* Group considered that this criterion was met.

AND STAGE 2

The identity of the *pathogenic agent* has been confirmed (Longyant *et al.*, 2006) in accordance with Article 1.5.5. The *ad hoc* Group considered that this criterion was met.

AND STAGE 3

There is evidence of *infection* with the *pathogenic agent* in the suspect host species (Longyant *et al.*, 2006) in accordance with criteria A to D in Article 1.5.6. The *ad hoc* Group considered that this criterion was met.

The *ad hoc* Group recommended to list ***Palaemonetes pugio*** as a species susceptible to YHV1 infection. The assessment undertaken by the *ad hoc* Group against the criteria is provided below.

STAGE 1

Transmission has been obtained by experimental procedures that mimic natural pathways for *infection* (Ma *et al.*, 2009) in accordance with Article 1.5.4. The *ad hoc* Group considered that this criterion was met.

AND STAGE 2

The identity of the *pathogenic agent* has been confirmed (Ma *et al.*, 2009) in accordance with Article 1.5.5. The *ad hoc* Group considered that this criterion was met.

AND STAGE 3

There is evidence of *infection* with the *pathogenic agent* in the suspect host species (Ma *et al.*, 2009) in accordance with criteria A to D in Article 1.5.6. The *ad hoc* Group considered that this criterion was met.

In addition:

The *ad hoc* Group recommended to remove ***Penaeus esculentus*** as a species susceptible to YHV1 infection as there is insufficient evidence to demonstrate susceptibility through the approach described in Article 1.5.3. The assessment undertaken by the *ad hoc* Group against the criteria is provided below.

STAGE 1

Transmission has only been obtained by experimental invasive procedures that do not mimic natural pathways for *infection* in accordance with Article 1.5.4. Further the literature only refers to studies using genotype 2 (GAV) so susceptibility to infection with the listed YHV1 cannot be confirmed (Spann *et al.*, 2000, 2003). The *ad hoc* Group considered that this criterion was not met.

AND STAGE 2

The identity of the *pathogenic agent* has not been confirmed in accordance with Article 1.5.5. Specifically, studies have not sufficiently demonstrated use of the agent YHV1 (Spann *et al.*, 2000, 2003). The *ad hoc* Group considered that this criterion was not met.

AND STAGE 3

There is not sufficient evidence of *infection* with the *pathogenic agent* in the suspect host species in accordance with criteria A to D in Article 1.5.6. Specifically, non-specific clinical signs were reported but internal pathology was not characteristic (Spann *et al.*, 2003). The *ad hoc* Group considered that this criterion was not met.

The *ad hoc* Group recommended to remove ***Penaeus japonicus*** as a species susceptible to YHV1 infection as there is insufficient evidence to demonstrate susceptibility through the approach described in Article 1.5.3. The assessment undertaken by the *ad hoc* Group against the criteria is provided below.

STAGE 1

Transmission has been obtained naturally for the *infection* (Wang *et al.*, 1996) in accordance with Article 1.5.4. The *ad hoc* Group considered that this criterion was met.

Annex 27 (contd)

AND STAGE 2

The identity of the *pathogenic agent* has not been confirmed (Wang *et al.*, 1996) in accordance with Article 1.5.5. Specifically, animals obtained from the outbreak under study were coinfected with WSSV and did not display typical clinical signs of YHV1. In addition, no typing of YHV1 in accordance with Section 7 of the appropriate *Manual* chapter was reported (Wang *et al.*, 1996). The *ad hoc* Group considered that this criterion was not met.

AND STAGE 3

There is evidence of *infection* with the *pathogenic agent* in the suspect host species (Wang *et al.*, 1996) in accordance with criteria A to D in Article 1.5.6. Specifically, although TEM was reported, the authors did not confirm that infection was caused by YHV1 (Wang *et al.*, 1996). The *ad hoc* Group considered that this criterion was met.

6. Review and finalise report of meeting

The *ad hoc* Group utilised an existing database of papers and reports pertaining to Yellowhead disease (YHV1, GAV and other known genotypes) (Dr Stentiford, Cefas). In addition, the *ad hoc* Group searched the CAB Abstracts database (time range 1968 and 2015) (EBSCO host) using the search terms "yellow head virus" (n=386) and "yellow baculovirus" (n=30). We limited our search from 2008 to 2015 to the key words "yellow head disease" (n=115) as the literature had been reviewed previous to 2009 (Stentiford *et al.*, 2009). Over 700 articles in the database were identified using these key words. The initial selection of articles was based on the relevance of the titles to the objectives of the terms of reference (host susceptibility). Selected titles were then evaluated to determine the route of infection, confirmation of the pathogen genotype, and the presence of viable replicating virus in the host. Once we had sufficient papers to assess that a host species was susceptible we did not search for other references pertaining to that crustacean host species. The search was broadened by using the Boolean operators "OR" and "AND", and other databases, such as Google Scholar and PubMed. Key terms searched included "YHV and Mosquito" (n=2), and "YHV and chinensis" (n=0), and "YHV and indicus" (n=1). A few "backward" searches on relevant articles were also conducted. Approximately 40 papers were downloaded for initial review. In total 18 papers were cited. Three proceedings and two peer-reviewed papers initially selected could not be accessed.

7. Summary

In summary, five crustacean host taxa fulfilled the criteria required for listing a species susceptible to infection with YHV1 according to Article 1.5 of *Aquatic Animal Health Code.* These Group 1 hosts were: *Penaeus monodon*, *Penaeus vannamei*, *Penaeus stylirostris*, *Palaemonetes pugio* and *Metapenaeus affinis*. In addition, a further nine crustacean host taxa fulfilled some of the criteria required for listing as susceptible but evidence was lacking to either confirm the identity of the pathogen under study as YHV1, to demonstrate a natural route of infection, or to definitively confirm an 'infected' status. These Group 2 hosts were: *Macrobrachium sintangense*, *Metapenaeus brevicornis*, *Palaemon serrifer*, *Palaemon styliferus*, *Penaeus aztecus*, *Penaeus duorarum*, *Penaeus japonicus*, *Penaeus merguiensis* and *Penaeus setiferus*. Finally, numerous taxa were classified in to a third group when either the viability of the virus within the host was not demonstrated, or where its status as a mechanical vector could not be ruled out. Detailed analysis of evidence pertaining to Group 1, 2 and 3 hosts is provided in Annex IV.

Annex 27 (contd)

Table 2. Susceptiblity of species to yellow head virus Genotype 1.

Species	Overall status
Metapenaeus affinis	1
Palaemonetes pugio	1
Penaeus monodon	1
Penaeus stylirostris	1
Penaeus vannamei	1
Macrobrachium sintangense	2
Metapenaeus brevicornis	2
Palaemon serrifer	2
Palaemon styliferus	2
Penaeus aztecus	2
Penaeus duorarum	2
Penaeus japonius	2
Penaeus merguiensis	2
Penaeus setiferus	2
Acetes sp.	3
Callinectes sapidus	3
Chelonibia patula	3
Ergasilus manicatus	3
Fundulus grandis	3
Metapenaeus bennettae	3
Metapenaeus ensis	3
Octolasmis muelleri	3
Penaeus esculentus	3

Annex 27 (contd)

8. Next meeting

Next meeting date to be confirmed after Aquatic Animals Health Standards Commission meeting in March 2015.

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Annex 27 (contd)

Annex I

MEETING OF THE OIE AD HOC GROUP ON SUSCEPTIBILITY OF CRUSTACEAN SPECIES TO INFECTION WITH OIE LISTED DISEASES

Paris, 10–12 February 2015

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Annex 27 (contd)

Annex II

MEETING OF *AD HOC* GROUP ON SUSCEPTIBILITY OF CRUSTACEAN SPECIES TO INFECTION WITH OIE LISTED DISEASES

Paris, 10–12 February 2015

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Agenda

1) Welcome and introduction
2) Objectives of the meeting
3) Terms of Reference
4) Discussion of working documents and other relevant documents
5) Outcome of the analysis
6) Review and finalise the report of the meeting
7) Summary
8) Next meeting

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Annex 27 (contd)

Annex III

AD HOC GROUP ON SUSCEPTIBILITY OF CRUSTACEAN SPECIES TO INFECTION WITH OIE LISTED DISEASES

Terms of Reference

Background

A new Chapter 1.5. 'Criteria for listing species as susceptible to infection with a specific pathogen' was introduced into the 2014 edition of the *Aquatic Code*. The purpose of this chapter is to provide criteria for determining which host species are listed as susceptible in Article X.X.2. of each disease-specific chapter in the *Aquatic Code.* The criteria are to be applied progressively to each disease-specific chapter in the *Aquatic Code*.

These assessments will be undertaken by *ad hoc* Groups and the assessments will be provided to Member Countries' for comment prior to any change in the list of susceptible species in Article X.X.2. of the disease-specific chapters in the *Aquatic Code.*

For species where there is some evidence of susceptibility but insufficient evidence to demonstrate susceptibility through the approach described in Article 1.5.3., information will be included in the relevant disease-specific chapter in the *Aquatic Manual*.

Purpose

The *ad hoc* Group on Susceptibility of crustacean species to infection with OIE listed diseases will undertake this task for OIE listed crustacean diseases.

Terms of Reference

1) Consider standards of evidence required to satisfy the criteria in Chapter 1.5.
2) Review relevant literature documenting susceptibility of species
3) Propose susceptible species for OIE listed diseases based on Article 1.5.7.
4) Propose susceptible species for OIE listed diseases based on Article 1.5.8.

Expected outputs of the *ad hoc* Group

1) Develop a list of susceptible species for inclusion in the relevant Articles of crustacean disease-specific chapters in the *Aquatic Code* and *Manual*, starting with Yellow Head Disease, and if time allows other OIE listed crustacean diseases.
2) Draft a report for consideration by the Aquatic Animals Commission at their March 2015 meeting.

Annex 27 (contd)

Annex IV

Evidence for susceptibility of host species to infection with yellow head virus according to Chapter 1.5. Criteria for listing species as susceptible to infection with a specific pathogen.

Genus	Species	Route of transmission	Pathogen id.	A	B	C	D	Reference	Outcome	Status
Acetes	*sp.*	N	suspected	no	yes	no	no	Flegel *et al.*, 1995	3	3
Callinectes	*sapidus*	EN	confirmed	no	no	no	yes	Ma *et al.*, 2009	3	3
Chelonibia	*patula*	EN	confirmed	no	no	no	no	Overstreet *et al.*, 2009	3	3
Ergasilus	*manicatus*	EN	confirmed	no	no	no	no	Overstreet *et al.*, 2009	3	3
Fundulus	*grandis*	EN	confirmed	no	no	no	no	Overstreet *et al.*, 2009	3	3
Metapenaeus	*bennettae*	EI	confirmed					Walker *et al.*, 2001	3	3
Macrobrachium	*sintangense*	EI	confirmed	yes	no	no	no	Longyant *et al.*, 2005	2	2
Metapenaeus	*affinis*	EN	confirmed	yes	no	yes	yes	Longyant *et al.*, 2006	1	1
Metapenaeus	*brevicornis*	EI	confirmed	yes	no	yes	yes	Longyant *et al.*, 2006	2	2
Metapenaeus	*ensis*	EI	suspected	no	no	no	no	Chantanchookin *et al.*, 1993	3	3
Octolasmis	*muelleri*	EN	confirmed	no	no	no	no	Overstreet *et al.*, 2009	3	3
Palaemon	*serrifer*	EI	confirmed	yes	no	no	no	Longyant *et al.*, 2005	2	2
Palaemon	*styliferus*	N	suspected	yes	yes	yes	yes	Flegel, 1997	2	2
		EI	confirmed	yes	no	no	no	Longyant *et al.*, 2005	2	
Palaemonetes	*pugio*	EN	confirmed	yes	no	yes	yes	Ma *et al.*, 2009	1	1
Penaeus	*aztecus*	EN	suspected	yes	no	yes	yes	Lightner *et al.*, 1998	2	2
Penaeus	*duorarum*	EN	suspected	yes	no	yes	yes	Lightner *et al.*, 1998	2	2
Penaeus	*esculentus*	N	confirmed	no	no	no	no	Walker *et al.*, 2001	3	3
		EI	suspected	no	no	yes	no	Spann *et al.*, 2000	3	
		EI	confirmed	no	no	yes	yes	Spann *et al.*, 2003	3	

Annex 27 (contd)

Annex IV (contd)

Genus	Species	Route of transmission	Pathogen id.	A	B	C	D	Reference	Outcome	Status
Penaeus	*japonius*	N	suspected	yes	no	yes	yes	Wang *et al.*, 1996	2	2
Penaeus	*merguiensis*	N	suspected	no	yes	yes	yes	Flegel 1997	2	2
		EI	suspected	no	no	no	no	Chantanchookin *et al.*, 1993	3	
Penaeus	*monodon*	N	confirmed	no	no	yes	yes	Wijegoonawardane *et al.*, 2008	1	1
		N	suspected	yes	no	yes	yes	Boonyaratpalin *et al.*, 1993	2	
		EI	suspected	no	yes	yes	no	Longyant *et al.*, 2006	2	
Penaeus	*setiferus*	EN	suspected	yes	no	yes	yes	Lightner *et al.*, 1998	2	2
Penaeus	*stylirostris*	N	confirmed	no	yes	yes	yes	R Castro-Longoria *et al.*, 2008	1	1
		EI	suspected	yes	no	yes	yes	Lu *et al.*, 1994	2	
Penaeus	*vannamei*	N	confirmed	no	yes	yes	yes	Songsuk *et al.*, 2011	1	1
		EN	suspected	yes	no	yes	yes	Lightner *et al.*, 1998	2	

Route of transmission: Natural (N), Experimental Non-invasive (EN), Experimental Invasive (EI);

Annex 28

AQUATIC ANIMALS COMMISSION WORK PLAN 2014–2015

Aquatic Code

	Task	**March 2015**	**May GS 2015**	**Sept 2015**
1	User's guide	Review Member Countries comments	Propose for adoption	
2	Glossary	Review Member Countries	Propose for adoption	
3	Article 1.1.5. (Chapter 1.1.)	Review Member Countries	Propose for adoption	
4	Chapter 1.2 – Criteria for listing	Consider *ad hoc* group report and circulate for Member Countries comments		Review Member comments
5	Revision of Section 4 to improve guidance on the control of disease	Develop a plan in light of Aquatic Conf. recommendations.		Agree on priorities in section 4
6	Chapter 4.3. – General recommendations on disinfection			Review AHG draft chapter and circulate for member.
7	Chapter 4.X. – Recommendations for surface disinfection of salmonid eggs	Review Member Countries comments	Propose for adoption	
8	Chapter 4.7. – Control of pathogenic agents in aquatic animal feed	Review Member Countries comments	Propose for adoption	
9	Chapter 6.6. – Risk analysis for antimicrobial resistance in aquaculture (new)	Review Member Countries comments	Propose for adoption	
10	Listing of susceptible species in disease-specific chapters	Review AHG report and propose amendments to 9.2.2		Review Member Countries' comments
11	AHPND	Review Member Countries comments	Propose for adoption	If adopted. AAC to develop new Chapter

Annex 28 (contd)

Aquatic Manual

	***Manual* tasks**	**Feb 2015**	**May GS 2015**	**Sept 2015**
12	Crustacean chapters (YHD, NHP, TSD, IHHN)	Review Member Countries comments	Propose for adoption	
13	Infection with *Perkinsus olseni*	Review Member Countries comments	Propose for adoption	
14	Chapter 1.1.3. – Methods for disinfection of aquaculture establishments	Propose deletion if new *Code* chapter 4.X. is adopted	Propose for deletion if new *Code* chapter 4.X. is adopted	
15	Listing of susceptible species in disease-specific chapters	Review AHG report and request amendments in section 2.2.1. of chapter 2.2.8. YHD by the author		Review and circulate for Member Countries' comments
16	AHPND chapter	Review AHG Report and draft chapter and circulate for Member Countries comments		Review Member Countries' comments
17	Test performance	Review progress		
18	Sections on agent stability (in connection with disinfection)	Review progress		

Other items

	Tasks	**Feb 2015**	**May GS 2015**	**Sept 2015**
19	OIE Global Aquatic Animal Health Conference	Discuss conference recommendation for review of work plan	Present conference recommendation	Discuss conference recommendation for establishing work plan
20	Fish-borne Zoonotic Trematodes (FZT)			Member Countries' comments