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**REPORT OF THE MEETING OF THE OIE  
AQUATIC ANIMAL HEALTH STANDARDS COMMISSION  
Paris, 3–7 March 2008**

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The OIE Aquatic Animal Health Standards Commission (hereafter referred to as the Aquatic Animals Commission) met at the OIE Headquarters from 3 to 7 March 2008.

Details of participants and the adopted agenda are given at Annexes I and II.

Dr Eva-Maria Bernoth opened the meeting and welcomed the participants. Dr Sarah Kahn, Head of the OIE International Trade Department, welcomed the Aquatic Animals Commission members on behalf of the Director General who was trading outside France. She noted that the agenda was very long and that a large number of Member comments on the report of the previous meeting (October 2007) had been received. She acknowledged the quality of work of the *ad hoc* Groups that had met since the last Aquatic Animals Commission meeting.

The Aquatic Animals Commission recognised the contribution of the following Members in providing comments: Australia, Belize, Canada, Chinese Taipei, European Union (EU), Japan, New Zealand, Norway, Switzerland, Thailand and the United States of America (USA).

The Aquatic Animals Commission reviewed various *Aquatic Animal Health Code* (hereafter referred to as *Aquatic Code*) draft texts from its October 2007 report in the light of Member comments. The outcome of the Aquatic Animals Commission's work is presented at Annexes III to XX in this report. Additions made during the October 2007 meeting are shown as double underlined text, with deleted text in ~~strikeout~~, and those made at this meeting (March 2008) in a similar fashion but with coloured background to distinguish the two groups of proposals.

Members are invited to submit their comments to the OIE on Annex XVII of this report prior to 12<sup>th</sup> September 2008. The comments should be sent preferably by electronic mail to the following address: [trade.dept@oie.int](mailto:trade.dept@oie.int). The Aquatic Animals Commission will address the comments received at its next meeting.

The table below summarises the texts that will be proposed – as presented in the Annexes III to XVI – to the OIE International Committee for adoption at the 76<sup>th</sup> General Session, texts for Member comment (Annex XVII) and texts for Members information (Annexes XVIII to XX).

<b>Annexes for adoption</b>	<b>Annex number</b>
Definitions (Ch. 1.1.1.)	Annex III
Diseases listed by the OIE (Ch. 1.2.3.)	Annex IV
General obligations (Ch. 1.3.1.)	Annex V
Guidelines for import risk analysis (Ch 1.4.2.)	Annex VI
Recommendations for transport (Ch 1.5.1.)	Annex VII
Infectious myonecrosis (Ch 2.3.9.)	Annex VIII
White tail disease (Ch 2.3.11.)	Annex IX
Infection with <i>Mikrocytos mackini</i> (Ch. 2.2.5.)	Annex X
Gyrodactylosis ( <i>Gyrodactylus salaris</i> )	Annex XI
Infection with <i>Batrachochytrium dendrobatidis</i> (Ch. 2.4.1.)	Annex XII
Infection with ranavirus (Ch. 2.4.2.)	Annex XIII
Introduction to guidelines for the welfare of farmed fish (Ch X.X.X.)	Annex XIV
Guidelines on the control of aquatic animal health hazards in aquatic animal feed (Ch. X.X.X.)	Annex XV
Guidelines for aquatic animal health surveillance (Ch. X.X.X.)	Annex XVI
<b>Annexes for Members' information and comment</b>	<b>Annex number</b>
Report of the <i>ad hoc</i> Group on the OIE List of Aquatic Animal Diseases – Mollusc Team	Annex XVII
<b>Annexes for Members' information</b>	<b>Annex number</b>
Report of the <i>ad hoc</i> Group on Aquatic Animal Surveillance	Annex XVIII
Summary of Dr Hill's presentation on "Update on developments in aquatic animal health" at the 9 <sup>th</sup> Conference of the OIE Regional Commission for the Middle East, 2007	Annex XIX
Work Plan	Annex XX

## 1. Activities and progress of *ad hoc* Groups

The Aquatic Animals Commission noted the progress made in two *ad hoc* Groups and the President thanked the chairmen of these Groups (Dr Franck Berthe and Dr Barry Hill) for their contributions.

- *Ad hoc* Group on the OIE List of Aquatic Animal Diseases – Mollusc Team, 25–27 January 2008

Dr Berthe, Chair of the *ad hoc* Group, acknowledged the Group's work and reported that it had achieved its two tasks: the first was the evaluation of the sabellid worm (*Terebrasabella heterouncinata*) for listing. The *ad hoc* Group recommended that the sabellid worm be considered for listing. The second task was to review the abalone mortality complex. The *ad hoc* Group concluded that it was difficult to differentiate this complex of diseases and recommended that it remains listed by the OIE. This complex would include abalone viral ganglioneuritis and abalone viral mortality. The *ad hoc* Group proposed a case definition for the complex that recognises two manifestations.

The Aquatic Animals Commission endorsed the recommendations of the *ad hoc* Group. Member comments are invited on the proposal to add the sabellid worm to the list of diseases (for a detailed justification see [Annex IV](#)). Regarding abalone viral mortality, the Aquatic Animals Commission requested that the *ad hoc* Group proceed by reviewing the disease card information, consider any Member comments received on the proposed case definition for abalone viral mortality (see [Annex VII](#) of the *ad hoc* Group's report) and draft disease chapters for the *Aquatic Code* and *Manual* prior to the next Aquatic Animals Commission meeting.

The full report of the *ad hoc* Group is provided for information at [Annex XVII](#).

Member comments are invited on [Annexes IV and VII](#) of the *ad hoc* Group report (refer to [Annex XVIII](#)).

- *Ad hoc* Group on Aquatic Animal Health Surveillance, 29 January–1 February 2008

Dr Hill, Chair of the *ad hoc* Group, reported on the outcomes of the group's meeting which had been very successful. The *ad hoc* Group reviewed Member comments on the draft *Aquatic Code* chapter on aquatic animal health surveillance and amended the text where appropriate (refer to [Annex IV](#) in the *ad hoc* Group report presented at [Annex XVIII](#)).

The *ad hoc* Group was also tasked with drafting disease-specific surveillance chapters, but identified the need for guidance from the Aquatic Animals Commission on a harmonised template for chapter authors, and which diseases required a specific surveillance chapter. The *ad hoc* Group advised that, in view of the scale of the task, it was not feasible in the short term to develop such chapters for all listed diseases, and that some prioritisation of the diseases to have specific surveillance chapters prepared was necessary. The *ad hoc* Group prepared a draft template for authors of the future disease-specific chapters, for consideration by the Aquatic Animals Commission (refer to [Annex V](#) in the *ad hoc* Group report presented at [Annex XVIII](#)). The Commission agreed to discuss the draft template at its October 2008 meeting. Dr Bernoth will raise with Delegates at the General Session the question how to prioritise diseases for preparation of the disease specific surveillance chapters.

Dr Hill reported that the *ad hoc* Group had made good progress on the Handbook on Aquatic Animal Health Surveillance. The *ad hoc* Group will meet in April and July to complete work on the manuscript by August 2008.

The full report of the *ad hoc* Group is provided for information at [Annex XVIII](#)

## 2. *Aquatic Animal Health Code* – Member comments on draft text

### 2.1. Disease chapters – general comments

The EU commented that there are different lists of susceptible species in the *Aquatic Code* and in the *Aquatic Manual*. The Aquatic Animals Commission pointed out that for any disease referred to in the *Aquatic Code*, the known susceptible species are listed in the relevant chapter in the *Aquatic Manual*. The disease chapters in the *Aquatic Code* make recommendations for international trade. The scope of each *Aquatic Code* chapter is therefore limited to those susceptible species that are traded internationally (as listed in Article 2 of each chapter). If Members feel that the scope should be expanded or narrowed, the Aquatic Animals Commission would welcome proposals with justification.

In response to EU comments on Article 8 of each chapter, the Aquatic Animals Commission deleted the words “international standards such as” to make it clear that the reference is to the ICES Code only. In the same Article, a web link is provided to the full text of the current version of the ICES Code.

The Aquatic Animals Commission took note of the EU suggestion that the Aquatic Animals Commission should include in its work programme consideration of how to provide guidelines for trade of aquaculture animals vaccinated against any of the currently listed OIE diseases. The Aquatic Animals Commission agreed that this is an issue that will require attention and added the task to its future work programme.

In response to the EU comment on Articles 4. and 5. regarding regaining disease free status in a compartment, the Aquatic Animals Commission believes that the approach suggested by the EU requires more detailed consideration (see item 4.2.).

## **2.2. Definitions (Chapter 1.1.1.)**

Norway and the USA raised concerns that there were many highly specialised terms related to statistics and risk analysis proposed as new definitions. The Aquatic Animals Commission believes that these definitions are needed for the proposed Chapter on Aquatic animal health surveillance (see Item 2.15.). Once the OIE Handbook on Aquatic Animal Health Surveillance is published (see Item 5.), the Aquatic Animals Commission will review the Chapter on Aquatic animal health surveillance in the *Aquatic Code* with a view to make the Chapter more concise and remove any unnecessary definitions.

The Aquatic Animals Commission also received the comment that definitions proposed for other draft chapters should appear in Article 1.1.1. of the *Aquatic Code* rather than those chapters. The Aquatic Animals Commission agreed and clarified that those definitions would be moved to Article 1.1.1. once those chapters are adopted.

The Aquatic Animals Commission identified a number of currently existing definitions in the *Aquatic Code* that are not cited in the text and proposes their deletion.

The EU had requested a definition for the term ‘vector’ that is used in Article 3 of all the disease chapters in the *Aquatic Code*. The Aquatic Animals Commission clarified that the defined term of ‘susceptible species’ already included the concept of a biological vector. The Aquatic Animals Commission proposes to insert the term ‘mechanical’ before the term ‘vector’ in all disease chapters to differentiate it from the concept of biological vector, but does not believe a separate definition is warranted.

The Aquatic Animals Commission received numerous comments on the proposed changes to the definition of ‘infestation’. The Aquatic Animals Commission noted that the term ‘infestation’ was introduced to increase accuracy of text on diseases caused by parasites (for example, Gyrodactylosis). However, the term is currently cross referenced only in other definitions. Also, with the exception of Abalone viral mortality, all the listed diseases of molluscs are caused by parasites, yet to date are referred to as “infection with”. The Commission therefore proposes to delete the term ‘infestation’ and modify the definition for ‘infection’ to encompass the concept of infestation where applicable. The Commission reminds Members that the definitions in the *Aquatic Code* are contextual (“for the purpose of the *Aquatic Code*”) and not stand-alone text book definitions.

Several comments were received on the proposed changes to the definition of ‘outbreak of disease’. The Aquatic Animals Commission agreed that this definition needed to remain consistent with that in the *Terrestrial Code* and therefore withdrew the proposal to change it.

It was noted that two definitions in connection with surveillance (target population and epidemiological unit) that appear in the *Aquatic Manual* are also appropriate for the *Aquatic Code*. These have been added to the Definitions chapter.

The updated Chapter on Definitions that will be proposed to the OIE International Committee for adoption at the 76<sup>th</sup> General Session in May 2008 is presented at [Annex III](#).

## **2.3. Diseases listed by the OIE (Chapter 1.2.3.)**

The Aquatic Animals Commission received only supportive comments on the proposed addition of two amphibian diseases to Chapter 1.2.3. of the *Aquatic Code*.

The updated Chapter on Diseases listed by the OIE that will be proposed to the OIE International Committee for adoption at the 76<sup>th</sup> General Session in May 2008 is presented at [Annex IV](#).

Thailand suggested the removal from the list of several crustacean diseases and provided supporting documentation. This will be referred to the *ad hoc* Group on the List of Diseases of Crustaceans, which will meet in June 2008.

#### **2.4. General obligations (Chapter 1.3.1.)**

A number of comments were received from Members. The Commission made some changes in line with Member comments.

The updated Chapter on General obligations that will be proposed to the OIE International Committee for adoption at the 76<sup>th</sup> General Session in May 2008 is presented at [Annex V](#).

#### **2.5. Guidelines for import risk analysis (Chapter 1.4.2.)**

New Zealand queried the proposed removal of the reference to spread and establishment of a hazard from the exposure assessment of the risk analysis. The Aquatic Animals Commission clarified that the risk assessment methodology needs to be consistent in the *Aquatic* and *Terrestrial Codes* and that spread or establishment of a hazard are understood to form part of the consequence assessment of the risk analysis in the *Terrestrial Code*. The Aquatic Animals Commission therefore maintains its proposal, which better aligns the two chapters.

The updated Chapter on Guidelines for import risk analysis that will be proposed to the OIE International Committee for adoption at the 76<sup>th</sup> General Session in May 2008 is presented at [Annex VI](#).

#### **2.6. Recommendations for transport (Chapter 1.5.1.)**

Some Members requested clarification on the scope of this chapter. The Aquatic Animals Commission confirmed that the scope of the chapter refers to measures to control the aquatic animal health risks associated with transport of live aquatic animals and aquatic animal products and does not include welfare aspects.

Currently, the guidelines focus on live aquatic animals but, in future, the Aquatic Animals Commission would consider expanding the guidelines to include more detail on aquatic animal products.

The Aquatic Animals Commission clarified that Article 1.5.1.7. refers only to the transport by well boat of live aquatic animals and not of aquatic animal products.

The EU suggested that a chapter be drafted addressing the specific requirements for transport by land. The Aquatic Animals Commission noted that the scope of the current chapter includes transport by land. The words 'by sea and by air' were deleted from Article 1.5.1.1. thus clarifying that the chapter covers safe transport by land, sea and air.

The Aquatic Animals Commission reviewed all the comments and made some minor editorial changes to improve the clarity of the text. The words 'safe' and 'aquatic animal products' were added to the title, which now is: Recommendations for Safe Transport of Aquatic Animals and Aquatic Animal Products.

The updated Chapter on Recommendations for transport that will be proposed to the OIE International Committee for adoption at the 76<sup>th</sup> General Session in May 2008 is presented at [Annex VII](#).

#### **2.7. Infectious myonecrosis (Chapter 2.3.9.) and White tail disease (Chapter 2.3.11.)**

Thailand proposed the listing of two commodities (de-headed and de-veined [intestine removed] crustaceans [chilled or frozen] and fillets, cutlets or meat [chilled or frozen]) under Articles 2.3.X.3. point 1b. The Aquatic Animals Commission noted that the risk management for the suggested commodities would not address the risks associated with the pathogens, which are primarily localised in the meat.

The Aquatic Animals Commission received a number of other comments from Members, which were of a horizontal nature. Minor amendments to the text were made.

The updated Chapters on Infectious myonecrosis and White tail disease that will be proposed to the OIE International Committee for adoption at the 76<sup>th</sup> General Session in May 2008 are presented at [Annexes VIII and IX](#).

#### **2.8. Infection with *Mikrocytos mackini* (Chapter 2.2.5.)**

In response to the comment from Thailand on the inconsistency in the list of safe commodities for molluscs, the Aquatic Animals Commission indicated that *Mikrocytos mackini* would infect muscular tissue that is strongly attached to the shell, and oyster shells with remnants of adductor muscle may still carry *M. mackini*. Therefore, half-shell oysters cannot be considered a safe commodity with respect to this disease.

The EU commented that larvae may not be a safe commodity and therefore should be removed from Article 2.2.5.3. The Aquatic Animals Commission recognised that although this particular live stage is unlikely to be infected, the current practice in hatcheries may not prevent contamination of a consignment. The Aquatic Animals Commission agreed to remove larvae from this Article in all mollusc chapters.

The Aquatic Animals Commission had received a number of conflicting views on the listing of ‘chemically preserved products (e.g. smoked, salted, pickled, marinated etc.)’ as safe commodities in this Chapter. The Aquatic Animals Commission decided not to include these commodities at this time and will await an OIE decision on the proposal to establish an *ad hoc* Group on Safe Commodities Derived from Aquatic Animals (see Item 3.1).

The updated Chapter on Infection with *Mikrocytos mackini* that will be proposed to the OIE International Committee for adoption at the 76<sup>th</sup> General Session in May 2008 is presented at [Annex X](#).

#### **2.9. Gyrodactylosis (*Gyrodactylus salaris*) (Chapter 2.1.14.)**

In response to a comment from the EU regarding the terms ‘other salmonid and freshwater fish species’ in Article 2.1.14.2, the Aquatic Animals Commission agreed to delete ‘other salmonid and freshwater’, thus clarifying the scope of the chapter.

Thailand and Norway had requested that ‘eviscerated fish’ be removed from Article 2.1.14.3. 1b). The Aquatic Animals Commission agreed that, as the disease is caused by an external parasite, evisceration is not a relevant risk mitigation measure.

The EU requested that a sentence be added to a paragraph in Article 2.1.14.4 to cover the case of *G. salaris* resistant stock. The Aquatic Animals Commission believes that such an addition is unnecessary as the issue is accounted for in point 2 (Article 2.1.14.4.), which requires no observed occurrence of the disease despite conditions that are conducive to its clinical expression. Such clinical expression would not occur in resistant stock.

While the surveillance guidelines generally require a period of 2 years for targeted surveillance to demonstrate freedom of a disease (see Articles 4 and 5 in each disease chapter), the Commission has received advice from the OIE expert that for gyrodactylosis, the period should be 5 years. This is based on the age of Atlantic salmon smolts when they leave a river, the 5 years consisting of the maximum age plus one year. Even if the maximum smolt age is only 2 or 3 years, this ensures a safety margin: some infected salmon yearlings may live in a hidden river tributary and during smoltification move downstream to the main river where the parasites may be spread into the established salmon parr population. Even if the parasite spreads relatively fast, it may take one year before it is observed in the targeted surveillance.

For consistency with the new surveillance guidelines (see Item 2.15.), the Aquatic Animals Commission changed the time period for self declaration of historical freedom in a country, zone or compartment from 25 years to 10 years.

The updated Chapter on Gyrodactylosis (*Gyrodactylus salaris*) that will be proposed to the OIE International Committee for adoption at the 76<sup>th</sup> General Session in May 2008 is presented at [Annex XI](#).

## **2.10. Infection with *Batrachochytrium dendrobatidis* (New chapter)**

For consistency with the new surveillance guidelines (see Item 2.15.), the Aquatic Animals Commission changed the time period for self declaration of historical freedom in a country, zone or compartment from 25 years to 10 years.

The EU had requested that the proposed treatment and testing prior to export of live aquatic animals from a country, zone or compartment not declared free from *Batrachochytrium dendrobatidis* be described in the chapter. The Aquatic Animals Commission clarified that the chapter does make reference to the *Aquatic Manual* for which the pertinent information is being developed.

The Aquatic Animals Commission agreed with the EU and USA comments concerning disinfection of amphibian eggs and therefore removed reference to this option in Articles 8 and 10 until such time as the methods have been described in the relevant chapter in the *Aquatic Manual* that is under development.

The Aquatic Animals Commission made some minor editorial changes to improve the clarity of the text and achieve consistency among the disease chapters.

The updated Chapter on Infection with *Batrachochytrium dendrobatidis* that will be proposed to the OIE International Committee for adoption at the 76<sup>th</sup> General Session in May 2008 is presented at [Annex XII](#).

## **2.11. Infection with ranavirus (New chapter)**

For consistency with the new surveillance guidelines, the Aquatic Animals Commission changed the time period for self declaration of historical freedom in a country, zone or compartment from 25 years to 10 years.

Australia and New Zealand commented on the appropriateness of the items proposed to be certified in a certificate for importation of live aquatic animals from a country, zone or compartment not declared free of disease. The Aquatic Animals Commission agreed that the requested certification is unclear and ambiguous, and removed the requirement for such a certificate from Articles 8 and 10.

The Aquatic Animals Commission made some minor editorial changes to improve the clarity of the text and achieve consistency among the disease chapters.

The updated Chapter on Infection with ranavirus that will be proposed to the OIE International Committee for adoption at the 76<sup>th</sup> General Session in May 2008 is presented at [Annex XIII](#).

## 2.12. Introduction to OIE Guidelines for the welfare of live aquatic animals (New chapter)

A number of comments had been received that reflected conflicting views regarding the fundamental principles and scope of the guidelines on welfare. The Aquatic Animals Commission clarified that the guidelines would relate to farmed fish only (excluding ornamental species) and amended the title accordingly.

In response to a comment from the EU on the use of the ‘three Rs’ (i.e. reduction, refinement and replacement) in animal experimentation, the Aquatic Animals Commission clarified that the scope of the guidelines is for transport, slaughter, and destruction for disease control purposes, and therefore there is no justification for inclusion of the ‘three Rs’ in the text.

The Aquatic Animals Commission revised the proposed Introduction to clearly separate it into considerations, guiding principles, and a scientific basis for the guidelines.

The updated Chapter on the Introduction to guidelines for the welfare of farmed fish that will be proposed to the OIE International Committee for adoption at the 76<sup>th</sup> General Session in May 2008 is presented at [Annex XIV](#). For Members’ convenience, the text is presented in two versions: one showing the text changes ([Annex XIVa](#)) and the other a clean copy ([Annex XIVb](#)).

Pending adoption of the Introduction, the Aquatic Animals Commission will prepare draft guidelines for welfare of farmed fish during transport, slaughter, and destruction for disease control purposes.

## 2.13. Guidelines for the control of aquatic animal health hazards in aquatic animal feed (New chapter)

The Aquatic Animals Commission received numerous comments on the draft chapter. A fundamental point raised by Australia was that some of the text of the current guidelines was confined to feed for food-producing animals while there were other uses of aquatic animal feed (e.g. live feeder fish and ornamental fish trade, and bait in commercial or recreational fisheries), which also constitute a significant aquatic animal health risk. The Aquatic Animals Commission confirmed that the scope states that the principles detailed in the guidelines could be applied to feed for aquatic animals used for purposes other than food. To improve clarity, the Aquatic Animals Commission revised the proposed definition of feed to ‘means any material (single or multiple), whether processed, semi-processed or raw that is intended to be fed directly to ~~food producing~~ aquatic animals’.

In response to a comment from New Zealand, the Aquatic Animals Commission clarified that the scope of the chapter extends beyond diseases that are listed in the *Aquatic Code*.

The EU also suggested additional wording on the authorisation to use terrestrial animal by-products in aquaculture. The Aquatic Animals Commission is unclear about the purpose of the addition and as none of the Members have seen these comments, the Aquatic Animals Commission does not accept to include them in the guidelines at this point and invites the EU to provide a more detailed explanation in time for the October meeting of the Aquatic Animals Commission.

The EU commented on inconsistency in the list of safe commodities. The Aquatic Animals Commission pointed out that the list in the guidelines comprises general categories of safe commodities; the list of those safe commodities that are specific for a given disease can be found in the corresponding disease chapter.

The EU requested that the guidelines contain reference to Articles 11 and 12 in the individual disease chapters regarding the importation of product from a country, zone or compartment declared free and not free, respectively. The Aquatic Animals Commission did not accept this request as they believed this was already covered in the Article given the last sentence in the Article which makes reference to the relevant disease chapters of the *Aquatic Code*.

The Aquatic Animals Commission deleted a number of definitions that do not appear in the Chapter and made minor editorial changes to improve the clarity of the text.

The updated Chapter on Guidelines for the control of aquatic animal health hazards in aquatic animal feed that will be proposed to the OIE International Committee for adoption at the 76<sup>th</sup> General Session in May 2008 is presented at [Annex XV](#).

Pending the adoption of this chapter, the proposed definitions will be transferred to Chapter 1.1.1. of the *Aquatic Code*, except for the definition for Hazard which will remain in the new Chapter as this definition is specific to this Chapter.

#### **2.14. Guidelines on handling and disposal of carcasses and wastes of aquatic animals (New chapter)**

A large number of Member comments had been received. The Aquatic Animals Commission deferred consideration of these comments to its October 2008 meeting.

#### **2.15. Guidelines for aquatic animal health surveillance (New chapter)**

The new Chapter on Guidelines for aquatic animal health surveillance that will be proposed for adoption and inclusion in the *Aquatic Code* contains a lot of technical information. Much of this information will be included in the OIE Handbook on Aquatic Animal Health Surveillance that is currently under preparation. Once the handbook is published (early 2009), the Aquatic Animals Commission will revise the surveillance chapter for the *Aquatic Code* to reduce the amount of technical information, thereby rendering the chapter more consistent with other chapters in the *Aquatic Code*.

In response to comments from the USA on the current inconsistency between the time periods required for demonstrating freedom from disease (e.g. 10 years for historical freedom in the guidelines versus 25 years in some of the disease chapters), the Aquatic Animals Commission clarified that if adopted, the guidelines would provide the default periods; deviation from this for specific diseases would only be proposed where this can be justified scientifically.

The EU made a number of suggestions for changes to Articles 6, 7 and 8 of the surveillance guidelines (pathways to demonstrate freedom from disease; maintenance of disease free status; and design of surveillance programmes to demonstrate freedom from disease) to be more suitable for the diseases Viral haemorrhagic septicaemia and Gyrodactylosis. The Aquatic Animals Commission believes that these comments should be taken into account in the specific disease chapters and not in these general guidelines.

The Aquatic Animals Commission reviewed the *ad hoc* Group's report on the surveillance guidelines and made some amendments in response to the *ad hoc* Group's queries. Some of the comments received from Members were of a highly technical nature and will be referred to the *ad hoc* Group for consideration at the next meeting in April 2008.

The updated Chapter on Guidelines for aquatic animal health surveillance that will be proposed to the OIE International Committee for adoption at the 76<sup>th</sup> General Session in May 2008 is presented at [Annex XVI](#).

### 3. *Aquatic Animal Health Code - other items*

#### 3.1. **Horizontal changes in disease chapters**

Dr Bernoth reminded the Aquatic Animals Commission that some of the changes made to the disease chapters adopted at the 75th General Session in May 2007 still needed to be made to all disease chapters in the *Aquatic Code*. These changes consisted of improving clarity to Article 3 on commodities and other minor editorial changes. The Aquatic Animals Commission had made further editorial changes to some of the chapters with its October 2007 report and will include all these in the 2008 edition of the *Aquatic Code*, provided that these are adopted.

The Aquatic Animals Commission noted comments from several Members on perceived inconsistencies in the listing of safe commodities in different disease chapters. The Aquatic Animals Commission clarified that because the chapters are disease specific, the list of safe commodities will not necessarily be the same for all diseases.

Thailand highlighted that in contrast to the fish disease chapters, no type of processed shrimp (chilled or frozen) is listed as a safe commodity under the category of products for human consumption and prepared and packaged for direct retail trade in four of the shrimp disease chapters. They queried why the risks of viral disease transmission from chilled and frozen fish product for human consumption can be considered negligible while those for shrimp are not. The Aquatic Animals Commission emphasised that the *Aquatic Code* chapters are written on a disease-by-disease basis and therefore treatment that renders a product safe for a fish disease does not necessarily render a similar product safe for a crustacean disease. However, the Aquatic Animals Commission welcomes any scientific evidence that demonstrates the safety of commodities and strongly encourages Members to make such information available to the Aquatic Animals Commission.

The Aquatic Animals Commission agrees that there is a need for further consideration of safe commodities based on the scientific evidence and will propose to the Director General to convene an *ad hoc* Group on Safe Commodities Derived from Aquatic Animals. This *ad hoc* Group should take account of any relevant work undertaken by the *ad hoc* Group on Trade in Terrestrial Animal Products ('commodities'). In the meantime, the Aquatic Animals Commission removed the listing of 'chemically preserved products (e.g. smoked, salted, pickled, marinated etc.)' as safe commodities from all disease chapters where it was included, because of the conflicting views expressed by Members.

#### 3.2. **Antimicrobial resistance in the field of aquatic animals**

Dr Tomoko Ishibashi, Deputy Director of the OIE Scientific and Technical Department, joined the Aquatic Animals Commission for this item. Dr Ishibashi provided an update on developments in this field. She explained that the fourth joint FAO/WHO/OIE Meeting on Critically Important Antimicrobials, held on 26 November 2007, was an important forum for discussing the appropriate balance between animal health needs and public health concerns in the use of antimicrobial products. Dr Ishibashi noted that one of the 15 experts selected to attend the joint meeting was an expert in aquatic animal health. She commented that the meeting had been very constructive, with all parties reaching agreement on the list of critically important antimicrobials. She noted that one of the recommendations from the meeting made reference to the aquatic environment, i.e. the need for a risk analysis on the release of human and animal effluents into aquatic environments which serve as the growing grounds of fisheries and aquaculture products. Dr Ishibashi indicated that the full report would be available on the OIE website shortly.

The Aquatic Animals Commission thanked Dr Ishibashi for this update and commented that the Commission would like to be involved in any future revisions on the critically important antimicrobials list to ensure antimicrobials in the aquatic sector are considered.

### **3.3. Crayfish plague (Chapter 2.3.7.)**

A revised version of the chapter on crayfish plague had been received from an OIE expert. The Aquatic Animals Commission will review this version at its October 2008 meeting.

## **4. Joint meeting with the President of the Terrestrial Animal Health Standards Commission**

### **4.1 Update on the new structure of the *Terrestrial Code***

Dr Alejandro Thiermann, President of the Terrestrial Animal Health Standards Commission (Code Commission) updated the Aquatic Animals Commission on the proposed revised structure of the *Terrestrial Code*. He explained that the *Terrestrial Code* would be divided into two volumes; the first would include all horizontal (generic) chapters and the second volume, all disease specific chapters. He reported that an OIE expert was working to harmonize many of the horizontal chapters. Dr Bernoth referred to the progress being made towards harmonisation of the two *Codes*. She commented that further amendments to the horizontal chapters in the *Aquatic Code* would await the division of the *Terrestrial Code* into two volumes and the revision of the horizontal chapters in the *Terrestrial Code*.

Dr Thiermann and Dr Bernoth pointed out that some Members provide comments only on proposed changes to the horizontal chapters of the *Terrestrial Code* and others only on those of the *Aquatic Code* when both Commissions have circulated proposed changes to the matching chapters, for example the chapter on General obligations. This makes harmonisation of the two *Codes* even more difficult. Members are encouraged therefore to bear both *Codes* in mind when sending comments on horizontal chapters.

### **4.2. Compartmentalisation**

Dr Thiermann informed the Aquatic Animals Commission of a Standards and Trade Development Facility (STDF) funded project that will be conducted in the next few months in Thailand and Brazil that will provide OIE expertise to those countries in the application of compartmentalisation for poultry diseases.

The Aquatic Animals Commission had received comments from the EU suggesting draft text for defining and re-establishing (after a breakdown) the disease free status of a compartment for all disease chapters proposed for comment. The Aquatic Animals Commission decided to await the outcomes of the proposed pilot projects in Thailand and Brazil before attempting to draft further text on compartmentalisation for the *Aquatic Code*. The Aquatic Animals Commission also draws Members' attention to the chapter on compartmentalisation in the special issue on "Changing Trends in Managing Aquatic Animal Disease Emergencies", in the OIE *Scientific and Technical Review* series, which will be published in April 2008 (see Item 5 below).

### **4.3. Model veterinary certificates**

Dr Thiermann provided an update on the recent meeting of the OIE *ad hoc* Group on the Revision of the OIE Model Certificates. The proposal is that all certificates currently in the *Terrestrial Code* (with two exceptions) will be replaced by the four model veterinary certificates developed by the *ad hoc* Group, as yet to be endorsed by the Terrestrial Animals Health Standards Commission. These model veterinary certificates have been harmonised with the Codex Alimentarius principles for certification. The Aquatic Animals Commission will await the adoption of the terrestrial model certificates before revising the aquatic model certificates. At that time, the Aquatic Animals Commission will also review Chapters 1.3.1 (General Obligations) and 1.3.2 (Certification procedures) for the *Aquatic Code*.

#### 4.4. Evaluation of Performance of Veterinary Services (OIE PVS Tool)

Dr Kahn updated the Aquatic Animals Commission on the new tool for the Evaluation of Performance of Veterinary Services (OIE PVS Tool), available on the OIE website, and noted that the introduction now makes reference to the application of the PVS Tool to the evaluation of aquatic animal health services.

The Aquatic Animals Commission reviewed a draft Annex to the PVS Tool prepared by Dr Keren Bar-Yaacov, CVO of Norway, on modifications of the approach that would be required for the evaluation of the performance of Competent Authorities responsible for aquatic animal health. The Aquatic Animals Commission appreciated this contribution and requested that work continue on the development of this Annex.

### 5. Joint meeting with the Publications Department

Prof. Paul-Pierre Pastoret, Head of OIE Publications Department, and Ms Annie Souryi, Deputy Head of OIE Publications Department, joined the Aquatic Animals Commission for an update on progress with the upcoming publication in the OIE Scientific and Technical Review series on “Changing Trends in Managing Aquatic Animal Disease Emergencies”. This issue of the Review is due for publication in April 2008 and will be available for the 76th General Session in May 2008.

The Publications Department confirmed that it would manage the publication of the Handbook on Aquatic Animal Health Surveillance (see Item 1.). It is envisaged that this would be published by early 2009.

### 6. The role and activities of the OIE in the field of aquatic animal health

#### 6.1. International meetings

##### 6.1.1. Regional Commission Conferences

Dr Hill attended the 9th Conference of the OIE Regional Commission for the Middle East (Damascus, Syria, 29 October-1 November 2007) and gave the Delegates an update on developments in aquaculture worldwide, with emphasis on the Middle Eastern region, and aquatic animal health initiatives of the Aquatic Animals Commission. A summary of his presentation can be found at [Annex XIX](#).

Dr Bernoth attended part of the 25<sup>th</sup> Conference of the OIE Regional Commission for Asia, the Far East and Oceania (Queenstown, New Zealand, 26-30 November 2007). She updated attendants on actions taken by the OIE and its Aquatic Animal Health Standards Commission to implement the recommendations on roles and responsibilities for aquatic animal health that had been adopted at the Commission’s 23<sup>rd</sup> Conference in 2003 (the “Nouméa Recommendations”). She drew participants’ attention to the OIE First Global Conference on Aquatic Animal Health that took place in October 2006, and to the upcoming issue on “Changing trends in managing aquatic animal disease emergencies” in the OIE *Scientific and Technical Review* series. Dr Bernoth also explained the implications of some important aquatic animal health decisions taken by the International Committee at the 75<sup>th</sup> General Session in May 2007, for example, the in-principle agreement to include amphibian diseases in the OIE’s remit, and some important draft text currently in the consultation process. She shared with Conference attendants thoughts about some challenges that lie ahead, for example the on-going ‘catch-up’ situation with emerging aquatic animal diseases in newly farmed species, wider animal production issues such as controls on availability and use of antimicrobials, closer scrutiny by trading partners of import measures, and consumer concerns about animal welfare, food safety and environmental protection.

The Aquatic Animals Commission noted the schedule for the upcoming Regional Commission Conferences and agreed the following representation for follow-up presentations on developments in aquatic animal health:

- 23rd Conference of the OIE Regional Commission for Europe (Vilnius, Lithuania, 16-19 September 2008): Dr Franck Berthe.
- 19<sup>th</sup> Conference of the OIE Regional Commission for the Americas (Havana, Cuba, 18-22 November 2008): Dr Ricardo Enriquez.
- 18<sup>th</sup> Conference of the OIE Regional Commission for Africa (N'Djamena, Chad, February 2009): Prof. Eli Katunguka-Rwakishaya.

### 6.1.2. Network of Aquaculture Centres in Asia-Pacific

In her role as the Aquatic Animals Commission's permanent representative on the Network of Aquaculture Centres in Asia-Pacific (NACA) Asia Regional Advisory Group (AG) on Aquatic Animal Health, Dr Bernoth attended the AG's 6th annual general meeting from 12 to 14 December 2007 at the NACA Headquarters in Bangkok, Thailand. Dr Bernoth had been Vice-Chair of the group since its first meeting in 2002 and at the 6th AGM was elected Chair. She provided an update on the latest (2007) edition of the OIE *Aquatic Code* and briefly explained some draft new or revised text that was sent to OIE Members for comment.

After receiving an update on the aquatic animal disease status in the region, the AG reviewed the regional OIE/NACA Quarterly Aquatic Animal Disease (QAAD) reporting list. Diseases de-listed from the OIE *Aquatic Code* were assessed against the OIE aquatic animal disease listing criteria applied in a regional rather than global context. The AG decided to retain viral encephalopathy and retinopathy, enteric septicaemia of catfish and Channel catfish virus disease on the regional list. Using the same set of criteria, the AG also decided to add the non-OIE-listed crustacean diseases *Monodon* slow growth syndrome and milky lobster disease; and the mollusc disease acute viral necrosis in scallops. The previously listed grouper iridoviral disease and the two mollusc diseases infection with *Marteilioides chungmuensis* and Akoya oyster diseases, which had never been listed in the *Aquatic Code*, were assessed and found to meet the listing criteria when applied regionally and hence maintained on the QAAD list.

Dr Karim Ben Jebara, Head of the OIE Animal Health Information Department, attended for part of the AGM and provided a brief explanation of the OIE's World Animal Health Information System (WAHIS) and its interface, the World Animal Health Information Database (WAHID). Dr Ben Jebara, Dr Sakurai from the OIE Regional Representation for Asia and the Pacific, and the AG agreed to a future aquatic animal disease reporting system for the region that allows full inclusion of QAAD reporting into the WAHIS six-monthly system, thereby avoiding the compilation of two data sets by countries. Information on OIE-listed diseases would be entered into WAHIS and be searchable in WAHID. However, the creation of a WAHIS/NACA Regional Core for Aquatic Animal Health would also allow entering information on non-OIE-listed diseases. Such information would not be displayed or searchable in WAHID globally, but would appear on the websites of NACA and OIE Asia Pacific. NACA and the OIE will expedite the necessary agreements between NACA and the OIE and technical specifications for the WAHIS/NACA Regional Core for Aquatic Animal Health.

### 6.1.3. OIE/NACA Regional Workshop on Aquatic Animal Health

Dr Bernoth reported on the upcoming OIE/NACA Regional Workshop on Aquatic Animal Health, which will be organised by the OIE and NACA and take place in Bangkok, Thailand, from 25 to 28 March 2008. The objectives of the Workshop are to recognise the importance of negative impacts of aquatic animal diseases, the need for their control and prevention, and the responsibilities of government authorities in this context; to provide updated information on emerging aquatic animal diseases in the region; to train national focal points on OIE aquatic animal health standards and on WAHIS (using computers); and to strengthen regional collaboration on aquatic animal disease control and prevention. The invited participants are national focal points for aquatic animal health in the countries which have participated in the QAAD reporting in the Asia-Pacific region, which has been implemented as a joint activity between NACA, FAO and OIE Regional Representation for Asia and the Pacific since 1998. Dr Bernoth reported that she has been invited by the OIE Regional Representation for Asia and the Pacific as a resource person to present on the introduction and use of OIE standards for aquatic animal health within the WTO-SPS Agreement framework and the OIE standards setting process.

### 6.1.4. Other meetings

The Third Meeting of the Inter-American Committee for Aquatic Animal Health will take place in Mexico in August 2008. Dr Enriquez will represent the Aquatic Animals Commission at this meeting, and will give an update on the activities of the Aquatic Animals Commission.

## 6.2. Cooperation with FAO

The Aquatic Animals Commission noted the proposed Regional Aquatic Biosecurity Framework Project for Africa, and agreed in principle to participate in this project, as appropriate. The Commission would welcome further information.

## 7. *Manual of Diagnostic Tests for Aquatic Animals*

### 7.1. Progress with 6<sup>th</sup> edition of the *Aquatic Manual* (2009)

The Aquatic Animals Commission was updated on progress with the 6<sup>th</sup> edition of the *Aquatic Manual*, which is due for publication in the third quarter of 2009. Authors had been invited to write chapters according to the revised template, and a number of drafts have been received. These have been sent to the Consultant Editor. Those authors who have not yet submitted a chapter will be reminded of the deadline. It is planned to circulate the chapters for comment to Members and reviewers in June this year. Members are reminded that the 6<sup>th</sup> edition will include updated chapters on de-listed diseases (these were not updated in the 5<sup>th</sup> edition).

In the report of its last meeting in October 2007, the Aquatic Animals Commission had requested Members to nominate experts who could be asked to update the chapters on Infectious pancreatic disease, Piscirickettsiosis (*Piscirickettsia salmonis*) and spawner-isolated mortality virus disease. No nominations were received. For the two fish diseases, Dr Ricardo Enriquez has contacted some experts he believes could assist with this task.

### 7.2. Update from the Consultant Editor

Dr David Alderman reported that he is still working on the chapter on disinfection. This will be circulated along with the disease chapters in June 2008 (see Item 7.1.).

### **7.3. OIE Procedure for validation and certification of diagnostic assays**

In April 2006 the OIE received an application for a test kit for white spot disease in crustaceans. Following the OIE procedure for validation and certification of diagnostic assays, the application was reviewed by experts. Based on the first report from the panel of experts, the applicant carried out additional studies and submitted a revised report, which again was assessed by the experts. In January 2008, the expert panel recommended that the kit ('IQ2000 WSSV PCR Detection and Prevention System') be included in the OIE Register as fit for the three purposes listed. The Aquatic Animals Commission found that the reviewers did a thorough job of evaluating the dossier and is in agreement with the conclusion that the kit should be registered for the three purposes listed. The President will recommend that this proposal be adopted at the next General Session.

## **8. OIE Reference Laboratories**

### **8.1. Updating the list of OIE Reference Laboratories**

The Aquatic Animals Commission had received two applications for OIE Reference Laboratory status: from the University of Arizona, USA, for its designation as an OIE Reference Laboratory for Infectious myonecrosis, with Prof. Donald Lightner as the designated expert; and from C. Abdul Hakeem College (Affiliated to Thiruvalluvar University, Tamil Nadu), India, for its designation as an OIE Reference Laboratory for White tail disease, with Dr A.Sait Sahul Hameed as the designated expert. The Aquatic Animals Commission will recommend their acceptance by the International Committee at the 76<sup>th</sup> General Session in May 2008.

### **8.2. Annual reports of OIE Reference Laboratory activities**

Reports had been received from all but three of the OIE Reference Laboratories 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 efforts.

## **9. Any other business**

### **9.1. Update of the Commission's web pages**

The meeting was joined by Dr Daniel Chaisemartin, Head of the OIE Administration and Management Systems Department. Dr Hill emphasised the need for easier direct access to the Aquatic Animals Commission's web pages from the OIE home page and suggested possible improvements. Dr Chaisemartin will explore possibilities to meet this request. The Aquatic Animals Commission identified a number of areas on the web pages that require updating and Dr Hill agreed to make these changes.

### **9.2. Review of the Aquatic Animals Commission's work plan for 2008-2009**

The Aquatic Animals Commission reviewed and updated its work plan which is attached at [Annex XX](#) for Members' information.

### **9.3. Date of the next meeting of the Aquatic Animals Commission**

The next meeting of the Aquatic Animals Commission will take place from 13 to 17 October 2008.

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.../Annexes



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

**Paris, 3-7 March 2008**

**List of participants**

**MEMBERS OF THE COMMISSION**

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

**Paris, 3-7 March 2008**

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**Adopted Agenda**

**Welcome from the Director General**

**Adoption of the Agenda**

**1. Activities and progress of *ad hoc* Groups**

- 1.1. Summary of *ad hoc* Groups - tasks and meetings
- 1.2. Report of the *ad hoc* Group on the OIE List of Aquatic Animal Diseases - Mollusc Team
- 1.3. Report of the *ad hoc* Group on Aquatic Animal Health Surveillance

**2. Aquatic Animal Health Code – Member comments on draft text**

- 2.1. Disease chapters – general comments
- 2.2. Definitions (Chapter 1.1.1.)
- 2.3. Diseases listed by the OIE (Chapter 1.2.3.)
- 2.4. General obligations (Chapter 1.3.1.)
- 2.5. Guidelines for import risk analysis (Chapter 1.4.2.)
- 2.6. Recommendations for transport (Chapter 1.5.1.)
- 2.7. Infectious myonecrosis (Chapter 2.3.9.) and White tail disease (Chapter 2.3.11.)
- 2.8. Infection with *Mikrocytos mackini* (Chapter 2.2.5.)
- 2.9. Gyrodactylosis (*Gyrodactylosis salaris*) (Chapter 2.1.14.)
- 2.10. Infection with *Batrachochytrium dendrobatidis* (New chapter)
- 2.11. Infection with ranavirus (New chapter)
- 2.12. Introduction to OIE guidelines for the welfare of live aquatic animals (New chapter)
- 2.13. Guidelines for the control of aquatic animal health hazards in aquatic animal feed (New chapter)
- 2.14. Guidelines on handling and disposal of carcasses and wastes of aquatic animals (New chapter)
- 2.15. Guidelines for aquatic animal health surveillance (New chapter)

Annex II (contd)**3. Aquatic Animal Health Code – other items**

- 3.1. Horizontal changes in disease chapters
- 3.2. Antimicrobial resistance in the field of aquatic animals
- 3.3. Crayfish plague (Chapter 2.3.7.)

**4. Joint meeting with the President of the Terrestrial Animal Health Standards Commission**

- 4.1. Update on the new structure of the *Terrestrial Code*
- 4.2. Compartmentalisation
- 4.3. Model veterinary certificates
- 4.4. Evaluation of Performance of Veterinary Services (OIE PVS Tool)

**5. Joint meeting with the Publications Department****6. The role and activities of the OIE in the field of aquatic animal health**

- 6.1. International meetings
  - 6.1.1. Regional Commission Conferences
  - 6.1.2. Network of Aquaculture Centres in Asia-Pacific
  - 6.1.3. OIE/NACA Regional Workshop on Aquatic Animal Health
  - 6.1.4. Other meetings
- 6.2. Cooperation with FAO

**7. Manual of Diagnostic Tests for Aquatic Animals**

- 7.1. Progress update on 6<sup>th</sup> edition of the *Aquatic Manual*
- 7.2. Update from the Consultant Editor
- 7.3. OIE Procedure for validation and certification of diagnostic assays

**8. OIE Reference Laboratories**

- 8.1. Updating the list of OIE Reference Laboratories
- 8.2. Annual reports of OIE Reference Laboratory activities

**9. Any other business**

- 9.1. Update of the Commission's web pages
- 9.2. Review of the Aquatic Animals Commission's work plan for 2008-2009

**10. Date of the next meeting**

## CHAPTER 1.1.1.

## DEFINITIONS

## Article 1.1.1.1.

***Aquatic animals***

means all life stages (including *eggs* and *gametes*) of fish, molluscs, ~~and~~ crustaceans, and amphibians originating from *aquaculture establishments* or removed from the wild, for farming purposes, for release into the aquatic environment or for human consumption.

***Aquatic animals for slaughter/harvest***

means aquatic animals that are destined to be transported or taken following arrival in the importing country under the control of the relevant Competent Authority, to a fish slaughtering premises or other processing plant preparing products for human consumption.

***Area of direct transit***

means a special area established in a transit country approved by the relevant Competent Authority where aquatic animals stay for a very short time, and where water changes may be made, before further transport to their final destination when passing through the transit territory.

**Bias**

A tendency of an estimate to differ in a non-random fashion from the true value of a *population* parameter.

**Case definition**

A *case definition* is a set of criteria used to distinguish a case animal or *epidemiological unit* from a non-case.

***Disease***

means clinical or non clinical *infection* ~~or infestation~~ with one or more of the aetiological agents of the *diseases* referred to in the *Aquatic Code*.

**Epidemiological unit**

A group of animals that share approximately the same risk of exposure to a *disease agent* with a defined location. This may be because they share a common aquatic environment (e.g. fish in a pond, caged fish in a lake), or because management practices make it likely that a *disease agent* in one group of animals would quickly spread to other animals (e.g. all the ponds on a farm, all the ponds in a village system).

**Incubation period**

means the period that elapses between the introduction of a *disease agent* into an aquatic animal population and the occurrence of the first clinical signs of the disease.

Annex III (contd)**Infection**

means the presence of a multiplying or otherwise developing or latent *disease agent* in a host. This term is understood to include infestation where the *disease agent* is a parasite in or on a host.

**Infestation**

means the presence in sufficient numbers of a multiplying of a notifiable parasitic, or commensal, agent on or in a host a host so as to cause damage or *disease*.

**Inspection**

means the control carried out by the *Competent Authority* in order to ensure that an *aquatic animal* is/*aquatic animals* are free from the *diseases* considered in the *Aquatic Code*; the *inspection* may call for clinical examination, laboratory tests and, generally, the application of other procedures that could reveal an *infection* or an *infestation* that may be present in an *aquatic animal population*.

**Offal**

means ~~visceral organs, cut offs, condemned raw material, organs, etc.~~ of aquatic animals.

**Probability sampling**

A sampling strategy in which every unit has a known non-zero probability of inclusion in the sample.

**Sensitivity**

the proportion of true positive tests given in a diagnostic test, i.e. the number of true positive results divided by the number of true positive and false negative results.

**Specificity**

the probability that absence of infection will be correctly identified by a diagnostic test, i.e. the number of true negative results divided by the number of true negative and false positive results.

**Stamping-out policy**

means the carrying out under the authority of the *Competent Authority*, on confirmation of a *disease*, of preventive *aquatic animal* health measures, consisting of killing the *aquatic animals* that are affected, those suspected of being affected in the *population* and those in other *populations* that have been exposed to *infection* ~~or infestation~~ by direct or indirect contact of a kind likely to cause the transmission of the *disease agent*. All these *aquatic animals*, vaccinated or unvaccinated, on an infected site should be killed and the carcasses destroyed by burning or burial, or by any other method that will eliminate the spread of *infection* ~~or infestation~~ through the carcasses or products of the *aquatic animals* destroyed.

This policy should be accompanied by cleansing and *disinfection* procedures as defined in the *Aquatic Code*. *Fallowing* should be for an appropriate period determined by *risk assessment*.

**Study population**

means the *population* from which surveillance data are derived. This may be the same as the *target population* or a subset of it.

**Subclinical**

means without clinical manifestations, for example a stage of *infection* ~~or infestation~~ at which signs are not apparent or detectable by clinical examination.

**Susceptible species**

means a species of *aquatic animal* in which *infection* ~~or infestation~~ has been demonstrated by natural cases or by experimental exposures to the *disease agent* that mimics the natural pathways for *infection* ~~or infestation~~. Each *disease* chapter in the *Aquatic Manual* contains a list of currently known *susceptible species*.

**Target population**

~~For the purposes of demonstrating freedom from *infection*, the *population* of interest, usually made up of all *aquatic animals* of species susceptible to a specified *disease agent* in a defined country, *zone* or *aquaculture establishment*.~~

**Targeted surveillance**

means *surveillance* targeted at a specific *disease*, or *infection* ~~or infestation~~.

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## CHAPTER 1.2.3.

**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 1.2.2.1.) or criteria for listing an *emerging aquatic animal disease* (see Article 1.2.2.2.).

## Article 1.2.3.1.

The following diseases of fish are listed by the OIE:

- Epizootic haematopoietic necrosis
- Infectious haematopoietic necrosis
- Spring viraemia of carp
- Viral haemorrhagic septicaemia
- Infectious salmon anaemia
- Epizootic ulcerative syndrome
- Gyrodactylosis (*Gyrodactylus salaris*)
- Red sea bream iridoviral disease
- Koi herpesvirus disease.

## Article 1.2.3.2.

The following diseases of molluscs are listed by the OIE:

- Infection with *Bonamia ostreae*
- Infection with *Bonamia exitiosa*
- Infection with *Marteilia refringens*
- Infection with *Perkinsus marinus*
- Infection with *Perkinsus olseni*
- Infection with *Xenohaliotis californiensis*
- Abalone viral mortality <sup>1</sup>.

## Article 1.2.3.3.

The following diseases of crustaceans are listed by the OIE:

- Taura syndrome
- White spot disease
- Yellowhead disease
- Tetrahedral baculovirus (*Baculovirus penaei*)
- Spherical baculovirus (*Penaeus monodon*-type baculovirus)
- Infectious hypodermal and haematopoietic necrosis
- Crayfish plague (*Aphanomyces astaci*)
- Necrotising hepatopancreatitis <sup>2</sup>
- Infectious myonecrosis
- White tail disease <sup>1</sup>
- Hepatopancreatic parvovirus disease <sup>2</sup>
- Mourilyan virus disease <sup>2</sup>.

Annex IV (contd)

Article 1.2.3.4

The following diseases of amphibians are listed by the OIE:

- Infection with *Batrachochytrium dendrobatidis*
- Infection with ranavirus

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1. Listed according to Article 1.2.2.2.
2. Listing of this disease is under study.

## CHAPTER 1.3.1.

### GENERAL OBLIGATIONS

#### Article 1.3.1.1.

~~A combination of health factors should be taken into account to ensure unimpeded international trade in aquatic animals and aquatic animal products without incurring unacceptable risks to human and aquatic animal health. International trade in aquatic animals and aquatic animal products depends on a combination of health factors that should be taken into account to ensure unimpeded trade, without incurring unacceptable risks to human and aquatic animal health. As a general principle, international trade in aquatic animals and their products from populations known to be infected with a listed disease and considered to be capable of transmitting the disease should only be done with the prior agreement of the importing and exporting countries.~~

Because of the likely variations in 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 that have to be met for trade. To maximise harmonisation of the aquatic animal health aspects of *international trade*, *Competent Authorities* of OIE Members ~~Countries~~ should base their import requirements on the OIE standards, guidelines and recommendations.

These requirements should be included in the model *international aquatic animal health certificates* approved by the OIE, which form Part 4. of the *Aquatic Code*.

Certification requirements should be exact and concise, and should clearly convey the wishes of the *importing country*. For this purpose, prior consultation between *Competent Authorities* of *importing* and *exporting countries* is useful and may be necessary. It enables the setting out of the exact requirements so that the ~~signing veterinarian or other~~ *certifying official* can, if necessary, be given a note of guidance explaining the understanding between the *Competent Authorities* involved.

When Members of, or representatives acting on behalf 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.

#### Article 1.3.1.2.

#### **Responsibilities of the importing country**

1. The import requirements included in the *international aquatic animal health certificate* should assure that *commodities* introduced into the *importing country* comply with the national level of protection. *Importing countries* should restrict their requirements to those justified for such level of protection. If these are more strict than the OIE standards, guidelines and recommendations, then they should be based on an import risk analysis.
2. The *international aquatic animal health certificate* should not include requirements for the exclusion of pathogens or *aquatic animal diseases* that are present within the *territory* of the *importing country* and are not subject to any official control programme ~~except when the strain of pathogen in the exporting country is of significantly higher pathogenicity and/or has a larger host range~~. The requirements applying to pathogens or *diseases* subject to official control programmes in a country, or *zone* should not provide a higher level of protection on imports than that provided for the same pathogens or *diseases* by the measures applied within that country, or *zone*.

## Annex V (contd)

3. The international aquatic animal health certificate should not include requirements for disease agents or diseases which are not OIE listed, unless the importing country has identified the disease agent as presenting a significant risk for that country, after conducting a scientifically based import risk analysis according to the guidelines in Section 1.4.

- 3.4. The transmission by the *Competent Authority* or *Veterinary Administration* of certificates or the communication of import requirements to persons other than the *Competent Authority* or *Veterinary Administration* of another country necessitates that copies of these documents be also sent to the *Competent Authority* or *Veterinary Administration*.

This important procedure avoids delays and difficulties that may arise between traders and *Competent Authorities* or *Veterinary Administrations* when the authenticity of the certificates or permits is not established.

This information is usually the responsibility of *Veterinary Administrations* or other *Competent Authorities* of the *exporting country*. However, it can be the responsibility of *Veterinary Authorities* or other *Competent Authorities* at the place of origin of the *aquatic animals*, if different from the *exporting country*, when it is agreed that the issue of certificates does not require the approval of the *Veterinary Administrations* or other *Competent Authorities*.

## Article 1.3.1.3.

**Responsibilities of the exporting country**

1. An *exporting country* should, on request, ~~be prepared to~~ supply the following ~~information~~ to *importing countries* ~~on request~~:
  - 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* that are free from ~~OIE-listed~~ OIE-listed diseases referred to in this Aquatic Code including the regulations and procedures in force to maintain its free status;
  - b) regular and prompt information on the occurrence of ~~transmissible listed~~ OIE-listed diseases referred to in this Aquatic Code;
  - c) for diseases not ~~listed~~ listed referred to in this Aquatic Code, information on if there are new findings that are of potential epidemiological significance to other countries;
  - d) details of the country's ability to apply measures to control and prevent ~~OIE-listed~~ OIE-listed diseases referred to in this Aquatic Code;
  - e) information on the structure of the *Competent Authority* and the authority that they exercise;
  - f) technical information, particularly on biological tests and vaccines applied in all or part of the national territory;
  - g) identification of the country or location of harvest or production of the product being exported.
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 covering possible suspension and termination of their appointment;
  - b) ensure that ~~the~~ relevant instructions and training are provided to *certifying officials*;

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

The Head of the *Competent Authority* of the *exporting country* is ultimately accountable for the *certifying official* used in *international trade*.

Article 1.3.1.4.

### **Responsibilities in case of an incident occurring after importation**

*International trade* involves a continuing ethical responsibility. Therefore, if within a reasonable period ~~the recognised infective periods of the various diseases~~ 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 *Authority* to notify the *importing country*, so that the imported *aquatic animals* may be inspected or tested and appropriate action be taken to limit the spread of the *disease* should it have been inadvertently introduced.

Equally, if a *disease* condition appears in imported *aquatic animals* within a reasonable time period after importation consistent with the recognised incubation period of the disease, 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*.

In case of suspicion, on reasonable grounds, that an official 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. The *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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## CHAPTER 1.4.2.

## GUIDELINES FOR IMPORT RISK ANALYSIS

## Article 1.4.2.1.

**Introduction**

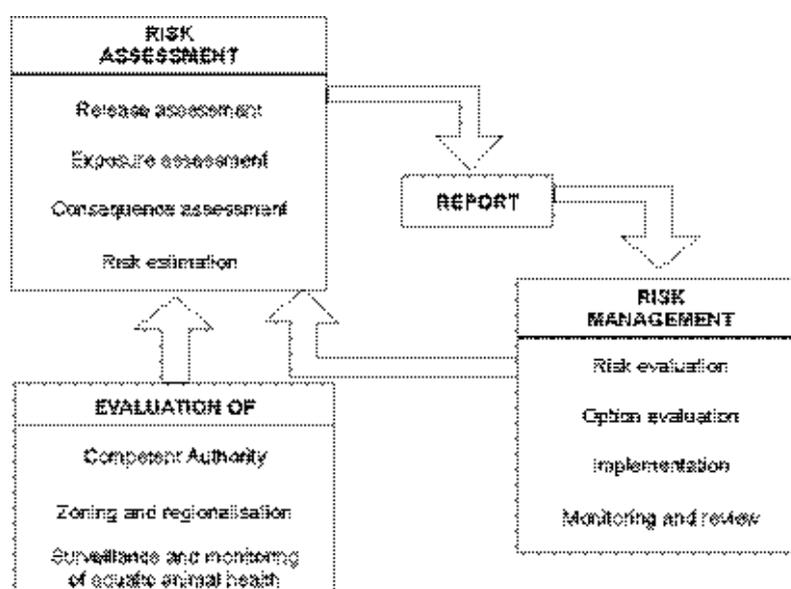
An import risk analysis begins with a description of the *commodity* proposed for import and the likely annual quantity of trade. It must be recognised that whilst an accurate estimate of the anticipated quantity of trade is desirable to incorporate into the risk estimate, it may not be readily available, particularly where such trade is new.

*Hazard identification* is an essential step that must be conducted before the *risk assessment*.

The *risk assessment* process consists of four interrelated steps. These steps clarify the stages of the *risk assessment*, describing them in terms of the events necessary for the identified potential *risk(s)* to occur, and facilitate understanding and evaluation of the conclusions (or 'outputs'). The product is the *risk assessment* report, which is used in *risk communication* and *risk management*.

The relationships between *risk assessment* and *risk management* processes are outlined in Figure 1.

**Fig. 1. The relationship between risk assessment and risk management processes**



## Article 1.4.2.2.

**Hazard identification**

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

The *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 an *OIE-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.

Annex VI (contd)

*Hazard identification* is a categorisation step, identifying biological agents dichotomously as *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 *Competent Authorities*, surveillance and control programmes, and *zoning* and regionalisation 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 1.4.2.3.

### **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* must be able to accommodate the variety of 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* and *quantitative* risk assessment methods are valid. Although quantitative analysis is recognised to provide deeper insights into a particular problem, qualitative methods may be more relevant when available data are limited as is often the case with aquatic species.
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 1.4.2.4.

### **Risk assessment steps**

#### 1. Release assessment

Release assessment consists of describing the biological pathway(s) necessary for an importation activity to 'release' (that is, introduce) a *hazard* into a particular environment, and estimating the likelihood of that complete process occurring. The release assessment describes the likelihood of the 'release' 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. Examples of the kind of inputs that may be required in the release 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/prevalence
  - Evaluation of *Competent Authorities*, surveillance and control programmes, and *zoning* 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 release 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 humans and aquatic and terrestrial animals in the *importing country* to the *hazards* and estimating the likelihood of these exposure(s) occurring, ~~and of the spread or establishment of the *hazard*~~.

The likelihood of exposure to the *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 human, *aquatic animal* or terrestrial animal populations exposed. Examples of the kind of inputs that may be required in the exposure assessment are:

- a) Biological factors
  - Presence of potential vectors or intermediate hosts
  - Genotype of host
  - Properties of the agent (e.g. virulence, pathogenicity and survival parameters).

Annex VI (contd)

## b) Country factors

- *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* should conclude at this step.

3. Consequence assessment

Consequence assessment consists of identifying the potential biological, environmental and economic consequences. A causal process must exist by which exposures to a *hazard* result in adverse health, environmental or socio-economic consequences. Examples of consequences include:

## a) Direct consequences

- *Aquatic animal infection, disease*, production losses and facility closures
- Adverse, and possibly irreversible, consequences to the environment
- Public health consequences.

## b) Indirect consequences

- Surveillance and control costs
- Compensation costs
- Potential trade losses
- Adverse consumer reaction.

#### 4. Risk estimation

Risk estimation consists of integrating the results of the release 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 1.4.2.5.

#### **Principles of risk management**

1. *Risk management* is the process of deciding upon and implementing measures to achieve the Member'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 or other recommendations of the SPS Agreement.

Article 1.4.2.6.

#### **Risk management components**

1. Risk evaluation - the process of comparing the *risk* estimated in the *risk assessment* with the Member'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 line with the Member's appropriate level of protection. The efficacy is the degree to which an option reduces the likelihood and/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 VI (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 1.4.2.7.

### **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* for obtaining a scientific critique aimed at ensuring that the data, information, methods and assumptions are the best available.

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## CHAPTER 1.5.1.

## RECOMMENDATIONS FOR **SAFE** TRANSPORT **OF** **AQUATIC ANIMALS AND** **AQUATIC ANIMAL PRODUCTS**

## Article 1.5.1.1.

**General considerations arrangements**

1. These considerations arrangements should be used as guidelines when countries introduce measures to control the aquatic animal health risks related to the transport of these aquatic animals and aquatic animal products. These guidelines do not address aquatic animal welfare, compulsory in all countries either by legislative or regulatory texts and methods of application should be described in a manual available to all concerned.
2. *Vehicles (or containers) used for the transport of aquatic animals shall be designed, constructed and fitted in such a way as to withstand the weight of the aquatic animals and water and to ensure their safety and welfare during transportation. Vehicles shall be thoroughly cleansed and disinfected before use according to the guidelines given in the Aquatic Code.*
3. *Vehicles (or containers) in which aquatic animals are confined during transport by sea or by air shall be secured to maintain optimal conditions for the aquatic animals during transport, and to allow easy access by the attendant.*

## Article 1.5.1.2.

**Particular considerations arrangements for containers**

1. The construction of *containers* intended for *transportation* of *aquatic animals* shall be such that the accidental release of water, etc., is prevented during *transport*.
2. In the case of the *transportation* of *aquatic animals*, provision shall be made to enable preliminary observation of the contents of *containers*.
3. *Containers* in transit in which there are *aquatic animal products* shall not be opened unless the *Competent Authorities* of the *transit country* consider it necessary. If this is the case, *containers* shall be subject to precautions to prevent contamination.
4. *Containers* shall be loaded only with one kind of product or, at least, with products not susceptible to contamination by one another.
5. It rests with each country to decide on the facilities it requires for the *transport* and importation of *aquatic animals* and *aquatic animal products* in *containers*.

## Article 1.5.1.3.

**Particular considerations arrangements for the transport of aquatic animals by air**

1. The stocking densities for the *transport* of *aquatic animals* in *containers* should be determined by taking the following into consideration when transporting by air:

Annex VII (contd)

- a) the total volume of available space for each type of *aquatic animal*;
- b) the oxygenation capacity available to supply the *containers* while on the ground and during all stages of the flight.

With regard to fish, molluscs and crustaceans, the space reserved for each *aquatic animal* species in *containers* that have been fitted for the separate *transportation* of several *aquatic animals* or for the *transportation* of groups of *aquatic animals* should comply with acceptable densities specified for the species in question.

2. The OIE approved International Air Transport Association (IATA) Regulations for live animals may be adopted if they do not conflict with national legislative arrangements. (Copies of these Regulations are obtainable from the International Air Transport Association, 800 Place Victoria, P.O. Box 113, Montreal, Quebec H4Z 1M1, Canada.)

Article 1.5.1.4.

**Disinfection and other sanitary measures**

1. *Disinfection* and all zoo-sanitary work should be carried out in order to:
  - a) avoid all unjustified inconvenience and to prevent damage or injury to the health of people and *aquatic animals*;
  - b) avoid damage to the structure of the *vehicle* or its appliances;
  - c) prevent, as far as possible, any damage to *aquatic animal products*.
2. On request, the *Competent Authority* shall issue the transporters with a certificate indicating the measures that have been applied to all *vehicles*, the parts of the *vehicle* that have been treated, the methods used and the reasons that led to the application of the measures.

In the case of aircraft, the certificate may be replaced, on request, by an entry in the General Declaration of the aircraft.

3. Likewise, the *Competent Authority* shall issue on request:
  - a) a certificate showing the date of arrival and departure of the *aquatic animals*;
  - b) a certificate to the shipper or exporter, the consignee and transporter or their representatives, indicating the measures applied.

Article 1.5.1.5.

**Treatment of transportation water**

Water to be used for *transportation* of *aquatic animals* should be appropriately treated after transport and/or before discharge in order to minimise the *risk* of transferring pathogens. The specific recommendations are provided in the chapter of the *Aquatic Code* on disinfection.

During *transportation* of *aquatic animals*, the transporter should not be permitted to evacuate and replace the water in the transport tanks except on specifically designated sites in the national *territory*. The waste and rinsing water should not be emptied into a drainage system that is directly connected to an aquatic environment where *aquatic animals* are present. The water from the tanks should therefore either be disinfected by a recognised process (for example, 50 mg iodine or chlorine/litre for one hour), or sprayed over land that does not directly drain into waters containing *aquatic animals*. Each country shall designate the sites in their national *territories* where these operations can be carried out.

~~This Article does not apply to treatment of transport water for transport by sea.~~

Article 1.5.1.6.

### **Discharge of infected material**

The *Competent Authority* shall take all practical measures to prevent the discharge of any untreated infective material, including transport water, into internal or territorial waters.

~~This Article does not apply to transport of aquatic animals by sea.~~

Article 1.5.1.7.

### **Particular considerations arrangements for the transport of live fish aquatic animals by well boat**

A well boat is a boat with integrated tanks to carry live fish in sea water that may operate with open valves to allow exchange of sea water. Therefore, well boats can present a biosecurity risk if the fish being carried are infected. Well boats are inherently difficult to disinfect.

1. Only healthy fish showing no clinical signs of disease on the day of loading should be transported. The well boat must have the capability of fully closed containment of fish during its operation if so required.
2. The stocking densities should be determined by taking both the total volume of available space for each species of fish and the oxygenation/aeration capacity available to supply the fish during all stages of transport into consideration.
23. In exceptional circumstances fish may be transported by well boat from an infected site if this is part of a disease response plan agreed to by the *Competent Authority*.
34. Provision shall be made to enable preliminary observation of the contents in the well, and monitoring equipment should be available where appropriate.
45. Access by farm staff to the vessel and from the vessel to the farm cages, including the equipment, should be restricted.
56. Transporting fish of different health status at the same time increases the risk of disease transfer between those fish and is discouraged. Well boats shall be loaded with only one type of fish at a time.
67. Well boats may operate with open valves and thereby exchange water in their tanks with the environment except in designated areas in proximity to aquaculture establishments or areas with protected wild populations. The *Competent Authority* should designate the areas based upon a risk assessment.
78. Multiple deliveries of fish during the same trip should be avoided. Where unavoidable the order of deliveries should be made to the youngest year class of fish first, taking into account health status. Deliveries should be made to sites of a higher health status first (e.g. youngest year class) to a single aquaculture establishment, or establishments of the same health status.
89. In the event of mortality occurring during transport, a contingency plan capable of dealing with full containment and disposal of dead fish, via an approved disposal method, should be available. This plan should be prepared according to the Guidelines on handling and disposal of carcasses and wastes of aquatic animals [in preparation].

Annex VII (contd)

910. Well boats should not operate in adverse inclement weather conditions that may force the operation to divert from the **agreed planned** route and schedule of transport.

1011. The well boat should be cleaned and **where required** disinfected to an acceptable standard before re-use. The level of disinfection should be proportional to the risk. Well boats should maintain a disinfection checklist which should be kept with the ship's log and should be open to audit. It is essential to ensure that all fish are removed from the system before cleaning. All organic matter should be removed through the process of cleaning before disinfection commences. The general principles and specific recommendations as outlined in the **Aquatic Manual** should be consulted for guidance.

1112. When travelling between areas and zones of different health levels, cleaning and **if required** disinfection procedures should be followed and implemented to a standard approved by the *Competent Authority*.

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## CHAPTER 2.3.9.

## INFECTIOUS MYONECROSIS

## Article 2.3.9.1.

For the purposes of the *Aquatic Code*, infectious myonecrosis (IMN) means *infection* with infectious myonecrosis virus (IMNV). This virus is similar to members of the family *Totiviridae*.

Methods for conducting surveillance and diagnosis of IMN are provided in the *Aquatic Manual* (under development).

## Article 2.3.9.2.

**Scope**

The recommendations in this Chapter apply to: Pacific white shrimp (*Penaeus vannamei*). These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

For the purposes of this Chapter, the terms shrimp and prawn are used interchangeably.

## Article 2.3.9.3.

**Commodities**

1. When authorising importation or transit of the following *commodities*, the *Competent Authorities* should not require any IMN related conditions, regardless of the IMN status of the *exporting country, zone or compartment*.
  - a) For the species referred to in Article 2.3.9.2. intended being used for any purpose:
    - i) commodities treated in a manner that inactivates the disease agent e.g. boiled, canned or pasteurised products and some ready to eat meals; and crustacean oil and crustacean meal intended for use in animal feeds commercially sterile canned products;
    - ii) ~~boiled products (e.g. boiled whole shrimp or tails, lobsters, crabs);~~
    - iii) chemically extracted chitin;
    - iv) ~~crustacean meals or by products made non infectious by heating or drying (e.g. flame dried or sun dried);~~
    - iiiv) crustacean products made non-infectious through processing as dry feeds (e.g. pelleted or extruded feeds);
    - ivi) biological samples preserved for diagnostic applications in such a manner as to inactivate the disease agent IMNV (e.g. formalin or alcohol preserved samples).
  - b) The following products destined for human consumption from species referred to in Article 2.3.9.2. which have been prepared and packaged for direct retail trade in such a way as to minimise the likelihood of alternative uses:

## Annex VIII (contd)

- i) ~~chemically preserved products (e.g. salted, pickled, marinated, pastes, etc.);~~
- ii) ~~products that have been heat treated or dried (e.g. ready prepared meals) in a manner to ensure the inactivation of the pathogen.~~

For the *commodities* listed in point 1b), Members ~~should~~ may wish to consider introducing internal measures to prevent the *commodity* being used for any purpose other than for human consumption. (under study)

2. When authorising the importation or transit of the *commodities* of a species referred to in Article 2.3.9.2., other than those listed in point 1 of Article 2.3.9.3., the *Competent Authorities* should require the conditions prescribed in Articles 2.3.9.7. to 2.3.9.11. relevant to the IMN status of the *exporting country, zone* or *compartment*.
3. When considering the importation/transit from an *exporting country, zone* or *compartment* not declared free of IMN of a ~~ny other~~ *commodity* of a species not covered in Article 2.3.9.2. but which could reasonably be expected to be a potential mechanical vector for IMNV ~~carrier~~ vector, the *Competent Authorities* should conduct a risk analysis in accordance with the recommendations in the Aquatic Code ~~of the risk of introduction, establishment and spread of IMNV, and the potential consequences, associated with the importation of the commodity prior to a decision.~~ The *exporting country* should be informed of the outcome of this assessment.

Article 2.3.9.4.

### Infectious myonecrosis free country

A country may make a *self-declaration of freedom* from IMN if it meets the conditions in points 1, 2, 3 or 4 below.

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from IMN if all the areas covered by the shared water are declared IMN free countries or *zones* (see Article 2.3.9.5.).

1. A country where none of the *susceptible species* referred to in Article 2.3.9.2. is present may make a *self-declaration of freedom* from IMN when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

2. A country where the *susceptible species* referred to in Article 2.3.9.2. are present but there has ~~never~~ been no ~~any~~ observed occurrence of the *disease* for at least the past 10 years despite conditions that are conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may make a *self-declaration of freedom* from IMN when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

3. A country where the last observed occurrence of the *disease* was within the past 10 years, or where the *infection status* prior to *targeted surveillance* was unknown, ~~for example (e.g. because of the absence of conditions conducive to its clinical expression, as described in Chapter X.X.X. of the Aquatic Manual),~~ may make a *self-declaration of freedom* from IMN when:
  - a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
  - b) *targeted surveillance*, as described in Chapters X.X.X. of the Aquatic Code 1.1.4 and X.X.X. of the *Aquatic Manual*, has been in place for at least the last 2 years without detection of IMNV.

OR

4. A country that has previously made a *self-declaration of freedom* from IMN but in which the *disease* is subsequently detected may ~~not~~ make a *self-declaration of freedom* from IMN again ~~until~~ when the following conditions have been met:
  - a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
  - c) *targeted surveillance*, as described in Chapters X.X.X. of the Aquatic Code 1.1.4 and X.X.X. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of IMNV; and
  - d) previously existing basic biosecurity conditions have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.

In the meantime, part of the non-affected area may be declared a free *zone* provided that they such part meets the conditions in point 3 of Article 2.3.9.5.

Article 2.3.9.5.

### **Infectious myonecrosis free zone or free compartment**

A *zone* or *compartment* within the *territory* of one or more countries not declared free from IMN may be declared free by the *Competent Authority(ies)* of the country(ies) concerned if the *zone* or *compartment* meets the conditions referred to in points 1, 2, 3 or 4 below.

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

1. A *zone* or *compartment* where none of the *susceptible species* referred to in Article 2.3.9.2. is present may be declared free from IMN when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

2. A *zone* or *compartment* where the *susceptible species* referred to in Article 2.3.9.2. are present but in which there has not been any observed occurrence of the *disease* for at least the past 10 years despite conditions that are conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may be declared free from IMN when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

3. A *zone* or *compartment* where the last observed occurrence of the *disease* was within the past 10 years, or where the *infection* status prior to *targeted surveillance* was unknown, ~~for example~~ because of the absence of conditions conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may be declared free from IMN when:
  - a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
  - b) *targeted surveillance*, as described in Chapters X.X.X. of the Aquatic Code 1.1.4 and X.X.X. of the *Aquatic Manual*, has been in place, through the *zone* or *compartment*, for at least the past 2 years without detection of IMNV.

Annex VIII (contd)

OR

4. A *zone* previously declared free from IMN but in which the *disease* is subsequently detected may ~~not~~ be declared free from IMN again ~~until~~ when the following conditions have been met:
- a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
  - c) *targeted surveillance*, as described in Chapters X.X.X. of the Aquatic Code 1.1.4 and X.X.X. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of IMNV; and
  - d) previously existing basic biosecurity conditions have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.

Article 2.3.9.6.

**Maintenance of free status**

A country, *zone* or *compartment* that is declared free from IMN following the provisions of points 1 or 2 of Articles 2.3.9.4. or 2.3.9.5. (as relevant) may maintain its status as IMN free provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from IMN following the provisions of point 3 of Articles 2.3.9.4. or 2.3.9.5. (as relevant) may discontinue *targeted surveillance* and maintain its status as IMN free provided that conditions that are conducive to clinical expression of IMN, as described in Chapter X.X.X. of the *Aquatic Manual*, exist, and *basic biosecurity conditions* are continuously maintained.

However, for declared free *zones* or *compartments* in infected countries and in all cases where conditions are not conducive to clinical expression of IMN, *targeted surveillance* needs to be continued at a level determined by the *Competent Authority* on the basis of the likelihood of *infection*.

Article 2.3.9.7.

**Importation of live aquatic animals from a country, zone or compartment declared free from infectious myonecrosis**

When importing live *aquatic animals* of species referred to in Article 2.3.9.2. from a country, *zone* or *compartment* declared free from IMN, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.3.9.4. or 2.3.9.5. (as applicable), the place of production of the aquatic animal commodity consignment is a country, *zone* or *compartment* declared free from IMN.

The *certificate* should be in accordance with the Model Certificate in Annex 4.1.3.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.9.3.

## Article 2.3.9.8.

**Importation of live aquatic animals for aquaculture from a country, zone or compartment not declared free from infectious myonecrosis**

1. When importing, for *aquaculture*, live *aquatic animals* of species referred to in Article 2.3.9.2. from a country, *zone* or *compartment* not declared free from IMN, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, apply the following risk mitigation measures ~~such as~~:
  - a) the direct delivery ~~into~~ and lifelong holding of the consignment in biosecure ~~quarantine~~ facilities for;
  - b) ~~the continuous isolation of the imported live aquatic animals and their first generation progeny from the local environment; and~~
  - e) b) the treatment of all effluent and waste materials ~~from the processing~~ in a manner that ensures inactivation of IMNV.
2. If the intention of the introduction is the establishment of a new stock genetic lines, international standards, such as the Guidelines Code of Practice on the Introductions and Transfers of Marine Organisms of the International Council for the Exploration of the Seas (ICES), should be followed.
3. For the purposes of the *Aquatic Code*, the ICES Guidelines Code (full version see: <http://www.ices.dk/indexfla.asp>) may be summarised to the following main points:
  - a) identify stock of interest (cultured or wild) in its current location;
  - b) evaluate stock health/disease history;
  - c) take and test samples for IMNV, pests and general health/disease status;
  - d) import and quarantine in a secure facility a founder (F-0) population;
  - 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 IMNV and perform general examinations for pests and general health/disease status;
  - g) if IMNV 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 IMN free or specific pathogen free (SPF) for IMNV;
  - h) release SPF F-1 stock from *quarantine* for *aquaculture* or stocking purposes in the country, *zone* or *compartment*.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.9.3.

## Article 2.3.9.9.

**Importation of live aquatic animals for human consumption from a country, zone or compartment not declared free from infectious myonecrosis**

When importing, for human consumption, live *aquatic animals* of species referred to in Article 2.3.9.2. from a country, *zone* or *compartment* not declared free from IMN, the *Competent Authority* of the *importing country* should assess the risk and, if justified, require that:

Annex VIII (contd)

1. the consignment be delivered directly to and held in isolation until **processing and /or** consumption; and
2. all effluent, dead *aquatic animals* and waste materials from the processing be treated in a manner that ensures inactivation of IMNV.

Members ~~should~~ may wish to consider introducing internal measures to prevent such *commodities* being used for any purpose other than for human consumption.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.9.3.

Article 2.3.9.10.

### **Importation of aquatic animal products from a country, zone or compartment declared free from infectious myonecrosis**

When importing *aquatic animal products* of species referred to in Article 2.3.9.2. from a country, *zone* or *compartment* declared free from IMN, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.3.9.4. or 2.3.9.5. (as applicable), the place of production of the consignment is a country, *zone* or *compartment* declared free from IMN.

The *certificate* should be in accordance with the Model Certificate in Annex 4.2.2.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.9.3.

Article 2.3.9.11.

### **Importation of aquatic animal products from a country, zone or compartment not declared free from infectious myonecrosis**

When importing *aquatic animal products* of species referred to in Article 2.3.9.2. from a country, *zone* or *compartment* not declared free from IMN, the *Competent Authority* of the *importing country* should assess the risk and apply appropriate risk mitigation measures.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.9.3.

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## CHAPTER 2.3.11.

## WHITE TAIL DISEASE

## Article 2.3.11.1.

For the purposes of the *Aquatic Code*, white tail disease (WTD) means *infection* with macrobrachium nodavirus (MrNV). This virus has yet to be formally classified.

Methods for conducting surveillance and diagnosis of WTD are provided in the *Aquatic Manual* (under development).

## Article 2.3.11.2.

**Scope**

The recommendations in this Chapter apply to: the giant fresh water prawn (*Macrobrachium rosenbergii*). Other common names are listed in the *Aquatic Manual*. These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

For the purposes of this Chapter, the terms shrimp and prawn are used interchangeably.

## Article 2.3.11.3.

**Commodities**

1. When authorising the importation or transit of the following *commodities*, the *Competent Authorities* should not require any WTD related conditions, regardless of the WTD status of the *exporting country, zone* or *compartment*.

a) For the species referred to in Article 2.3.11.2. intended ~~being used~~ for any purpose:

- i) commodities treated in a manner that inactivates the disease agent e.g. boiled, canned or pasteurised products and some ready to eat meals; and crustacean oil and crustacean meal intended for use in animal feeds ~~commercially sterile canned products;~~
- ii) ~~boiled products (e.g. boiled whole shrimp or tails, lobsters, crabs);~~
- iii) chemically extracted chitin;
- iv) ~~crustacean meals or by products made non infectious by heating or drying (e.g. flame dried or sun dried);~~
- iii) crustacean products made non-infectious through processing as dry feeds (e.g. pelleted or extruded feeds);
- iv) biological samples preserved for diagnostic applications in such a manner as to inactivate the disease agent MrNV (e.g. formalin or alcohol preserved samples).

b) The following products destined for human consumption from species referred to in Article 2.3.11.2. which have been prepared and packaged for direct retail trade in such a way as to minimise the likelihood of alternative uses:

## Annex IX (contd)

- i) ~~chemically preserved products (e.g. salted, pickled, marinated, pastes, etc.);~~
- ii) ~~products that have been heat treated or dried (e.g. ready prepared meals) in a manner to ensure the inactivation of the pathogen.~~

For the *commodities* listed in point 1b), Members ~~should~~ may wish to consider introducing internal measures to prevent the *commodity* being used for any purpose other than for human consumption. (under study)

2. When authorising the importation or transit of the *commodities* of a species referred to in Article 2.3.11.2., other than those listed in point 1 of Article 2.3.11.3., the *Competent Authorities* should require the conditions prescribed in Articles 2.3.11.7. to 2.3.11.11. relevant to the WTD status of the *exporting country, zone* or *compartment*.
3. When considering the importation/transit from an *exporting country, zone* or *compartment* not declared free of WTD of ~~any other commodity~~ of a species not covered in Article 2.3.11.2. but which could reasonably be expected to be a potential mechanical vector for MrNV ~~carrier~~ vector, the *Competent Authorities* should conduct a risk analysis in accordance with the recommendations in the Aquatic Code of the risk of introduction, establishment and spread of MrNV, and the potential consequences, associated with the importation of the commodity prior to a decision. The *exporting country* should be informed of the outcome of this assessment.

Article 2.3.11.4.

### White tail disease free country

A country may make a *self-declaration of freedom* from WTD if it meets the conditions in points 1, 2, 3 or 4 below.

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from WTD if all the areas covered by the shared water are declared WTD free countries or *zones* (see Article 2.3.11.5.).

1. A country where none of the *susceptible species* referred to in Article 2.3.11.2. is present may make a *self-declaration of freedom* from WTD when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

2. A country where the *susceptible species* referred to in Article 2.3.11.2. are present but there has ~~never~~ been no ~~any~~ observed occurrence of the *disease* for at least the past 10 years despite conditions that are conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may make a *self-declaration of freedom* from WTD when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

3. A country where the last observed occurrence of the *disease* was within the past 10 years, or where the *infection* status prior to *targeted surveillance* was unknown, ~~for example~~ because of the absence of conditions conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may make a *self-declaration of freedom* from WTD when:
  - a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
  - b) *targeted surveillance*, as described in Chapters X.X.X of the Aquatic Code 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the last 2 years without detection of MrNV.

OR

4. A country that has previously made a *self-declaration of freedom* from WTD but in which the *disease* is subsequently detected may ~~not~~ make a *self-declaration of freedom* from WTD again ~~until~~ when the following conditions have been met:
- a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
  - c) *targeted surveillance*, as described in Chapters X.X.X of the Aquatic Code 1.1.4 and X.X.X. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of MrNV; and
  - d) *previously existing basic biosecurity conditions have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.*

In the meantime, part of the non-affected area may be declared a free *zone* provided that ~~they~~ such part meets the conditions in point 3 of Article 2.3.11.5.

Article 2.3.11.5.

### **White tail disease free zone or free compartment**

A *zone* or *compartment* within the *territory* of one or more countries not declared free from WTD may be declared free by the *Competent Authority(ies)* of the country(ies) concerned if the *zone* or *compartment* meets the conditions referred to in points 1, 2, 3 or 4 below.

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

1. A *zone* or *compartment* where none of the *susceptible species* referred to in Article 2.3.11.2. is present may be declared free from WTD when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

2. A *zone* or *compartment* where the *susceptible species* referred to in Article 2.3.11.2. are present but in which there has not been any observed occurrence of the *disease* for at least the past 10 years despite conditions that are conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may be declared free from WTD when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

3. A *zone* or *compartment* where the last observed occurrence of the *disease* was within the past 10 years, or where the *infection* status prior to *targeted surveillance* was unknown, ~~for example (e.g. because of the absence of conditions conducive to its clinical expression, as described in Chapter X.X.X. of the Aquatic Manual),~~ may be declared free from WTD when:
  - a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
  - b) *targeted surveillance*, as described in Chapters X.X.X of the Aquatic Code 1.1.4 and X.X.X. of the *Aquatic Manual*, has been in place, through the *zone* or *compartment*, for at least the past 2 years without detection of MrNV.

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OR

4. A *zone* previously declared free from WTD but in which the *disease* is subsequently detected may ~~not~~ be declared free from WTD again ~~until~~ when the following conditions have been met:
- a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
  - c) *targeted surveillance*, as described in Chapters X.X.X of the Aquatic Code 1.1.4 and X.X.X. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of MrNV; and
  - d) previously existing basic biosecurity conditions have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.

Article 2.3.11.6.

**Maintenance of free status**

A country, *zone* or *compartment* that is declared free from WTD following the provisions of points 1 or 2 of Articles 2.3.11.4. or 2.3.11.5. (as relevant) may maintain its status as WTD free provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from WTD following the provisions of point 3 of Articles 2.3.11.4. or 2.3.11.5. (as relevant) may discontinue *targeted surveillance* and maintain its status as WTD free provided that conditions that are conducive to clinical expression of WTD, as described in Chapter X.X.X. of the *Aquatic Manual*, exist, and *basic biosecurity conditions* are continuously maintained.

However, for declared free *zones* or *compartments* in infected countries and in all cases where conditions are not conducive to clinical expression of WTD, *targeted surveillance* needs to be continued at a level determined by the *Competent Authority* on the basis of the likelihood of *infection*.

Article 2.3.11.7.

**Importation of live aquatic animals from a country, zone or compartment declared free from white tail disease**

When importing live *aquatic animals* of species referred to in Article 2.3.11.2. from a country, *zone* or *compartment* declared free from WTD, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.3.11.4. or 2.3.11.5. (as applicable), the place of production of the aquatic animal commodity is a country, *zone* or *compartment* declared free from WTD.

The *certificate* should be in accordance with the Model Certificate in Annex Appendix 4.1.3.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.11.3.

## Article 2.3.11.8.

**Importation of live aquatic animals for aquaculture from a country, zone or compartment not declared free from white tail disease**

1. When importing, for *aquaculture*, live *aquatic animals* of species referred to in Article 2.3.11.2. from a country, *zone* or *compartment* not declared free from WTD, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, apply the following risk mitigation measures ~~such as~~:
  - a) the direct delivery ~~into~~ and lifelong holding of the consignment in biosecure ~~quarantine~~ facilities for;
  - b) ~~the continuous isolation of the imported live aquatic animals and their first generation progeny from the local environment; and~~
  - e) b) the treatment of all effluent and waste materials ~~from the processing~~ in a manner that ensures inactivation of MrNV.
2. If the intention of the introduction is the establishment of a new stock genetic lines, international standards, such as the Guidelines Code of Practice on the Introductions and Transfers of Marine Organisms of the International Council for the Exploration of the Seas (ICES), should be followed.
3. For the purposes of the *Aquatic Code*, the ICES Guidelines Code (full version see: <http://www.ices.dk/indexfla.asp>) may be summarised to the following main points:
  - a) identify stock of interest (cultured or wild) in its current location;
  - b) evaluate stock's health/disease history;
  - c) take and test samples for MrNV, pests and general health/disease status;
  - d) import and quarantine in a secure facility a founder (F-0) population;
  - 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 MrNV and perform general examinations for pests and general health/disease status;
  - g) if MrNV 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 WTD free or specific pathogen free (SPF) for MrNV;
  - h) release SPF F-1 stock from *quarantine* for *aquaculture* or stocking purposes in the country, *zone* or *compartment*.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.11.3.

## Article 2.3.11.9.

**Importation of live aquatic animals for human consumption from a country, zone or compartment not declared free from white tail disease**

When importing, for human consumption, live *aquatic animals* of species referred to in Article 2.3.11.2. from a country, *zone* or *compartment* not declared free from WTD, the *Competent Authority* of the *importing country* should assess the risk and, if justified, require that:

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1. the consignment be delivered directly to and held in isolation until **processing and /or** consumption; and
2. all effluent, dead *aquatic animals* and waste materials from the processing be treated in a manner that ensures inactivation of MrNV.

Members ~~should~~ may wish to consider introducing internal measures to prevent such *commodities* being used for any purpose other than for human consumption.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.11.3.

Article 2.3.11.10.

### **Importation of aquatic animal products from a country, zone or compartment declared free from white tail disease**

When importing *aquatic animal products* of species referred to in Article 2.3.11.2. from a country, *zone* or *compartment* declared free from WTD, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.3.11.4. or 2.3.11.5. (as applicable), the place of production of the consignment is a country, *zone* or *compartment* declared free from WTD.

The *certificate* should be in accordance with the Model Certificate in **Appendix Annex** 4.2.2.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.11.3.

Article 2.3.11.11.

### **Importation of aquatic animal products from a country, zone or compartment not declared free from white tail disease**

When importing *aquatic animal products* of species referred to in Article 2.3.11.2. from a country, *zone* or *compartment* not declared free from WTD, the *Competent Authority* of the *importing country* should assess the risk and apply appropriate risk mitigation measures.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.11.3.

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## CHAPTER 2.2.5.

INFECTION WITH *MIKROCYTOS MACKINI*

## Article 2.2.5.1.

For the purposes of the *Aquatic Code*, infection with *Mikrocytos mackini*<sup>1</sup> means infection only with *M. mikrocytos mackini*.

Methods for conducting surveillance, diagnosis and confirmatory identification of infection with *M. mikrocytos mackini* are provided in the *Aquatic Manual* (under study).

## Article 2.2.5.2.

**Scope**

The recommendations in this Chapter apply to: European flat oyster (*Ostrea edulis*), Olympia oyster (*O. conchaphila*), Pacific oyster (*Crassostrea gigas*) and Eastern oyster (*C. virginica*). These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

## Article 2.2.5.3.

**Commodities**

1. When authorising the importation or transit of the following *commodities*, the *Competent Authorities* should not require any *M. mikrocytos mackini* related conditions, regardless of the *M. mikrocytos mackini* status of the *exporting country, zone* or *compartment*:
  - a) For the species referred to in Article 2.2.5.2. intended ~~being used~~ for any purpose:
    - i) *commodities* treated in a manner that kills the host (and thereby inactivates the *disease agent*) e.g. canned or pasteurised products; ~~chemically preserved products (e.g. smoked, salted, pickled, marinated, etc.);~~
    - ii) ~~larvae;~~
    - iii) biological samples preserved for diagnostic applications in such a manner as to inactivate the *disease agent*.
  - b) All *commodities* from *Panope abrupta*, including the live *aquatic animal*.
  - c) The following *commodities* destined for human consumption from the species referred to in Article 2.2.5.2. which have been prepared and packaged for direct retail trade:
    - i) off the shell (chilled or frozen).

For the *commodities* referred to in point 1c), Members may wish to ~~should~~ consider introducing internal measures to prevent the *commodity* being used for any purpose other than for human consumption.

2. When authorising the importation or transit of *commodities* of a species referred to in Article 2.2.5.2., other than *commodities* referred to in point 1 of Article 2.2.5.3., the *Competent Authorities* should require the conditions prescribed in Articles 2.2.5.7. to 2.2.5.11. relevant to the *M. mikrocytos mackini* status of the *exporting country, zone* or *compartment*.

Annex X (contd)

3. When considering the importation/transit from an *exporting country, zone or compartment* not declared free of infection with *Mikrocytes mackini* of a *commodity* from bivalve species not covered in Article 2.2.5.2. or in point 1b) of Article 2.2.5.3. but which could reasonably be expected to be a potential **mechanical vector for Mikrocytes mackini vector**, the *Competent Authorities* should conduct a *risk analysis* in accordance with the recommendations in *the Aquatic Code*. The *exporting country* should be informed of the outcome of this assessment.

## Article 2.2.5.4.

***Mikrocytes mackini* free country**

A country may make a *self-declaration of freedom* from *Mikrocytes mackini* if it meets the conditions in points 1, 2, 3 or 4 below.

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from *Mikrocytes mackini* if all the areas covered by the shared water are declared *Mikrocytes mackini* free *zones* (see Article 2.2.5.5.).

1. A country where none of the *susceptible species* referred to in Article 2.2.5.2. is present may make a *self-declaration of freedom* from *Mikrocytes mackini* when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

2. A country where any *susceptible species* referred to in Article 2.2.5.2. are present but there has ~~never~~ been no ~~any~~ observed occurrence of the *disease* for at least the past 10 years despite conditions – in all areas where the species are present – that are conducive to its clinical expression, as described in Chapter 2.2.5. of the *Aquatic Manual*, may make a *self-declaration of freedom* from *Mikrocytes mackini* when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years and infection with *Mikrocytes mackini* is not known to be established in wild populations.

OR

3. A country where the last known clinical occurrence was within the past 10 years, or where the *infection* status prior to *targeted surveillance* was unknown (e.g. because of the absence of conditions conducive to clinical expression as described in Chapter 2.2.5. of the *Aquatic Manual*), may make a *self-declaration of freedom* from *Mikrocytes mackini* when:
  - a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
  - b) *targeted surveillance*, as described in Chapters **X.X.X of the Aquatic Code 1.1.4** and 2.2.5. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of *Mikrocytes mackini*.

OR

4. A country that has previously made a *self-declaration of freedom* from *Mikrocytes mackini* but in which the *disease* is subsequently detected may make a *self-declaration of freedom* from *Mikrocytes mackini* again when the following conditions have been met:
  - a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
- c) *targeted surveillance*, as described in Chapters X.X.X of the *Aquatic Code 1.1.4* and 2.2.5. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of *Mikrocytes mackini*; and
- d) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.

In the meantime, part of the non-affected area may be declared a free *zone* provided that such part meets the conditions in point 3 of Article 2.2.5.5.

Article 2.2.5.5.

### ***Mikrocytes mackini* free zone or free compartment**

A *zone* or *compartment* free from *Mikrocytes mackini* may be established within the *territory* of one or more countries of infected or unknown status for infection with *Mikrocytes mackini* and declared free by the *Competent Authority(ies)* of the country(ies) concerned if the *zone* or *compartment* meets the conditions referred to in points 1, 2, 3 or 4 below.

If a *zone* or *compartment* extends over more than one country, it can only be declared a *Mikrocytes mackini* free *zone* or *compartment* if the conditions outlined below apply to all areas of the *zone* or *compartment*.

1. In a country of unknown status for *Mikrocytes mackini*, a *zone* or *compartment* where none of the *susceptible species* referred to in Article 2.2.5.2. is present may be declared free from *Mikrocytes mackini* when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

2. In a country of unknown status for *Mikrocytes mackini*, a *zone* or *compartment* where any *susceptible species* referred to in Article 2.2.5.2. are present but there has never been any observed occurrence of the *disease* for at least the past 10 years despite conditions – in all areas where the species are present – that are conducive to its clinical expression, as described in Chapter 2.2.5. of the *Aquatic Manual*, may be declared free from *Mikrocytes mackini* when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years and infection with *Mikrocytes mackini* is not known to be established in wild populations.

OR

3. A *zone* or *compartment* where the last known clinical occurrence was within the past 10 years, or where the *infection* status prior to *targeted surveillance* was unknown (e.g. because of the absence of conditions conducive to clinical expression as described in Chapter 2.2.5. of the *Aquatic Manual*), may be declared free from *Mikrocytes mackini* when:
  - a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
  - b) *targeted surveillance*, as described in Chapters X.X.X of the *Aquatic Code 1.1.4* and 2.2.5. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of *Mikrocytes mackini*.

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OR

4. A *zone* previously declared free from *Mikrocytes mackini* but in which the *disease* is subsequently detected may be declared free from *Mikrocytes mackini* again when the following conditions have been met:
- a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
  - c) *targeted surveillance*, as described in Chapters X.X.X of the *Aquatic Code 1.1.4*, and 2.2.5. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of *Mikrocytes mackini*; and
  - d) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.

Article 2.2.5.6.

**Maintenance of free status**

A country, *zone* or *compartment* that is declared free from *Mikrocytes mackini* following the provisions of points 1 or 2 of Articles 2.2.5.4. or 2.2.5.5. (as relevant) may maintain its status as *Mikrocytes mackini* free provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from *Mikrocytes mackini* following the provisions of point 3 of Articles 2.2.5.4. or 2.2.5.5. (as relevant) may discontinue *targeted surveillance* and maintain its status as *Mikrocytes mackini* free provided that conditions that are conducive to clinical expression of infection with *Mikrocytes mackini*, as described in Chapter 2.2.5. of the *Aquatic Manual*, exist and *basic biosecurity conditions* are continuously maintained.

However, for declared free *zones* or *compartments* in infected countries and in all cases where conditions are not conducive to clinical expression of infection with *Mikrocytes mackini*, *targeted surveillance* needs to be continued at a level determined by the *Competent Authority* on the basis of the likelihood of *infection*.

Article 2.2.5.7.

**Importation of live aquatic animals from a country, zone or compartment declared free from *Mikrocytes mackini***

When importing live *aquatic animals* of species referred to in Article 2.2.5.2. from a country, *zone* or *compartment* declared free from *Mikrocytes mackini*, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country*.

This *certificate* must certify, on the basis of the procedures described in Articles 2.2.5.4. or 2.2.5.5. (as applicable), whether the place of production of the *commodity aquatic animal* is a country, *zone* or *compartment* declared free from *Mikrocytes mackini*.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.1.2.

This Article does not apply to *commodities* referred to in point 1 of Article 2.2.5.3.

Article 2.2.5.8.

**Importation of live aquatic animals for aquaculture from a country, zone or compartment not declared free from *Mikrocytes mackini***

1. When importing, for *aquaculture*, live *aquatic animals* of species referred to in Article 2.2.5.2. from a country, zone or compartment not declared free from *Mikrocytes mackini*, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, apply the following *risk* mitigation measures:
  - a) the direct delivery to and lifelong holding of the consignment in biosecure facilities for continuous isolation from the local environment; and
  - b) the treatment of all effluent and waste material in a manner that ensures inactivation of *Mikrocytes mackini*.
2. If the intention of the introduction is the establishment of a new stock, *international standards, such as 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 followed.
3. For the purposes of the *Aquatic Code*, the ICES Code *(full version see: <http://www.ices.dk/indexfla.asp>)* may be summarised to the following main points:
  - a) identify stock of interest (cultured or wild) in its current location;
  - b) evaluate stock health/disease history;
  - c) take and test samples for *Mikrocytes mackini*, pests and general health/disease status;
  - d) import and quarantine in a secure facility a founder (F-0) population;
  - 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 *Mikrocytes mackini* and perform general examinations for pests and general health/disease status;
  - g) if *Mikrocytes mackini* 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 free of infection with *Mikrocytes mackini* or specific pathogen free (SPF) for *Mikrocytes mackini*;
  - h) release SPF F-1 stock from *quarantine* for *aquaculture* or stocking purposes in the country, zone or compartment.

This Article does not apply to *commodities* referred to in point 1 of Article 2.2.5.3.

Article 2.2.5.9.

**Importation of live aquatic animals for processing for human consumption from a country, zone or compartment not declared free from *Mikrocytes mackini***

When importing, for processing for human consumption, live *aquatic animals* of species referred to in Article 2.2.5.2. from a country, zone or compartment not declared free from *Mikrocytes mackini*, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, require that:

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1. the consignment be delivered directly to and held in *quarantine* facilities until processing and/or consumption; and
2. all effluent and waste material from the processing be treated in a manner that ensures inactivation of *M.ikrocytes mackini*.

This Article does not apply to *commodities* referred to in point 1 of Article 2.2.5.3.

Article 2.2.5.10.

**Importation of aquatic animal products from a country, zone or compartment declared free from *M.ikrocytes mackini***

When importing *aquatic animal products* of species referred to in Article 2.2.5.2. from a country, *zone* or *compartment* declared free from *M.ikrocytes mackini*, 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*.

This *certificate* must certify, on the basis of the procedures described in Articles 2.2.5.4. or 2.2.5.5. (as applicable), whether or not the place of production of the consignment is a country, *zone* or *compartment* declared free from *M.ikrocytes mackini*.

The *certificate* should be in accordance with the Model Certificate in Appendix X.X.X. (under study).

This Article does not apply to *commodities* referred to in point 1 of Article 2.2.5.3.

Article 2.2.5.11.

**Importation of aquatic animal products from a country, zone or compartment not declared free from *M.ikrocytes mackini***

When importing *aquatic animal products* of species referred to in Article 2.2.5.2. from a country, *zone* or *compartment* not declared free from *M.ikrocytes mackini*, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

This Article does not apply to *commodities* referred to in point 1 of Article 2.2.5.3.

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1. This disease does not meet the listing criteria in Chapter 1.2.2. Nevertheless, reporting requirements for non listed diseases apply in regard to significant epidemiological events (see point 1e) of Article 1.2.1.3.).

CHAPTER 2.1.14.

**GYRODACTYLOSIS**  
(*Gyrodactylus salaris*)

Article 2.1.14.1.

For the purposes of the *Aquatic Code*, gyrodactylosis means *infestation* with the viviparous freshwater ectoparasite *Gyrodactylus salaris* (*G. salaris*) (Phylum Platyhelminthes; Class Monogenea).

Methods for conducting surveillance and diagnosis of gyrodactylosis are provided in the *Aquatic Manual*.

Article 2.1.14.2.

**Scope**

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

Article 2.1.14.3.

**Commodities**

1. When authorising the importation or transit of the following *commodities*, the *Competent Authorities* should not require any gyrodactylosis related conditions, regardless of the gyrodactylosis status of the *exporting country, zone* or *compartment*:
  - a) For the species referred to in Article 2.1.14.2. intended for any purpose:
    - i) *commodities* treated in a manner that inactivates the **disease agent kills *G. salaris*** e.g. leather made from fish skin, pasteurised products and **some** ready to eat meals; and fish oil and fish meal intended for use in animal feeds;
    - ii) chilled products of fish, where the head, fins and skin has been removed
    - iii) biological samples preserved for diagnostic applications in such a manner as to inactivate **the disease agent *G. salaris***.
  - b) The following *commodities* destined for human consumption from the species referred to in Article 2.1.14.2. that have been prepared and packaged for direct retail trade:
    - i) **viscerated** fish (chilled **or** frozen);
    - ii) fillets or cutlets (chilled or frozen);
    - iii) dried **viscerated** fish (including air dried, flame dried and sun dried);
    - iv) smoked salmonids.

Annex XI (contd)

For the *commodities* referred to in point 1b), OIE Members may wish to consider introducing internal measures to prevent the *commodity* being used for any purpose other than for human consumption.

2. When authorising the importation or transit of *commodities* of a species referred to in Article 2.1.14.2., other than those referred to in point 1 of Article 2.1.14.3., the *Competent Authorities* should require the conditions prescribed in Articles 2.1.14.7. to 2.1.14.11. relevant to the gyrodactylosis status of the *exporting country, zone or compartment*.
3. When considering the importation/transit from an *exporting country, zone or compartment* not declared free of gyrodactylosis of any live *commodity* of a species not covered in Article 2.1.14.2. but which could reasonably be expected to be a potential **mechanical vector for *G. salaris* vector**, the *Competent Authorities* should conduct a *risk analysis* in accordance with the recommendations in the *Aquatic Code*. The *exporting country* should be informed of the outcome of this assessment.

Article 2.1.14.4.

### **Gyrodactylosis free country**

A country may make a *self-declaration of freedom* from gyrodactylosis if it meets the conditions in points 1, 2, 3 or 4 below.

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from gyrodactylosis if all the areas covered by the shared watercourse(s) are declared gyrodactylosis free countries or *zones* (see Article 2.1.14.5.).

1. A country where none of the *susceptible species* referred to in Article 2.1.14.2. is present may make a *self-declaration of freedom* from gyrodactylosis when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

2. A country where the *susceptible species* referred to in Article 2.1.14.2. are present but there has been no observed occurrence of the *disease* for at least the past **25 10** years despite conditions that are conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may make a *self-declaration of freedom* from gyrodactylosis when *basic biosecurity conditions* have been continuously met in the country for at least the past 10 years.

OR

3. A country where the last observed occurrence of the *disease* was within the past **25 10** years, or where the infestation status prior to *targeted surveillance* was unknown (e.g. because of the absence of conditions conducive to its clinical expression as described in Chapter X.X.X. of the *Aquatic Manual*), may make a *self-declaration of freedom* from gyrodactylosis when:
  - a) *basic biosecurity conditions* have been continuously met for at least the past 10 years; and
  - b) *targeted surveillance*, as described in Chapters **X.X.X of the Aquatic Code 1.1.4.** and X.X.X. of the *Aquatic Manual*, has been in place for at least the last 5 years without detection of *G. salaris*.

OR

4. A country that has previously made a *self-declaration of freedom* from gyrodactylosis but in which the *disease* is subsequently detected may make a *self-declaration of freedom* from gyrodactylosis again when the following conditions have been met:

- a) on detection of the *disease*, the affected area was declared an infested zone and a *buffer zone* was established; and
- b) infested populations have been destroyed or removed from the infested zone by means that minimise the risk of further spread of the *disease*, and the appropriate *disinfestation* procedures (see *Aquatic Manual*) have been completed, or the waters containing the infested fish have been treated by chemicals that kill the parasite **without affecting the wild or farmed host**; and
- c) *targeted surveillance*, as described in Chapters **X.X.X of the Aquatic Code 1-1.4** and X.X.X. of the *Aquatic Manual*, has been in place for at least the last 5 years without detection of *G. salaris*; and
- d) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place for at least the past 5 years.

In the meantime, part of the non-affected area may be declared a free *zone* provided that such part meets the conditions in point 3 of Article 2.1.14.5.

Article 2.1.14.5.

### **Gyrodactylis free zone or free compartment**

A *zone* or *compartment* within the *territory* of one or more countries not declared free from gyrodactylis may be declared free by the *Competent Authority(ies)* of the country(ies) concerned if the *zone* or *compartment* meets the conditions referred to in points 1, 2, 3 or 4 below.

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

1. A *zone* or *compartment* where none of the *susceptible species* referred to in Article 2.1.14.2. is present may be declared free from gyrodactylis when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

2. A *zone* or *compartment* where the *susceptible species* referred to in Article 2.1.14.2. are present but there has never been any observed occurrence of the *disease* for at least the past **2510** years despite conditions that are conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may be declared free from gyrodactylis when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 10 years.

OR

3. A *zone* or *compartment* supplied with seawater with a salinity of at least 25 parts per thousand and into which no live aquatic animals of species referred to in Article 2.1.14.2 have been introduced for the previous 14 days from a site of a lesser health status **for *G. salaris***.

OR

4. A *zone* or *compartment* where the last observed occurrence of the *disease* was within the past **2510** years, or where the infestation status prior to *targeted surveillance* was unknown (e.g. because of the absence of conditions conducive to its clinical expression as described in Chapter X.X.X. of the *Aquatic Manual*), may be declared free from gyrodactylis when:

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- a) *basic biosecurity conditions* have been continuously met for at least the past 10 years; and
- b) *targeted surveillance*, as described in Chapters X.X.X of the Aquatic Code 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the last 5 years without detection of *G. salaris*.

OR

5. A *zone* previously declared free from gyrodactylosis but in which the *disease* is subsequently detected may be declared free from gyrodactylosis again when the following conditions have been met:
  - a) on detection of the *disease*, the affected area was declared an *infested zone* and a *buffer zone* was established; and
  - b) infested populations have been destroyed or removed from the *infested zone* by means that minimise the risk of further spread of the *disease*, and the appropriate disinfestation procedures (see *Aquatic Manual*) have been completed, or the waters containing the infested fish have been treated by chemicals that kill the parasite without affecting the wild or farmed host; and
  - c) *targeted surveillance*, as described in Chapters X.X.X of the Aquatic Code 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the last 5 years without detection of *G. salaris*; and
  - d) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place for at least the past 5 years.

Article 2.1.14.6.

**Maintenance of free status**

A country, *zone* or *compartment* that is declared free from gyrodactylosis following the provisions of points 1 or 2 of Articles 2.1.14.4. or 2.1.14.5. (as relevant) may maintain its status as gyrodactylosis free provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from gyrodactylosis following the provisions of point 3 of Articles 2.1.14.4. or 2.1.14.5. (as relevant) may discontinue *targeted surveillance* and maintain its status as gyrodactylosis free provided that conditions that are conducive to clinical expression of gyrodactylosis, as described in Chapter X.X.X. of the *Aquatic Manual*, exist, and *basic biosecurity conditions* are continuously maintained.

However, for declared free *zones* or *compartments* in infested countries and in all cases where conditions are not conducive to clinical expression of gyrodactylosis, *targeted surveillance* needs to be continued at a level determined by the *Competent Authority* on the basis of the likelihood of infestation.

Article 2.1.14.7.

**Importation of live aquatic animals from a country, zone or compartment declared free from gyrodactylosis**

When importing live *aquatic animals* of species referred to in Article 2.1.14.2. from a country, *zone* or *compartment* declared free from gyrodactylosis, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.1.14.4. or 2.1.14.5. (as applicable), the place of production of the commodity aquatic animal is a country, *zone* or *compartment* declared free from gyrodactylosis.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.1.1.

This Article does not apply to *commodities* referred to in point 1 of Article 2.1.14.3.

Article 2.1.14.8.

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

1. When importing, for *aquaculture*, live *aquatic animals* of species referred to in Article 2.1.14.2. from a country, *zone* or *compartment* not declared free from gyrodactylosis, the *Competent Authority* of the *importing country* should:
    - a) require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* attesting that:
      - i) the *aquatic animals* have been held, immediately prior to export, in water with a salinity of at least 25 parts per thousand for a continuous period of at least 14 days; and
      - ii) no other live *aquatic animals* of the species referred to in Article 2.1.14.2. have been introduced during that period;

OR

    - iii) in the case of eyed eggs, the eggs have been disinfected by a method demonstrated to be effective against *G. salaris*;

OR

  - b) assess the *risk* and apply risk mitigation measures such as:
    - i) the direct delivery to and lifelong holding of the consignment in biosecure facilities for continuous isolation from the local environment;
    - ii) if breeding from the imported fish, disinfection of the fertilised eggs by a method demonstrated to be effective against *G. salaris*, and complete separation of the hatched progeny from the imported animals;
    - iii) the treatment of all effluent and waste materials in a manner that ensures inactivation of *G. salaris*.
2. If the intention of the introduction is the establishment of a new stock, **international standards, such as 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 followed.
3. For the purposes of the *Aquatic Code*, the ICES Code **(full version see: <http://www.ices.dk/indexfla.asp>)** may be summarised to the following main points:
  - a) identify stock of interest (cultured or wild) in its current location;
  - b) evaluate stock's health/disease history;
  - c) take and test samples for *G. salaris*, pests and general health/disease status;

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- d) import and quarantine in a secure facility a founder (F-0) population;
- 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 *G. salaris* and perform general examinations for pests and general health/disease status;
- g) if *G. salaris* 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 gyrodactylosis free or specific pathogen free (SPF) for *G. salaris*;
- h) release SPF F-1 stock from *quarantine* for *aquaculture* or stocking purposes in the country, *zone* or *compartment*.

This Article does not apply to *commodities* referred to in point 1 of Article 2.1.14.3.

## Article 2.1.14.9.

**Importation of live aquatic animals for processing for human consumption from a country, zone or compartment not declared free from gyrodactylosis**

When importing, for processing for human consumption, live *aquatic animals* of species referred to in Article 2.1.14.2. from a country, *zone* or *compartment* not declared free from gyrodactylosis, 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 held, immediately prior to export, in water with a salinity of at least 25 parts per thousand for a continuous period of at least 14 days, and no other live fish of the species listed in Article 2.1.14.2. have been introduced during that period;

OR

2. require that the consignment be delivered directly to and held in *quarantine* facilities for slaughter and processing to one of the products referred to in point 1 of Article 2.1.14.3. or other products authorised by the *Competent Authority*, and all effluent and waste materials be treated in a manner that ensures inactivation of *G. salaris*.

This Article does not apply to *commodities* referred to in point 1 of Article 2.1.14.3.

## Article 2.1.14.10.

**Importation of live aquatic animals intended for use in animal feed, or for agricultural, industrial or pharmaceutical use, from a country, zone or compartment not declared free from gyrodactylosis**

When importing, for use in animal feed, or for agricultural, industrial or pharmaceutical use, live *aquatic animals* of species referred to in Article 2.1.14.2. from a country, *zone* or *compartment* not declared free from gyrodactylosis, 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 held, immediately prior to export, in water with a salinity of at least 25 parts per thousand for a continuous period of at least 14 days, and no other live *aquatic animals* of the species referred to in Article 2.1.14.2. have been introduced during that period;

OR

2. require that the consignment be delivered directly to and held in *quarantine* facilities for slaughter and processing to one of the products referred to in point 1 of Article 2.1.14.3. or other products authorised by the *Competent Authority*, and all effluent and waste materials be treated in a manner that ensures inactivation of *G. salaris*.

This Article does not apply to *commodities* referred to in point 1 of Article 2.1.14.3.

Article 2.1.14.11.

### **Importation of aquatic animal products from a country, zone or compartment declared free from gyrodactylosis**

When importing *aquatic animal products* of species referred to in Article 2.1.14.2. from a country, *zone* or *compartment* declared free from gyrodactylosis, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.1.14.4. or 2.1.14.5. (as applicable), the place of production of the consignment is a country, *zone* or *compartment* declared free from gyrodactylosis. The *certificate* should be in accordance with the Model Certificate in Appendix 4.2.1.

This Article does not apply to *commodities* referred to in point 1 of Article 2.1.14.3.

Article 2.1.14.12.

### **Importation of aquatic animal products from a country, zone or compartment not declared free from gyrodactylosis**

When importing *aquatic animal products* of species referred to in Article 2.1.14.2. from a country, *zone* or *compartment* not declared free from gyrodactylosis, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate risk mitigation measures.

1. In the case of dead *aquatic animals*, whether *eviscerated* or *uneviscerated*, such risk mitigation measures may include:
  - a) the direct delivery into and holding of the consignment in biosecure facilities for processing to one of the products referred to in point 1 of Article 2.1.14.3. or other products authorised by the *Competent Authority*,
  - b) the treatment of all effluent and waste materials in a manner that ensures inactivation of *G. salaris*.

OR

2. The *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued from the *Competent Authority* of the *exporting country* attesting that the product was derived from *aquatic animals* which had been held, immediately prior to processing, in water with a salinity of at least 25 parts per thousand for a continuous period of 14 days, and no other live *aquatic animals* of the species referred to in Article 2.1.14.2. have been introduced during that period.

This Article does not apply to *commodities* referred to in point 1 of Article 2.1.14.3.

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CHAPTER 2.4.1.

**INFECTION WITH  
BATRACHOCHYTRIUM DENDROBATIDIS**

Article 2.4.1.1.

For the purposes of the *Aquatic Code*, infection with *Batrachochytrium dendrobatidis* means infection with the freshwater fungus *B. atrachochytrium dendrobatidis* (Fungi, Chytridiomycota, Rhizophydiales).

Methods for conducting surveillance and diagnosis of infection with *B. atrachochytrium dendrobatidis* are provided in the *Aquatic Manual* (under development).

Article 2.4.1.2.

**Scope**

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

Article 2.4.1.3.

**Commodities**

2. When authorising the importation or transit of the following *commodities*, the *Competent Authorities* should not require any *B. atrachochytrium dendrobatidis* related conditions, regardless of the *B. atrachochytrium dendrobatidis* status of the *exporting country, zone or compartment*:
  - a) For the species referred to in Article 2.4.1.2. intended for any purpose:
    - i) *commodities* treated in a manner that *inactivates kills* the *disease agent* e.g. canned products; leather made from amphibian skin; dried amphibian products (including air dried, flame dried and sun dried);
    - ii) biological samples preserved for diagnostic applications in such a manner as to inactivate the *disease agent*.
  - b) The following *commodities* destined for human consumption from the species referred to in Article 2.4.1.2. which have been prepared and packaged for direct retail trade:
    - i) skinned frog legs with feet removed;
    - ii) skinned amphibian ~~carcasses or~~ meat ~~or carcasses~~, with ~~heads~~, hands and feet removed.

For the *commodities* referred to in point 1b), Members may wish to consider introducing internal measures to prevent the *commodity* being used for any purpose other than for human consumption.

2. When authorising the importation or transit of *commodities* of a species referred to in Article 2.4.1.2., other than those referred to in point 1 of Article 2.4.1.3., the *Competent Authorities* should require the conditions prescribed in Articles 2.4.1.7. to 2.4.1.12. relevant to the *B. atrachochytrium dendrobatidis* status of the *exporting country, zone or compartment*.

## Annex XII (contd)

3. When considering the importation/transit from an *exporting country, zone or compartment* not declared free of *B. atrachoetryrium dendrobatidis* of any live *commodity* of a species not covered in Article 2.4.1.2. but which could reasonably be expected to be a potential *mechanical vector for B. atrachoetryrium dendrobatidis vector*, the *Competent Authorities* should conduct a *risk analysis* in accordance with the recommendations in the *Aquatic Code*. The *exporting country* should be informed of the outcome of this assessment.

## Article 2.4.1.4.

***B. atrachoetryrium dendrobatidis* free country**

A country may make a *self-declaration of freedom* from *B. atrachoetryrium dendrobatidis* if it meets the conditions in points 1, 2, 3 or 4 below.

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from *B. atrachoetryrium dendrobatidis* if all the areas covered by the *zone* are declared *B. atrachoetryrium dendrobatidis* free (see Article 2.4.1.5.).

1. A country where none of the *susceptible species* referred to in Article 2.4.1.2. is present may make a *self-declaration of freedom* from *B. atrachoetryrium dendrobatidis* when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

2. A country where the *susceptible species* referred to in Article 2.4.1.2. are present but there has been no observed occurrence of the *disease* for at least the past ~~25~~ 10 years despite conditions that are conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may make a *self-declaration of freedom* from *B. atrachoetryrium dendrobatidis* when *basic biosecurity conditions* have been continuously met in the country for at least the past 10 years.

OR

3. A country where the last observed occurrence of the *disease* was within the past ~~25~~ 10 years, or where the *infection status* prior to *targeted surveillance* was unknown (e.g. because of the absence of conditions conducive to its clinical expression as described in Chapter X.X.X. of the *Aquatic Manual*), may make a *self-declaration of freedom* from *B. atrachoetryrium dendrobatidis* when:

- a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
- b) *targeted surveillance*, as described in Chapters ~~X.X.X of the Aquatic Code 1.1.4.~~ and X.X.X. of the *Aquatic Manual*, has been in place for at least the last 2 years without detection of *B. atrachoetryrium dendrobatidis*.

OR

4. A country that has previously made a *self-declaration of freedom* from *B. atrachoetryrium dendrobatidis* but in which the *disease* is subsequently detected may make a *self-declaration of freedom* from *B. atrachoetryrium dendrobatidis* again when the following conditions have been met:
  - a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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* (see *Aquatic Manual*) have been completed; and

- c) *targeted surveillance*, as described in Chapters X.X.X of the Aquatic Code 1.1.4 and X.X.X. of the *Aquatic Manual* (under development), has been in place for at least the last 2 years without detection of *B. atrachochytrium dendrobatidis*, and
- d) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.

In the meantime, part of the non-affected area may be declared a free *zone* provided that such part meets the conditions in point 3 of Article 2.4.1.5.

Article 2.4.1.5.

### ***B. atrachochytrium dendrobatidis* free zone or free compartment**

A *zone* or *compartment* within the *territory* of one or more countries not declared free from *B. atrachochytrium dendrobatidis* may be declared free by the *Competent Authority(ies)* of the country(ies) concerned if the *zone* or *compartment* meets the conditions referred to in points 1, 2, 3 or 4 below.

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

1. A *zone* or *compartment* where none of the *susceptible species* referred to in Article 2.4.1.2. is present may be declared free from *B. atrachochytrium dendrobatidis* when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

2. A *zone* or *compartment* where the *susceptible species* referred to in Article 2.4.1.2. are present but there has never been any observed occurrence of the *disease* for at least the past 25 10 years despite conditions that are conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual* (under development), may be declared free from *B. atrachochytrium dendrobatidis* when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 10 years.

OR

3. A *zone* or *compartment* where the last observed occurrence of the *disease* was within the past 25 10 years, or where the *infection status* prior to *targeted surveillance* was unknown (e.g. because of the absence of conditions conducive to its clinical expression as described in Chapter X.X.X. of the *Aquatic Manual*, under development), may be declared free from *B. atrachochytrium dendrobatidis* when:
  - a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
  - b) *targeted surveillance*, as described in Chapters X.X.X of the Aquatic Code 1.1.4 and X.X.X. of the *Aquatic Manual* (under development), has been in place for at least the last 2 years without detection of *B. atrachochytrium dendrobatidis*.

OR

4. A *zone* previously declared free from *B. atrachochytrium dendrobatidis* but in which the *disease* is subsequently detected may be declared free from *B. atrachochytrium dendrobatidis* again when the following conditions have been met:

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- a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
- c) *targeted surveillance*, as described in Chapters X.X.X of the Aquatic Code 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the last 2 years without detection of *B. atrachochytrium dendrobatidis*; and
- d) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.

Article 2.4.1.6.

**Maintenance of free status**

A country, *zone* or *compartment* that is declared free from *B. atrachochytrium dendrobatidis* following the provisions of points 1 or 2 of Articles 2.4.1.4. or 2.4.1.5. (as relevant) may maintain its status as *B. atrachochytrium dendrobatidis* free provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from *B. atrachochytrium dendrobatidis* following the provisions of point 3 of Articles 2.4.1.4. or 2.4.1.5. (as relevant) may discontinue *targeted surveillance* and maintain its status as *B. atrachochytrium dendrobatidis* free provided that conditions that are conducive to clinical expression of *B. atrachochytrium dendrobatidis*, as described in Chapter X.X.X. of the *Aquatic Manual*, exist, and *basic biosecurity conditions* are continuously maintained.

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

Article 2.4.1.7.

**Importation of live aquatic animals from a country, zone or compartment declared free from *B. atrachochytrium dendrobatidis***

When importing live *aquatic animals* of species referred to in Article 2.4.1.2. from a country, *zone* or *compartment* declared free from *B. atrachochytrium dendrobatidis*, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.4.1.4. or 2.4.1.5. (as applicable), the place of production of the *aquatic animal commodity* is a country, *zone* or *compartment* declared free from *B. atrachochytrium dendrobatidis*.

The *certificate* should be in accordance with the Model Certificate (under study) in Annex 4.X.1.

This Article does not apply to *commodities* referred to in point 1 of Article 2.4.1.3.

Article 2.4.1.8.

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

1. When importing live aquatic animals of species referred to in Article 2.4.1.2. from a country, *zone* or *compartment* not declared free from *B. atrachochytrium 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:

i) the *aquatic animals* of the species referred to in Article 2.4.1.2 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* (under development); and

ii) no other live aquatic animals of the species referred to in Article 2.4.1.2, have been introduced during that period;

OR

iii) in the case of eggs, the eggs have been disinfected;

OR

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

- i) the direct delivery to and lifelong holding of the consignment in biosecure facilities for continuous isolation from the local environment;
- ii) the treatment of all effluent and waste materials in a manner that kills *B. atrachochytrium dendrobatidis*.

2. If the intention of the introduction is the establishment of a new stock, 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 followed.

23. For the purposes of the Aquatic Code, the ICES Code (full version see: <http://www.ices.dk/indexfla.asp>) may be summarised to the following main points:

For the purposes of the Aquatic Code the following steps should be taken if the importation is for the establishment of a new stock:

- a) identify stock of interest (cultured or wild) in its current location;
- b) evaluate stock's health/disease history;
- c) take and test samples for *B. atrachochytrium dendrobatidis*, pests and general health/disease status;
- d) import and quarantine in a secure facility a founder (F-0) population;
- 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 *B. atrachochytrium dendrobatidis* and perform general examinations for pests and general health/disease status;
- g) if *B. atrachochytrium 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 *B. atrachochytrium dendrobatidis* free or specific pathogen free (SPF) for *B. atrachochytrium dendrobatidis*;

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

This Article does not apply to *commodities* referred to in point 1 of Article 2.4.1.3.

## Article 2.4.1.9.

**Importation of live aquatic animals for processing for human consumption from a country, zone or compartment not declared free from *B. atrachochytrium dendrobatidis***

When importing, for processing for human consumption, live *aquatic animals* of species referred to in Article 2.4.1.2. from a country, *zone* or *compartment* not declared free from *B. atrachochytrium dendrobatidis*, the *Competent Authority* of the *importing country* should require that the consignment be delivered directly to and held in *quarantine* facilities for slaughter and processing to one of the products referred to in point 1 of Article 2.4.1.3. or other products authorised by the *Competent Authority*, and all effluent and waste materials be treated in a manner that **ensure inactivation of kills *B. atrachochytrium dendrobatidis***.

This Article does not apply to *commodities* referred to in point 1 of Article 2.4.1.3.

## Article 2.4.1.10.

**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 *B. atrachochytrium dendrobatidis***

When importing live *aquatic animals* of species referred to in Article 2.4.1.2. from a country, *zone* or *compartment* not declared free from *B. atrachochytrium 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:
  - a) the *aquatic animals* have been appropriately treated to eradicate infection and have been subsequently tested to confirm absence of the diseases according to specifications provided in the relevant chapter in the *Aquatic Manual* **(under development); and**
  - ~~b) no other live *aquatic animals* of the species referred to in Article 2.4.1.2. have been introduced during that period;~~

**OR**

  - ~~c) in the case of eggs, the eggs have been disinfected;~~

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 all effluent and waste materials in a manner that kills *B. atrachochytrium dendrobatidis*.

This Article does not apply to *commodities* referred to in point 1 of Article 2.4.1.3.

Article 2.4.1.11.

**Importation of aquatic animal products from a country, zone or compartment declared free from *B. atrachochytrium dendrobatidis***

When importing *aquatic animal products* of species referred to in Article 2.4.1.2. from a country, *zone* or *compartment* declared free from *B. atrachochytrium dendrobatidis*, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.4.1.4. or 2.4.1.5. (as applicable), the place of production of the consignment is a country, *zone* or *compartment* declared free from *B. atrachochytrium dendrobatidis*.

The *certificate* should be in accordance with the Model Certificate (under study) in Annex 4.X.X.

This Article does not apply to *commodities* referred to in point 1 of Article 2.4.1.3.

Article 2.4.1.12.

**Importation of aquatic animal products from a country, zone or compartment not declared free from *B. atrachochytrium dendrobatidis***

1. When importing *aquatic animal products* of species referred to in Article 2.4.1.2. from a country, *zone* or *compartment* not declared free from *B. atrachochytrium dendrobatidis*, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate risk mitigation measures.
2. In the case of dead *aquatic animals* of species referred to in Article 2.4.1.2., whether *eviscerated* or *uneviscerated*, such risk mitigation measures may include:
  - a) the direct delivery into and holding of the consignment in biosecure facilities for processing to one of the products referred to in point 1 of Article 2.4.1.3. or other products authorised by the *Competent Authority*;
  - b) the treatment of all effluent and waste materials in a manner that kills *B. atrachochytrium dendrobatidis*.

This Article does not apply to *commodities* referred to in point 1 of Article 2.4.1.3.

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## CHAPTER 2.4.2.

## INFECTION WITH RANAVIRUS

## Article 2.4.2.1.

For the purposes of the *Aquatic Code*, infection with ranavirus means infection with any **members virus species** of the genus *Ranavirus* in the family Iridoviridae with the exception of epizootic haematopoietic necrosis virus and European catfish virus.

Methods for conducting surveillance and diagnosis of infection with ranavirus are provided in the *Aquatic Manual* **(under development)**.

## Article 2.4.2.2.

**Scope**

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

## Article 2.4.2.3.

**Commodities**

1. When authorising the importation or transit of the following *commodities*, the *Competent Authorities* should not require any ranavirus related conditions, regardless of the ranavirus status of the *exporting country, zone or compartment*:
  - a) For the species referred to in Article 2.4.2.2. intended for any purpose:
    - i) *commodities* treated in a manner that **inactivates kills** the *disease agent* e.g. canned products; leather made from amphibian skin;
    - ~~ii-iii)~~ **ii-iii)** biological samples preserved for diagnostic applications in such a manner as to inactivate the *disease agent*.
  - b) The following *commodities* destined for human consumption from the species referred to in Article 2.4.2.2. which have been prepared and packaged for direct retail trade:
    - i) skinned frog legs;
    - ii) skinned amphibian carcasses or meat.

For the *commodities* referred to in point 1b), Members may wish to consider introducing internal measures to prevent the *commodity* being used for any purpose other than for human consumption.

2. When authorising the importation or transit of *commodities* of a species referred to in Article 2.4.2.2., other than those referred to in point 1 of Article 2.4.2.3., the *Competent Authorities* should require the conditions prescribed in Articles 2.4.2.7. to 2.4.2.12. relevant to the ranavirus status of the *exporting country, zone or compartment*.

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3. When considering the importation/transit from an *exporting country, zone* or *compartment* not declared free of ranavirus of any live *commodity* of a species not covered in Article 2.4.2.2. but which could reasonably be expected to be a potential **mechanical vector for** ranavirus **vector**, the *Competent Authorities* should conduct a *risk analysis* in accordance with the recommendations in the *Aquatic Code*. The *exporting country* should be informed of the outcome of this assessment.

## Article 2.4.2.4.

**Ranavirus free country**

A country may make a *self-declaration of freedom* from ranavirus if it meets the conditions in points 1, 2, 3 or 4 below.

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from ranavirus if all the areas covered by the *zone* are declared ranavirus free (see Article 2.4.2.5.).

1. A country where none of the *susceptible species* referred to in Article 2.4.2.2. is present may make a *self-declaration of freedom* from ranavirus when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

2. A country where the *susceptible species* referred to in Article 2.4.2.2. are present but there has been no observed occurrence of the *disease* for at least the past **15 10** years despite conditions that are conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual* (under development), may make a *self-declaration of freedom* from ranavirus when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

3. A country where the last observed occurrence of the *disease* was within the past **25 10** years, or where the *infection* status prior to *targeted surveillance* was unknown (e.g. because of the absence of conditions conducive to its clinical expression as described in Chapter X.X.X. of the *Aquatic Manual*, under development), may make a *self-declaration of freedom* from ranavirus when:
  - a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
  - b) *targeted surveillance*, as described in Chapters **X.X.X of the Aquatic Code 1.1.4.** and X.X.X. of the *Aquatic Manual* (under development), has been in place for at least the last 2 years without detection of ranavirus.

OR

4. A country that has previously made a *self-declaration of freedom* from ranavirus but in which the *disease* is subsequently detected may make a *self-declaration of freedom* from ranavirus again when the following conditions have been met:
  - a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and

- c) *targeted surveillance*, as described in Chapters X.X.X of the Aquatic Code 1.1.4 and X.X.X. of the *Aquatic Manual* (under development), has been in place for at least the last 2 years without detection of ranavirus; and
- d) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.

In the meantime, part of the non-affected area may be declared a free *zone* provided that such part meets the conditions in point 3 of Article 2.4.2.5.

Article 2.4.2.5.

### **Ranavirus free zone or free compartment**

A *zone* or *compartment* within the *territory* of one or more countries not declared free from ranavirus may be declared free by the *Competent Authority(ies)* of the country(ies) concerned if the *zone* or *compartment* meets the conditions referred to in points 1, 2, 3 or 4 below.

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

1. A *zone* or *compartment* where none of the *susceptible species* referred to in Article 2.4.2.2. is present may be declared free from ranavirus when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

2. A *zone* or *compartment* where the *susceptible species* referred to in Article 2.4.2.2. are present but there has never been any observed occurrence of the *disease* for at least the past 25 10 years despite conditions that are conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual* (under development), may be declared free from ranavirus when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 10 years.

OR

3. A *zone* or *compartment* where the last observed occurrence of the *disease* was within the past 25 10 years, or where the *infection status* prior to *targeted surveillance* was unknown (e.g. because of the absence of conditions conducive to its clinical expression as described in Chapter X.X.X. of the *Aquatic Manual*, under development), may be declared free from ranavirus when:
  - a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
  - b) *targeted surveillance*, as described in Chapters X.X.X of the Aquatic Code 1.1.4 and X.X.X. of the *Aquatic Manual* (under development), has been in place for at least the last 2 years without detection of ranavirus.

OR

4. A *zone* previously declared free from ranavirus but in which the *disease* is subsequently detected may be declared free from ranavirus again when the following conditions have been met:
  - a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer zone* was established; and

Annex XIII (contd)

- 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 (see *Aquatic Manual*) have been completed; and
- c) *targeted surveillance*, as described in Chapters X.X.X of the Aquatic Code 1.1.4 and X.X.X. of the *Aquatic Manual* (under development), has been in place for at least the last 2 years without detection of ranavirus; and
- d) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.

Article 2.4.2.6.

**Maintenance of free status**

A country, *zone* or *compartment* that is declared free from ranavirus following the provisions of points 1 or 2 of Articles 2.4.2.4. or 2.4.2.5. (as relevant) may maintain its status as ranavirus free provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from ranavirus following the provisions of point 3 of Articles 2.4.2.4. or 2.4.2.5. (as relevant) may discontinue *targeted surveillance* and maintain its status as ranavirus free provided that conditions that are conducive to clinical expression of ranavirus, as described in Chapter X.X.X. of the *Aquatic Manual* (under development), exist, and *basic biosecurity conditions* are continuously maintained.

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

Article 2.4.2.7.

**Importation of live aquatic animals from a country, zone or compartment declared free from ranavirus**

When importing live *aquatic animals* of species referred to in Article 2.4.2.2. from a country, *zone* or *compartment* declared free from ranavirus, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.4.2.4. or 2.4.2.5. (as applicable), the place of production of the aquatic animal commodity is a country, *zone* or *compartment* declared free from ranavirus.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.X.X.

This Article does not apply to *commodities* referred to in point 1 of Article 2.4.2.3.

Article 2.4.2.8.

**Importation of live aquatic animals for farming from a country, zone or compartment not declared free from ranavirus**

1. When importing live *aquatic animals* of species referred to in Article 2.4.2.2. from a country, *zone* or *compartment* not declared free from ranavirus, 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 no other live *aquatic animals* of the species referred to in Article 2.4.2.2. have been introduced during that period;

OR

- b) assess the *risk* and apply risk mitigation measures such as:
- i) the direct delivery to and lifelong holding of the consignment in biosecure facilities for continuous isolation from the local environment;
  - ii) the treatment of all effluent and waste materials in a manner that kills ranavirus.

2. If the intention of the introduction is the establishment of a new stock, 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 followed.

32. For the purposes of the Aquatic Code, the ICES Code (full version see: <http://www.ices.dk/indexfla.asp>) may be summarised to the following main points:

2. For the purposes of the Aquatic Code the following steps should be taken if the importation is for the establishment of a new stock:

- a) identify stock of interest (cultured or wild) in its current location;
- b) evaluate stock's health/disease history;
- c) take and test samples for ranavirus, pests and general health/disease status;
- d) import and quarantine in a secure facility a founder (F-0) population;
- 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 ranavirus and perform general examinations for pests and general health/disease status;
- g) if ranavirus 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 ranavirus free or specific pathogen free (SPF) for ranavirus;
- h) release SPF F-1 stock from *quarantine* for *aquaculture* or stocking purposes in the country, *zone* or *compartment*.

This Article does not apply to *commodities* referred to in point 1 of Article 2.4.2.3.

Article 2.4.2.9.

### **Importation of live aquatic animals for processing for human consumption from a country, zone or compartment not declared free from ranavirus**

When importing, for processing for human consumption, live *aquatic animals* of species referred to in Article 2.4.2.2. from a country, *zone* or *compartment* not declared free from ranavirus, the *Competent Authority* of the *importing country* should require that the consignment be delivered directly to and held in *quarantine* facilities for slaughter and processing to one of the products referred to in point 1 of Article 2.4.2.3. or other products authorised by the *Competent Authority*, and all effluent and waste materials be treated in a manner that ensures inactivation of kills ranavirus.

Annex XIII (contd)

This Article does not apply to *commodities* referred to in point 1 of Article 2.4.2.3.

## Article 2.4.2.10.

**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 ranavirus**

**1.** When importing live *aquatic animals* of species referred to in Article 2.4.2.2. from a country, *zone* or *compartment* not declared free from ranavirus, 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 no other live *aquatic animals* of the species referred to in Article 2.4.2.2. have been introduced during that period;~~

**OR**

**b)** 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 kills ranavirus.

This Article does not apply to *commodities* referred to in point 1 of Article 2.4.2.3.

## Article 2.4.2.11.

**Importation of aquatic animal products from a country, zone or compartment declared free from ranavirus**

When importing *aquatic animal products* of species referred to in Article 2.4.2.2. from a country, *zone* or *compartment* declared free from ranavirus, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.4.2.4. or 2.4.2.5. (as applicable), the place of production of the consignment is a country, *zone* or *compartment* declared free from ranavirus.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.X.X.

This Article does not apply to *commodities* referred to in point 1 of Article 2.4.2.3.

## Article 2.4.2.12.

**Importation of aquatic animal products from a country, zone or compartment not declared free from ranavirus**

1. When importing *aquatic animal products* of species referred to in Article 2.4.2.2. from a country, *zone* or *compartment* not declared free from ranavirus, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate risk mitigation measures.

2. In the case of dead *aquatic animals*, whether *eviscerated* or *uneviscerated*, such risk mitigation measures may include:

- a) the direct delivery into and holding of the consignment in biosecure facilities for processing to one of the products referred to in point 1 of Article 2.4.2.3. or other products authorised by the *Competent Authority*;
  - b) the treatment of all effluent and waste materials in a manner that kills ranavirus.
3. This Article does not apply to *commodities* referred to in point 1 of Article 2.4.2.3.

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## INTRODUCTION TO OIE GUIDELINES FOR THE WELFARE OF LIVE AQUATIC ANIMALS FARMED FISH

Article X.X.X.1.

### Guiding principles for aquatic animal welfare

Considering that:

1. That there is a critical relationship between aquatic animal health and aquatic animal welfare. The use of fish in harvest or capture fisheries, in research and for recreation (e.g. ornamental fish and aquaria) makes a major contribution to the wellbeing of people; and
2. That the use of aquatic animals in aquaculture, harvest or capture fisheries, research and for recreation (eg ornamentals and aquaria), makes a major contribution to the wellbeing of people. There is a critical relationship between fish health and fish welfare; and
3. That the use of aquatic animals carries with it an ethical responsibility to ensure the welfare of such animals to the greatest extent practicable. Improvements in farmed fish welfare can often improve productivity and hence lead to economic benefits.
4. That improvements in aquatic animal welfare can often improve productivity and hence lead to economic benefits.
5. That the internationally recognised 'five freedoms' (freedom from hunger, thirst and malnutrition; freedom from fear and distress; freedom from physical and thermal discomfort; freedom from pain, injury and disease; and freedom to express normal patterns of behaviour) provide valuable guidance in aquatic animal welfare.
6. That the scientific assessment of aquatic animal welfare involves both scientifically derived data and value based assumptions which need to be considered together, and the process of making these assessments should be made as explicit as possible.
7. That equivalent outcomes based on performance criteria, rather than identical systems based on design criteria, be the basis for comparison of aquatic animal welfare standards and guidelines.

The OIE will develop guidelines for the welfare of farmed fish (excluding ornamental species) during transport, slaughter, and destruction for disease control purposes. In developing these, the following principles will apply:

1. The use of fish carries with it an ethical responsibility to ensure the welfare of such animals to the greatest extent practicable.
2. The scientific assessment of fish welfare involves both scientifically derived data and value-based assumptions that need to be considered together, and the process of making these assessments should be made as explicit as possible.

**Scientific basis for guidelines**

The scientific assessment of *aquatic animal* welfare has progressed rapidly in recent years and forms the basis of these guidelines. Many areas of *aquatic animal* welfare require further research to understand in full the ability of *aquatic animals* to feel pain and to be sentient. [To be developed]

1. The basic requirements for the welfare of farmed fish include handling methods appropriate to the biological characteristics of the fish and a suitable environment to fulfil their needs.
2. There are many species of fish in farming systems and these have different biological characteristics. It is not practicable to develop specific guidelines for each of these species. These OIE guidelines therefore address the welfare of farmed fish at a general level.

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## INTRODUCTION TO GUIDELINES FOR THE WELFARE OF FARMED FISH

Article X.X.X.1.

### **Guiding principles**

Considering that:

1. The use of fish in harvest or capture fisheries, in research and for recreation (eg ornamentals and aquaria), makes a major contribution to the wellbeing of people; and
2. There is a critical relationship between fish health and fish welfare; and
3. Improvements in farmed fish welfare can often improve productivity and hence lead to economic benefits.

The OIE will develop guidelines for the welfare of farmed fish (excluding ornamental species) during transport, slaughter, and destruction for disease control purposes. In developing these, the following principles will apply:

1. The use of fish carries with it an ethical responsibility to ensure the welfare of such animals to the greatest extent practicable.
2. The scientific assessment of fish welfare involves both scientifically derived data and value-based assumptions that need to be considered together, and the process of making these assessments should be made as explicit as possible.

Article X.X.X.2.

### **Scientific basis for guidelines**

1. The basic requirements for the welfare of farmed fish include handling methods appropriate to the biological characteristics of the fish and a suitable environment to fulfil their needs.
2. There are many species of fish in farming systems and these have different biological characteristics. It is not practicable to develop specific guidelines for each of these species. These OIE guidelines therefore address the welfare of farmed fish at a general level.

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## APPENDIX X.X.X.

## DRAFT GUIDELINES FOR THE CONTROL OF AQUATIC ANIMAL HEALTH HAZARDS IN AQUATIC ANIMAL FEED

Article X.X.X.1.

### Introduction

One of the key objectives of the *Aquatic Code* is to help OIE Members trade safely in *aquatic animals* and *aquatic animal* ~~their~~ *products* by developing relevant *aquatic animal* health measures. These guidelines address *aquatic animal* health hazards in *aquatic animal feed*. A key objective is to prevent the spread, via *aquatic animal* *feed*, of diseases from an infected country, zone or compartment to a free country, zone or compartment.

These guidelines do not ~~for the moment~~ address food safety issues in detail as this is not within the mandate of the ~~OIE Aquatic Code Animal Health Standards Commission hereafter referred to as the Aquatic Animals Commission~~.

These Guidelines should be read in conjunction with relevant recommendations of the ~~OIE Terrestrial Animal Health Code (under study Appendix containing recommendations on animal feed)~~. The Food and Agriculture Organization of the United Nations (FAO) has published recommendations relevant to terrestrial and aquatic animal *feed* (Technical Guidelines for Responsible Fisheries – Aquaculture Development: 1. Good aquaculture feed manufacturing practice. FAO 2001; Draft Good Practices for the Animal Feed Industry – Implementing the Codex Alimentarius' Code of Practice on Good Animal Feeding, IFIF/FAO [*in preparation*]) and there is a Codex Alimentarius Commission (CAC) standard (Code of Practice on Good Animal Feeding [CAC/RCP 54-2004]). Members are encouraged to consult these publications.

Key considerations relevant to *aquatic animal feeds* are as follows:

1. Concentration of *aquaculture establishments* ~~and intensive rearing causes a concentration of aquatic animals, feed and faecal matter in time and space and this~~ heightens the risk of disease transmission, whether the pathogen enters the culture system via *feed* or other means.
2. For many *aquatic animal* species, predation (including cannibalism) is their natural way of feeding in their natural habitat.
3. Historically, animal proteins used in *feed* were mainly sourced from the marine environment, due to the nutritional needs of *aquatic animals* and for reasons of economy. This practice increases the ~~risk of~~ disease ~~risks~~ ~~transmission~~, especially when *aquatic animals* are fed ~~with~~ live or whole *aquatic animals* of the same or related species. There are many examples of this type of practice, e.g. early stage crustaceans fed on *Artemia* species and *aquaculture* tuna fed on whole wild caught fish.

Annex XV (contd)

4. The usage of *feed* in moist form (moisture content equal to or greater than 70%), semi-moist form (moisture content between 15 and 70%), and dry form (a moisture content equal to or less than 15%) implies different levels of risk due to the processing applied to the *feed*.
5. With the increasing number of species being farmed (especially marine finfish), the use of *live feed* and moist *feed* has increased. It is likely that these industries will in future use formulated *feed* as appropriate technologies are developed.
6. *Hazards* may be transmitted from *feed* to *aquatic animals* via direct or indirect means. Direct transmission occurs when the cultured species consumes *feed* containing a pathogenic agent (e.g. shrimp larvae consuming rotifer infected with white spot syndrome virus) while indirect transmission refers to pathogens in *feed* entering the aquatic environment or infecting non target species, and thereby establishing a mechanism for indirect infection of the species of commercial interest. Pathogens that are less host-specific (e.g. white spot syndrome virus, *Vibrio* species) present a greater risk of indirect transmission as they can establish reservoirs of infection in multiple species.
7. As new species become the subject of *aquaculture*, new pathogens emerge in association with these hosts. The expression of disease may be facilitated by culturing species under intensive and novel conditions. Also, it is necessary to conduct research and develop new *feed* (and *feed ingredients*) that are appropriate to the species and its culture system. As more and more *aquatic animal* species are being cultured, it is difficult to make recommendations for all disease agent/host species combinations.

Article X.X.X.2.

## Scope

These guidelines document risk mitigation measures, including traceability and certification, to deal with aquatic animal health risks associated with trade in *aquatic animal feeds* and *feed ingredients*. They recommend the control of *hazards* through adherence to recommended practices during the production (harvest, handling, storage, processing and distribution) and use of both commercial and on-farm produced *feed* (and *feed ingredients*) for *aquatic animals*. *Hazards* include pathogens that cause OIE-listed diseases referred to in this Aquatic Code and other agents that cause an adverse effect on animal and/or public health. While *aquatic animals* grown for food are the main focus, the same principles apply to *feed* for *aquatic animals* used for other purposes.

Article X.X.X.3.

## Definitions

### Dry feed

mMeans *feed* that has a moisture content equal to or less than 15%.

**Feed**

**m**Means any material (single or multiple), whether processed, semi-processed or raw that is intended to be fed directly to **food-producing aquatic animals**.

**Feed additives**

**m**Means any ingredient intentionally added in micro-amounts not normally consumed as *feed* by itself, whether or not it has nutritional value, which affects the characteristics of *feed* or animal products. Micro-organisms, enzymes, acidity regulators, trace elements, vitamins, substances used to attract *aquatic animals* to feed and promote *feed* intake, pigments, synthetic binders, synthetic amino acids, antioxidants and other products fall within the scope of this definition, depending on the purpose of use and method of administration. This excludes veterinary drugs.

**Feed ingredient**

**m**Means a component, part or constituent of any combination or mixture making up a *feed*, including *feed additives*, whether or not it has a nutritional value in the animal's diet. Ingredients may be of terrestrial or aquatic, plant or animal origin and may be organic or inorganic substances.

**Fish solubles**

**m**Means a by product of the fish oil production system, comprising the product remaining when water is drawn off (evaporated) from the residual aqueous phase.

**Hazard**

**m**Means a biological, chemical or physical agent in a *feed* or a *feed ingredient* with the potential to cause an adverse effect on animal or public health.

**Live feed**

**m**Means live farmed or wild caught animals and algae used as *feed* for *aquatic animals*. *Live feed* is often fed to *aquatic animal* species at an early life-stage and to *aquatic animal* species that have been cultured for a relatively short time.

**Meal**

**m**Means a product derived from an *aquatic animal* that has been ground and heat processed to reduce the moisture content to less than 10%.

**Medicated feed**

**m**Means any *feed* which contains a veterinary drug administered to food producing animals, for therapeutic or prophylactic purposes or for modification of physiological functions.

**Moist (or wet) feed**

**m**Means *feed* that has a moisture content equal to or greater than 70%.

**Semi-moist feed**

**m**Means *feed* that has a moisture content between 15 and 70%.

## General principles

### 1. Roles and responsibilities

The *Competent Authority* has the legal power to set and enforce regulatory requirements related to animal *feed*, and has final responsibility for verifying that these requirements are met. The *Competent Authority* may establish regulatory requirements for relevant parties, including requirements to provide information and assistance.

It is a particular responsibility of the *Competent Authority* to set and enforce the regulatory requirements pertaining to the use of veterinary drugs, *aquatic animal* disease control and the food safety aspects that relate to the management of *live aquatic animals* on farm.

Those involved in the production and use of animal *feed* and *feed ingredients* have the responsibility to ensure that these products meet regulatory requirements. All personnel involved in the harvest, manufacture, storage and handling of *feed* and *feed ingredients* should be adequately trained and aware of their role and responsibility in preventing the spread of *hazards*. Appropriate contingency plans should be developed in case of a *feed-borne disease outbreak of disease*. Equipment for producing, storing and transporting *feed* should be kept clean and maintained in good working order.

Private *veterinarians* and others (e.g. laboratories) 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).

### 2. Regulatory standards for feed safety

All *feed* and *feed ingredients* should meet regulatory standards for *feed* safety. In defining limits and tolerances for *hazards*, scientific evidence, including the sensitivity of analytical methods, and on the characterisation of risks, should be taken into account.

### 3. Risk analysis

Internationally accepted principles and practices for *risk analysis* (see Section 1.4. of the *Aquatic Code* and relevant Codex texts) should be used in developing and applying the regulatory framework.

A generic *risk analysis* framework should be applied to provide a systematic and consistent process for managing *hazards*.

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<sup>1</sup> *if at the national level, there are specific food safety or animal health regulations related to genetically modified organisms, these should be taken into account in relation to feed and feed ingredients as these products form an important part of the food chain.*

#### 4. Good practices

Where national guidelines exist, good *aquaculture* practices and good manufacturing practices (including good hygienic practices) should be followed. Countries without such guidelines are encouraged to develop them.

Where appropriate, Hazard Analysis and Critical Control Point<sup>2</sup> (HACCP; as defined in the Annex to the Recommended International Code of Practice on General Principles of Food Hygiene [CAC/RCP 1-1969]) principles should be followed to control *hazards* that may occur in *feed*.

#### 5. Relationship between prions and aquatic animal species

Scientific knowledge is lacking on the relationship between prions and *aquatic animal* species. There is no evidence to suggest that the use of terrestrial animal by-products as ingredients in *aquatic animal feed* gives rise to risks in respect of prion diseases. More scientific information is desirable to enable *aquaculture* industries to utilise more terrestrial animal by-products as a means of reducing dependency on aquatic protein and lipid sources.

#### 6. Bioaccumulation

Heavy metals, dioxins and polychlorinated biphenyls (PCB) persist in fatty tissues and therefore tend to accumulate through the food chain.

#### 7. Geographic and environmental considerations

Aquatic and terrestrial harvest areas for *feed* should not be located in proximity to sources of animal health or food safety *hazards*. Where this cannot be avoided, preventive measures should be applied to control risk. The same recommendations apply for the processing of *feed* and the location of *aquaculture establishments*.

*Aquatic animal* health considerations include factors such as disease status, location of quarantined premises, existence of processing plants without proper biosecurity measures and the existence of *zones/compartments* of specified health status.

Public health considerations include factors such as industrial operations and waste treatment plants that generate pollutants and other hazardous products. The potential accumulation of pollutants in the food chain through *feed* needs to be considered.

#### 8. Zoning and compartmentalisation

*Feed* is an important components of biosecurity and needs to be considered when defining a *compartment* or *zone* in accordance with Chapter 1.4.4. of the *Aquatic Code*.

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<sup>2</sup> Hazard Analysis and Critical Control Point, as defined in the Annex to the Recommended International Code of Practice on General Principles of Food Hygiene (CAC/RCP 1-1969).

Annex XV (contd)9. Sampling and analysis

Sampling and analytical protocols **for feed** should be based on scientific principles and procedures, and OIE standards where applicable.

10. Labelling

Labelling should be clear and informative on how the *feed* and *feed ingredients* should be handled, stored and used and should comply with regulatory requirements. Labelling should provide for trace-back.

See Section 4.2. of the Codex Code of Practice on Good Animal Feeding (CAC/RCP 54-2004).

11. Design and management of inspection programmes

In meeting animal and public health objectives prescribed in national legislation or required by *importing countries*, *Competent Authorities* contribute through the direct performance of some tasks or through the auditing of animal and public health activities conducted by other agencies or the private sector.

Operators in the *feed* and *feed ingredients* business and other relevant industries should implement procedures to ensure compliance with regulatory standards for harvest, handling, storage, processing, distribution and use of *feed* and *feed ingredients*. Operators have the primary responsibility for implementing systems for process control. Where such systems are applied, the *Competent Authority* should verify that they meet all regulatory requirements.

12. Assurance and certification

*Competent Authorities* are responsible for providing assurances domestically and to trading partners that regulatory requirements have been met.

13. Hazards associated with aquatic animal feeda) Biological hazards

Biological hazards that may occur in *feed* and *feed ingredients* include agents such as bacteria, viruses, fungi and parasites. The scope of these guidelines **covers is limited to the OIE-listed diseases referred to in this Aquatic Code and other agents that cause an adverse effect on animal and/or public health.**

b) Chemical hazards

Chemical hazards that may occur in *feed* and *feed ingredients* include naturally occurring chemicals (such as mycotoxins, gossypol and free radicals), industrial and environmental contaminants (such as heavy metals, dioxins and PCBs), residues of veterinary drugs and pesticides and radionuclides.

c) Physical hazards

Physical hazards that may occur in *feed* and *feed ingredients* include foreign objects (such as pieces of glass, metal, plastic or wood).

14. Cross-contamination

It is important to avoid cross-contamination during the manufacture, storage, distribution (including transport) and use of *feed* and *feed ingredients*. Appropriate provisions should be included in the regulatory framework. Scientific evidence, including the sensitivity of analytical methods and on the characterisation of risks, should be drawn upon in developing this framework.

Procedures such as flushing, sequencing and physical clean-out should be used to avoid cross-contamination between batches of *feed* or *feed ingredients*. National regulations should be followed in order to avoid the use of unauthorised *feed ingredients* with a risk of cross-contamination.

15. Antimicrobial resistance

Concerning the use of antimicrobials in animal *feed* refer to Section X.X.X. of the *Aquatic Code* **(under study)**.

16. Management of information

The *Competent Authority* should establish requirements for the provision of information by the private sector in accordance with the ~~on~~ regulatory framework.

The private sector should maintain records, in a readily accessible form, on the production, distribution, importation and use of *feed* and *feed ingredients*. These records are required to facilitate the prompt trace-back of *feed* and *feed ingredients* to the immediate previous source, and trace-forward to the next/subsequent recipients, to address *aquatic animal* health and/or public health concerns. The private sector should provide information to the *Competent Authority* in accordance with the regulatory framework.

Animal identification (in the case of *aquatic animals* this will normally be on a group basis) and traceability are tools for addressing animal health and food safety risks arising from animal *feed* (see Section 3.5. of the *Terrestrial Animal Health Code*, Section 4.3 of CAC/RCP 54-2004).

Article X.X.X.5.

**Pathogens in feed**1. Pathogens can be introduced into *feed* in the following ways:

- a) via the harvest of infected *aquatic animals*;
- b) during storage, processing and transport, due to poor hygienic practices, the presence of pests, or residues of previous batches of *feed* remaining in processing lines, containers or transport vehicles

Annex XV (contd)2. *Aquatic animals* can be exposed to pathogens in *feed* in the following ways:

## a) Direct exposure

The use of unprocessed *feed* derived from *aquatic animals* to feed *aquatic animals* presents a direct route of exposure, particularly when feeding whole *aquatic animals* and unprocessed products of *aquatic animals* to animals of the same species. For example feeding salmonid *offal* to salmonids or feeding rotifers or *Artemia* species to crustaceans presents a heightened risk of disease transmission.

## b) Indirect exposure

Pathogens in *feed* may be transmitted to *aquatic animals* in *aquaculture* and wild *aquatic animals* via contamination of the environment or *infection* of non-target species.

Article X.X.X.6.

**Chemical agents in feed**

[under study]

Article X.X.X.7.

**Physical agents in feed**

[under study]

Article X.X.X.8.

**Recommended approaches to risk mitigation**1. Commodities

## a) Safe commodities

The following *commodities* undergo extensive processing such as heat treatment, acidification, extrusion and extraction. There is a negligible risk that pathogens will survive in such products if they have been produced in accordance with normal commercial practice:

- i) fish oil;
- ii) crustacean oil;
- iii) *fish solubles*; a by-product of the fish oil production system, comprising the product remaining when water is drawn off (evaporated) from the residual aqueous phase.
- iv) fish *meal*;
- v) crustacean *meal*;
- vi) squid *meal* and squid liver-*meal*;
- vii) bivalve *meal*;
- viii) finished *feed* (e.g. flake, pelleted and extruded *feed*).

For these *commodities*, *Competent Authorities* should not require conditions in relation to *aquatic animal diseases*, regardless of the *aquatic animal* health status of the *exporting country, zone* or *compartment*.

b) Other commodities

*Competent Authorities* should consider the following risk mitigation measures:

- i) sourcing *feed* and *feed ingredients* from a disease *free country, free zone* or *free compartment*; or
- ii) confirmation (e.g. by testing) that pathogens are not present in the *commodity*; or
- iii) treatment (e.g. by heat or acidification) of the *commodity* using a method approved by the *Competent Authority* to inactivate pathogens; or
- iv) use of *feed* only in *populations* that are not susceptible to the pathogen(s) in question and where *aquatic animals* that are susceptible to the pathogen(s) in question will not come into contact with the *feed* or its waste products.

In addition, risks associated with the disposal of effluents and waste material from *feed* processing plants and *aquaculture establishments* should be considered.

c) Whole fish (fresh or frozen)

The practice of trading fresh or frozen whole marine fish for use as *aquatic animal feed* presents a risk of introducing diseases into *populations*. Risk mitigation measures include sourcing fish only from stocks where there is no evidence of infection with any of the OIE-listed diseases or treatments that inactivate *aquatic animal* pathogens. Given the difficulty of imposing effective risk mitigation measures, this practice is not recommended.

2. Feed production

To prevent contamination by pathogens during production, storage and transport of *feed* and *feed ingredients*

- a) flushing, sequencing or physical clean-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) in particular, *feed* manufacturing plants should be designed and operated to avoid cross-contamination between batches;
- d) processed *feed* and *feed ingredients* should be stored separately from unprocessed *feed ingredients*, under appropriate storage conditions;

Annex XV (contd)

- e) *feed* and *feed ingredients*, manufacturing equipment, storage facilities and their immediate surroundings should be kept clean and pest control programmes should be implemented;
- f) measures to inactivate pathogens, such as heat treatment or the addition of authorised chemicals, should be used where appropriate. Where such measures are used, the efficacy of treatments should be monitored at appropriate stages in the manufacturing process;
- g) labelling should provide for the identification of *feed* and *feed ingredients* as to the batch/lot and place and date of production. To assist in tracing *feed* and *feed ingredients* as may be required to deal with animal **disease** incidents, labelling should provide for identification by batch/lot and place and date of production.

3. Importing countries

*Competent Authorities* should consider the following measures:

- i) imported *feed* and *feed ingredients* should be delivered to feed manufacturing plants or *aquaculture* facilities for processing and use under conditions approved by the *Competent Authority*;
- ii) effluent and waste material from feed manufacturing plants and *aquaculture* facilities should be managed under conditions approved by the *Competent Authority*, including, where appropriate, treatment before discharge into the aquatic environment;
- iii) *feed* that is known to contain pathogens should only be used in a *zone* or *compartment* that does not contain species susceptible to the *disease* in question;
- iv) the importation of raw unprocessed *feed* derived from *aquatic animals* to feed *aquatic animal* species should be avoided where possible.

Article X.X.X.9.

**Certification procedures for feeds and feed ingredients of aquatic animal origin**

When importing *feed* and *feed ingredients* of aquatic animal origin other than those mentioned in Article X.X.X.8, X. (see ~~Article with safe commodities, currently~~ point 1a) ~~of Article X.X.X.8,~~ 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*).

This certificate should certify:

- a) that *feed* and *feed ingredients* of aquatic animal origin were obtained from a country, *zone* or *compartment* that is free from relevant aquatic animal diseases<sup>3</sup>; or

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<sup>3</sup> Conditions agreed between the Competent Authorities of the importing and exporting countries in accordance with the recommendations of the OIE Aquatic Animal Health Code.

- b) that *feed* and *feed ingredients* of **aquatic animal** origin were tested for relevant **aquatic animal diseases**<sup>4</sup> and shown to be free of these **diseases**; or
- c) that *feed* and *feed ingredients* of **aquatic animal** origin have been processed to ensure that they are free of relevant **aquatic animal diseases**.

Specific provisions for **OIE-listed diseases referred to in this Aquatic Code** may be found in relevant *disease* chapters of the *Aquatic Code*.

Article X.X.X.10.

### **Risk chart of pathogen transmission and contamination through harvest, manufacture and use of aquatic **animal** feed**

Figure 1 illustrates the possible pathways for transmission of pathogens within the feed production and utilisation process.

*Feed ingredients* of aquatic origin used in *aquaculture* can be a source of pathogens (viruses, bacteria and parasites) to cultured *aquatic animal* species. In *aquaculture establishments* pathogens in *feed* can infect the animals directly (via consumption of *feed*) or indirectly via environmental sources. *Live feed* and moist *feed* are more likely to contain pathogens because their ingredients are either in a raw state or subject to minimal treatment.

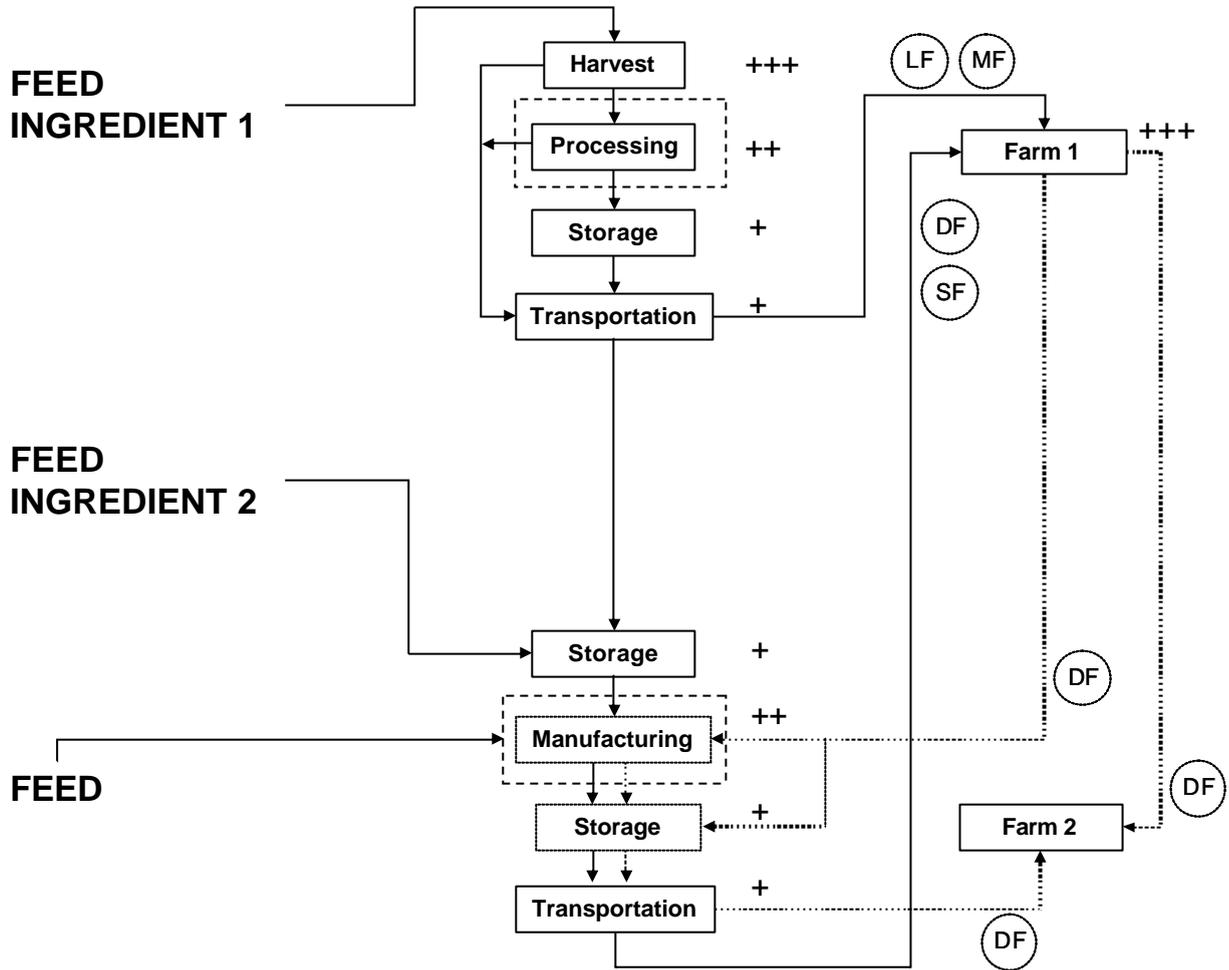
*Feed* and *feed ingredients* harvested from infected countries, *zones* or *compartments* may have a high pathogen load. *Feed* and *feed ingredients* from these sources should be processed (e.g. using heat or chemical treatments) to reduce, or eliminate, the pathogen load. After processing care should be taken to avoid post processing contamination during storage and transportation of these *commodities*. For example, when two or more batches of ingredients of different sanitary status are handled, stored and/or transported together without appropriate biosecurity measures, there is a risk of cross-contamination of the *feed*.

An *aquaculture* facility can also be a source of pathogens in **aquatic animal** *feed*. For example, *feed* can be contaminated with pathogens through poor hygiene practices at an infected *aquaculture establishment*. If the *feed* is redistributed from the *aquaculture* facility to the manufacturing facility for recycling, or distributed to another farm, pathogens can be transferred to other *aquaculture establishments*.

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<sup>4</sup> **Conditions agreed between the Competent Authorities of the importing and exporting countries in accordance with the recommendations of the OIE Aquatic Animal Health Code.**

Figure 1: **RISK CHART OF PATHOGEN TRANSMISSION AND CONTAMINATION THROUGH HARVEST, MANUFACTURE AND USE OF AQUATIC ANIMAL FEED**



LF: Live feed MF: Moist feed SF: Semi-moist feed DF: Dry feed	Possibility for risk reduction
+++: High risk of pathogen presence ++: Moderate risk of pathogen presence +: Low risk of pathogen presence	Redistribution or recycling of finished feed

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## APPENDIX X.X.X.

**GUIDELINES FOR AQUATIC ANIMAL  
HEALTH SURVEILLANCE**

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Article x.x.x.1.

**Introduction and objectives**

- 1 Surveillance activities may be performed to achieve any of the following objectives:
  - a) demonstrating the absence of *disease*,
  - b) identifying events requiring notification as listed in Article 1.2.1.3. of the *Aquatic Code*.
  - c) determining the occurrence or distribution of endemic *disease*, including changes to their incidence or prevalence (or its contributing factors), in order to:
    - i) provide information for domestic *disease* control programmes,
    - ii) provide relevant *disease* occurrence information to be used by trading partners for qualitative and quantitative risk assessment.

The type of surveillance applied depends on the desired outputs needed to support decision-making. Surveillance data determine the quality of *disease* status reports and should satisfy information requirements for accurate risk analysis both for *international trade* as well as for national decision-making. Surveillance of endemic diseases provides valuable information for day-to-day health management and can act as the foundation for detecting outbreaks of exotic disease and demonstrating specific disease freedom.

Surveillance systems described in this chapter should also be used to generate information for decisions on prescribed disease prevention and control programmes. However, the actual strategies for prevention and control are beyond the scope of this chapter on surveillance guidelines.

Having a suitable management strategy to respond to surveillance data is of utmost importance for the successful implementation of surveillance systems.

2. Essential prerequisites to enable a Member to provide information for the evaluation of its animal health status are:
  - a) that the particular Member complies with the provisions of Chapter 1.4.3. of the *Aquatic Code* on the quality and evaluation of the *Competent Authorities*,
  - b) that, where possible, surveillance data be complemented by other sources of information (e.g. scientific publications, research data, documented field observations and other non-survey data);

Annex XVI (contd)

- c) that transparency in the planning and execution of surveillance activities and the analysis and availability of data and information, be maintained at all times, in accordance with Chapter 1.2.1. of the *Aquatic Code*.
3. The following guidelines may be applied to all *diseases*, their agents, and susceptible species as listed in the *Aquatic Manual*, and are designed to assist with the development of surveillance methodologies. Where possible, the development of surveillance systems using these guidelines should be based on the relevant information in the individual *disease* chapters in the *Aquatic Manual*. These guidelines are also applicable to ~~other non OIE-listed diseases that are not included in the Aquatic Code but which~~ may be of importance to a country or region, such as new or emerging diseases. There is sometimes a perception that surveillance can only be conducted using sophisticated methodologies. However, an effective surveillance system can also be developed by making use of gross observations and already available resources.
4. It would be impractical to try to develop a surveillance system for all the known *aquatic animal* diseases for which a country has susceptible species. Therefore prioritising the diseases to be included in a surveillance system should be conducted considering:
- the needs to provide assurance of disease status for trade purposes
  - the resources of the country
  - the financial impact or threat posed by the different diseases
  - the importance of an industry-wide disease control programme within a country or region.
5. More detailed information in each ~~disease~~ chapter (where it exists) of the *Aquatic Manual* may be used to further refine the general approaches described in this chapter. Where detailed *disease* specific information is not available, surveillance can also be conducted following the guidelines in this chapter. Access to epidemiological expertise would be invaluable for the design, implementation of the system and interpretation of results derived from a surveillance system.

Article x.x.x.2.

**Principles of surveillance**

1. Surveillance may be based on many different data sources and can be classified in a number of ways, including:
- a) the means by which data are collected (targeted versus non-targeted);
  - b) the *disease* focus (pathogen-specific versus general surveillance); and
  - c) the way in which *units* for observation are selected (~~structured~~ surveys versus non-random data sources).
2. Surveillance activities include:
- a) ~~structured~~ population-based surveys, such as:
    - i) systematic sampling at slaughter;
    - ii) random surveys;

- b) ~~structured~~ non-random surveillance activities, such as:
- i) *disease* reporting or notifications;
  - ii) control programmes/health schemes;
  - iii) targeted testing/screening;
  - iv) ante-mortem and post-mortem inspections;
  - v) laboratory investigation records;
  - vi) biological specimen banks;
  - vii) sentinel *units*;
  - viii) field observations;
  - ix) farm production records.
3. In addition, surveillance data should be supported by related information, such as:
- a) data on the epidemiology of the *disease*, including environmental, and host and wild reservoir population distributions;
  - b) data on farmed and wild animal movements and trading patterns for *aquatic animals* and *aquatic animal products*, including potential for exposure to populations of wild aquatic animals ~~populations~~, water sources or other contacts;
  - c) national animal health regulations, including information on compliance with them and their effectiveness;
  - d) history of imports of potentially infected material; and
  - e) biosecurity measures in place.
4. The sources of evidence should be fully described. ~~In the case of a structured~~ A survey, ~~this~~ should include a description of the sampling strategy used for the selection of *units* for testing. For ~~structured~~ non-random data sources, a full description of the system is required including the source(s) of the data, when the data were collected, and a consideration of any *biases* that may be inherent in the system.

Article x.x.x.3.

### Critical elements of surveillance

In assessing the quality of a surveillance system, the following critical elements need to be addressed in conjunction with an evaluation of the *Competent Authority* (Chapter 1.4.3.).

#### 1. Populations

Ideally, surveillance should be carried out in such a way as to take into account all animal species susceptible to the *disease* in a country, *zone* or *compartment*. The surveillance activity may cover all individuals in the *population* or part of them. Estimates of total *population* at risk for each species are required. When surveillance is conducted only on a *subpopulation*, care should be taken regarding the inferences made from the results.

For OIE-listed diseases, ~~d~~Definitions of appropriate *populations* should be based on the specific recommendations of the *disease* chapters of the *Aquatic Manual*.

Annex XVI (contd)2. Epidemiological unit

The relevant *epidemiological unit* for the surveillance system should be defined and documented to ensure that it is representative of the *population* or targeted *subpopulations* that would generate the most useful inferences about *disease* patterns. Therefore, it should be chosen taking into account factors such as carriers, reservoirs, vectors, immune status, genetic resistance and age, sex, and other host criteria.

3. Clustering

*Disease* in a country, *zone* or *compartment* usually clusters rather than being uniformly or randomly distributed through a *population*. Clustering of *disease* may occur in space (e.g. tank, pond, farm, or *compartment*), time (e.g. season), or animal subgroups (e.g. age, physiological condition). Clustering should be taken into account in the design of surveillance activities and interpretation of surveillance data.

4. Case and outbreak definitions

Clear and unambiguous case and outbreak definitions should be developed and documented for each *disease* under surveillance, using, where they exist, the standards in this Appendix and the *Aquatic Manual*.

5. Analytical methodologies

Surveillance data should be analysed using appropriate methodologies, and at the appropriate organisational levels to facilitate effective decision making, whether it be planning interventions or demonstrating status.

Methodologies for the analysis of surveillance data should be flexible to deal with the complexity of real life situations. No single method is applicable in all cases. Different methodologies may be needed to accommodate the relevant pathogens, varying production and surveillance systems, and types, quality, and amounts of data ~~and~~ information available.

The methodology used should be based on the best available information that is in accord with current scientific thinking. The methodology should be in accordance with this Appendix and fully documented, and supported by reference to the scientific literature and other sources, including expert opinion. Sophisticated mathematical or statistical analyses should only be carried out when justified by the proper amount and quality of field data.

Consistency in the application of different methodologies should be encouraged and transparency is essential in order to ensure fairness and rationality, consistency in decision making and ease of understanding. The uncertainties, assumptions made, and the effect of these on the final conclusions should be documented.

## 6. Testing

Surveillance involves the detection of *disease* by the use of appropriate *case definitions* based on the results of one or more tests for evidence of *disease* status. In this context, a test may range from detailed laboratory examinations to field observations and the analysis of production records. The performance of a test at the *population* level (including field observations) may be described in terms of its *sensitivity* and *specificity* and predictive values. Imperfect *sensitivity* and/or *specificity* will have an impact on the conclusions from surveillance. Therefore, these parameters should be taken into account in the design of surveillance systems and analysis of surveillance data as described in this Appendix.

Although not determined for many *aquatic animal diseases*, *sensitivity* and *specificity* should be estimated as best as possible for a specific testing situation. Alternatively, where values for *sensitivity* and/or *specificity* for a particular test and testing situation are estimated in the disease chapter in the *Aquatic Manual*, these values may be used as a guide.

Samples from a number of *aquatic animals* or *units* may be pooled and subjected to a testing protocol. The results should be interpreted using *sensitivity* and *specificity* values that have been determined or estimated for that particular pool size and testing procedure.

## 7. Quality assurance

Surveillance systems should incorporate the principles of quality assurance and be subjected to periodic auditing to ensure that all components of the system function and provide verifiable documentation of procedures and basic checks to detect significant deviations of procedures from those documented in the design.

## 8. Validation

Results from animal health surveillance systems are subject to one or more potential *biases*. When assessing the results, care should be taken to identify potential *biases* that can inadvertently lead to an over-estimate or an under-estimate of the parameters of interest.

## 9. Data collection and management

The success of a surveillance system is dependent on a reliable process for data collection and management. The process may be based on paper records or computerised. Even where data are collected for non-survey purposes (e.g. during *disease* control interventions, inspections for movement control or during *disease* eradication schemes), the consistency and quality of data collection and event reporting in a format that facilitates analysis, is critical. Factors influencing the quality of collected data include:

- a) the distribution of, and communication between, those involved in generating and transferring data from the field to a centralised location;
- b) motivation of the people involved in the surveillance system;
- c) the ability of the data processing system to detect missing, inconsistent or inaccurate data, and to address these problems;
- d) maintenance of disaggregated data rather than the compilation of summary data;
- e) minimisation of transcription errors during data processing and communication.

## **Structured ~~p~~Population-based surveys**

In addition to the principles for surveillance discussed in article 6, the following guidelines should be used when planning, implementing and analysing surveys.

### 1. Types of surveys

Surveys may be conducted on the entire *target population* (i.e. a census) or on a sample. Periodic or repeated surveys conducted in order to document *disease* freedom should be done using probability based sampling methods (simple random selection, cluster sampling, stratified sampling, systematic sampling) so that data from the *study population* can be extrapolated to the *target population* in a statistically valid manner. Non-probability based sampling methods (convenience, expert choice, quota) can also be used. Recognising the inherent impracticalities in sampling from some *aquatic animal populations*, non-probability based sampling could be used when *biases* are recognised and used to optimise detection.

The sources of information should be fully described and should include a detailed description of the sampling strategy used for the selection of *units* for testing. Also, consideration should be made of any *biases* that may be inherent in the survey design.

### 2. Survey design

The *population* of epidemiological *units* should first be clearly defined; hereafter sampling *units* appropriate for each stage, depending on the design of the survey, should be defined.

The design of the survey will depend on the size and structure of the *population* being studied, the epidemiology of the *disease* and the resources available.

### 3. Sampling

The objective of sampling from a *population* is to select a subset of *units* from the *population* that is representative of the *population* with respect to the object of the study such as the presence or absence of *disease*. Sampling should be carried out in such a way as to provide the best likelihood that the sample will be representative of the *population*, within the practical constraints imposed by different environments and production systems. In order to detect the presence of a *disease* in a *population* of unknown *disease* status, ~~targeted~~ sampling methods that optimise the detection of *disease* can be used. In such cases, care should be taken regarding the inferences made from the results.

### 4. Sampling methods

When selecting *epidemiological units* from within a *population* the objectives of the surveillance system should be considered. In general, *probability sampling* (e.g. simple random selection) is preferable. When this is not possible, sampling should provide the best practical chance of generating optimal inferences about *disease* patterns in the *target population*.

In any case, the sampling method used at all stages should be fully documented and justified.

## 5. Sample size

In general, surveys are conducted either to demonstrate the presence or absence of a factor (e.g. *disease*) or to estimate a parameter (e.g. the prevalence of *disease*). The method used to calculate sample size for surveys depends on the purpose of the survey, the expected prevalence (also referred to as the threshold prevalence), the level of confidence desired of the survey results and the performance (e.g. sensitivity and specificity estimates) of the tests used.

Article x.x.x.5.

## ~~Structured~~ Non-random data sources used in surveillance

Surveillance systems routinely use ~~structured~~ non-random data, either alone or in combination with surveys.

### 1. Common non-random surveillance data sources

A wide variety of non-random surveillance data sources may be available. These vary in their primary purpose and the type of surveillance information they are able to provide. Some surveillance systems are primarily established as *early detection systems*, but may also provide valuable information to demonstrate freedom from *disease*. Other systems provide cross-sectional information suitable for prevalence estimation, either once or repeatedly, while yet others provide continuous information, suitable for the estimate of incidence data (e.g. *disease* reporting systems, sentinel sites, testing schemes).

#### a) Disease reporting or notification systems

Data derived from *disease* reporting systems can be used in combination with other data sources to substantiate claims of animal health status, to generate data for risk analysis, or for early detection. The first step of a *disease* reporting or notification system is often based on the observation of abnormalities (e.g. clinical signs, reduced growth, elevated mortality rates, behavioural changes, etc.), which can provide important information about the occurrence of endemic, exotic or new *diseases*. Effective laboratory support is however, an important component of most reporting systems. Reporting systems relying on laboratory confirmation of suspect clinical cases should use tests that have a high *specificity*. Reports should be released by the laboratory in a timely manner, with the amount of time from *disease* detection to report generation minimised.

#### b) Control programmes/health schemes

Animal *disease* control programmes or health schemes, while focusing on the control or eradication of specific *diseases*, should be planned and structured in such a manner as to generate data that are scientifically verifiable and contribute to ~~structured~~ surveillance.

#### c) Targeted ~~testing/screening~~ sampling

This may involve sampling testing targeted to selected sections of the *population* (*subpopulations*), in which *disease* is more likely to be introduced or found. Examples include selecting testing culled and dead animals for testing, animals exhibiting clinical signs, animals located in a defined geographical area and specific age or commodity group.

Annex XVI (contd)

## d) Post-harvest inspections

Inspections of *aquatic animal* slaughter premises or processing plants may provide valuable surveillance data provided diseased *aquatic animals* survive to slaughter. Post-harvest inspections are likely to provide good coverage only for particular age groups and geographical areas. Post-harvest surveillance data are subject to obvious *biases* in relation to *target* and *study populations* (e.g. only animals of a particular class and age may be slaughtered for human consumption in significant numbers). Such *biases* need to be recognised when analysing surveillance data.

Both for traceback in the event of detection of *disease* and for analysis of spatial and *population*-level coverage, there should be, if possible, an effective identification system that relates each animal in the slaughter premises/processing plant to its locality of origin.

## e) Laboratory investigation records

Analysis of laboratory investigation records may provide useful surveillance information. The coverage of the system will be increased if analysis is able to incorporate records from national, accredited, university and private sector laboratories. Valid analysis of data from different laboratories depends on the existence of standardised diagnostic procedures and standardised methods for interpretation and data recording. If available, the method listed in the *Aquatic Manual* in relation to the purpose of testing should be used. As with post-harvest inspections, there needs to be a mechanism to relate specimens to the farm of origin. It must be recognised that laboratory submissions may not accurately reflect the *disease* situation on the farm.

## f) Biological specimen banks

Specimen banks consist of stored specimens, gathered either through representative sampling or opportunistic collection or both. Specimen banks may contribute to retrospective studies, including providing support for claims of historical freedom from *disease*, and may allow certain studies to be conducted more quickly and at lower cost than alternative approaches.

g) Sentinel *units*

Sentinel *units*/sites involve the identification and regular testing of one or more of animals of known health/exposure status in a specified geographical location to detect the occurrence of *disease*. They are particularly useful for surveillance of *diseases* with a strong spatial component, such as vector-borne *diseases*. Sentinel *units* provide the opportunity to *target surveillance* depending on the likelihood of *disease* (related to vector habitats and host *population* distribution), cost and other practical constraints. Sentinel *units* may provide evidence of freedom from *disease*, or provide data on prevalence and incidence as well as the distribution of *disease*. Cohabitation of sentinel *units* (preferably of the most susceptible species and life stage) with a susceptible *population* should be considered for testing *disease* in *populations* of valuable animals, the lethal sampling of which may be unacceptable (e.g. ornamental fish) or in animal *subpopulations* where sampling techniques are incapable of detecting the presence of disease or infection (e.g. where vaccination means that serological tests are inapplicable).

## h) Field observations

Clinical observations of epidemiological *units* in the field are an important source of surveillance data. The *sensitivity* and/or *specificity* of field observations may be relatively low, but these can be more easily determined and controlled if a clear, unambiguous and easy to apply standardised *case definition* is applied. Education of potential field observers in application of the *case definition* and reporting is an important component. Ideally, both the number of positive observations and the total number of observations should be recorded.

i) Farm production records

Systematic analysis of farm production records may be used as an indicator of the presence or absence of *disease* at the *population* level. If production records are accurate and consistently maintained, the *sensitivity* of this approach may be quite high (depending on the *disease*), but the *specificity* is often quite low.

2. Critical elements for ~~structured~~ non-random data used in surveillance

There ~~is~~are a number of critical factors that should be taken into account when using ~~structured~~ non-random surveillance data such as coverage of the *population*, duplication of data, and *sensitivity* and *specificity* of tests that may give rise to difficulties in the interpretation of data. Surveillance data from non-random data sources may increase the level of confidence or be able to detect a lower level of prevalence with the same level of confidence compared to ~~structured~~ surveys.

3. Analytical methodologies

Different scientifically valid methodologies may be used for the analysis of non-random surveillance data. This most often requires information on parameters of importance to the surveillance system, such as *sensitivity* and *specificity* and prior probabilities of infection, i.e., apparent prevalences (e.g. for ~~negative~~ predictive value calculations). Where no such data are available, estimates based on expert opinions, gathered and combined using a formal, documented and scientifically valid methodology may be used.

4. Combination of multiple sources of data

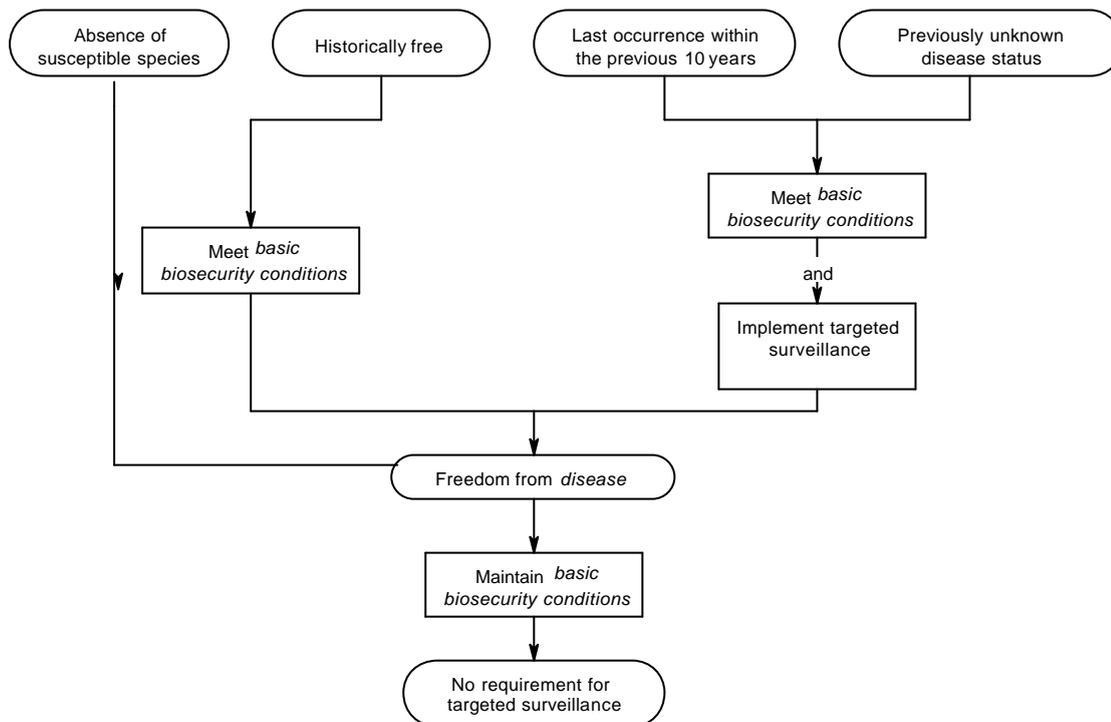
The methodology used to combine the evidence from multiple or recurrent (e.g. time series) data sources should be scientifically valid, and fully documented including references to published material.

Surveillance information gathered from the same country, *zone* or *compartment* at different times (e.g. repeated annual surveys) may provide cumulative evidence of animal health status. Such evidence gathered over time may be combined to provide an overall level of confidence. However, a single larger survey, or the combination of data collected during the same time period from multiple random or non-random sources, may be able to achieve the same level of confidence in a shorter period of time.

Analysis of surveillance information gathered intermittently or continuously over time should, where possible, incorporate the time of collection of the information to take into account the decreased value of older information. The *sensitivity*, *specificity* and completeness of data from each source should also be taken into account for the final overall confidence level estimation.

## Pathways to demonstrate freedom from disease

The different paths to declaration of freedom from *disease* are summarised in the diagram below.



### 1. Absence of susceptible species

Unless otherwise specified in the relevant *disease* chapter, a country, *zone* or *compartment* may be recognised as being free from *disease* without applying *targeted surveillance* if there are no *susceptible species* (as listed in the relevant chapter of this *Aquatic Manual*, or in the scientific literature) present in that country, *zone* or *compartment*.

### 2. Historically free

Unless otherwise specified in the relevant *disease* chapter, a country, *zone* or *compartment* may be declared free from *disease* without formally applying a pathogen-specific surveillance programme when:

- a) there has never been a substantiated occurrence of *disease* reported officially or in the scientific literature (peer reviewed), or
- b) *disease* has not occurred for at least 10 years, provided that the *disease* agents are likely to produce identifiable clinical signs in observable susceptible animals.

and for at least the past 10 years:

- c) the *basic biosecurity conditions* are in place and effectively enforced;

- d) no vaccination against the *disease* has been carried out unless otherwise allowed for in the *Aquatic Code*;
- e) *disease* is not known to be established in wild *aquatic animals* within the country or *zone* intended to be declared free. (A country or *zone* cannot apply for historical freedom if there is any evidence of *disease* in wild *aquatic animals*. However, specific surveillance in wild *aquatic animals* is not necessary.)

A country, *zone* or *compartment* that was *self-declared* free on the basis of the absence of susceptible species, but subsequently introduces any of the susceptible species as listed in the *Aquatic Manual*, may be considered historically free from the *disease* provided that:

- f) the country, *zone* or *compartment* of origin was declared free of the *disease* at the time of introduction;
- g) *basic biosecurity conditions* were introduced prior to the introduction;
- h) no vaccination against the *disease* has been carried out unless otherwise allowed for in the *disease* specific chapter of this *Aquatic Code*.

### 3. Last occurrence within the previous 10 years/previously unknown status

Countries, *zones* or *compartments* that have achieved eradication (or in which the *disease* has ceased to occur) within the previous 10 years or where the *disease* status is unknown, should follow the pathogen-specific surveillance requirements in the *Aquatic Manual* if they exist. In the absence of *disease* specific information to aid the development of a surveillance system, declaration of *disease* freedom should follow at least 2 surveys per year (for at least 2 consecutive years) to be conducted 3 or more months apart, on the appropriate species, at the appropriate life stage and at times of the year when temperature and season offer the best opportunity to detect the pathogen. Surveys should be designed to provide an overall 95% confidence or greater and with a design prevalence at the animal and higher levels of aggregation (i.e. pond, farm, village, etc.) levels being of 2% or lower (this value may be different for different *diseases* and may be provided in the specific *disease* chapter in the *Aquatic Manual*). Such surveys should not be based on voluntary submission and should be developed following the guidelines provided in the *Aquatic Manual*. Survey results will provide sufficient evidence of *disease* freedom provided that for at least the past 10 years these additional criteria are met:

- a) the *basic biosecurity conditions* are in place and effectively enforced;
- b) no vaccination against the *disease* has been carried out unless otherwise provided in the *Aquatic Code*;
- c) *disease* is not known to be established in wild *aquatic animals* within the country or *zone* intended to be declared free. (A country or *zone* cannot apply for freedom if there is any evidence of *disease* in wild *aquatic animals*. Specific surveillance in wild *aquatic animals* of susceptible species is necessary to confirm absence.)

Article x.x.x.7.

### **Maintenance of disease free status**

A country or *zone* that has been declared free from *disease* following the provisions of the *Aquatic Code* may discontinue pathogen-specific surveillance while maintaining the *disease* free status provided that:

Annex XVI (contd)

1. if present, the pathogen is likely to produce identifiable clinical signs in observable *susceptible species*;
2. the *basic biosecurity conditions* are in place and effectively enforced;
3. no vaccination against the *disease* has been carried out unless otherwise provided in the *Aquatic Code*;
4. where applicable, surveillance has previously demonstrated that *disease* is not present in populations of wild aquatic animal populations of susceptible species.

A special case can be made for a disease free compartment located in a country or zone that is not declared disease free, ~~proven to be free from disease~~ if surveillance should be ~~is~~ maintained at a level commensurate with the degree of risk and exposure to potential sources of *disease* is prevented.

Article x.x.x.8.

### **Design of surveillance programmes to demonstrate freedom from disease**

A surveillance programme to demonstrate freedom from *disease* should meet the following requirements in addition to the general requirements for surveillance outlined in this Appendix.

Freedom from *disease* implies the absence of the pathogenic agent in the country, *zone* or *compartment*. Scientific methods cannot provide absolute certainty of the absence of *disease*. Demonstrating freedom from *disease* involves providing sufficient evidence to demonstrate (to a level of confidence acceptable to Members) that *disease* with a specified pathogen is not present in a *population*. In practice, it is not possible to prove (i.e. be 100% confident) that a *population* is free from *disease*. Instead, the aim is to provide adequate evidence (to an acceptable level of confidence), that *disease*, if present, is present in less than a specified proportion of the *population* (i.e., threshold prevalence).

However, apparent *disease* at any level in the *target population* automatically invalidates any freedom from *disease* claim unless the positive test results are accepted as false positives based on *specificity* values described in the relevant *disease* chapter.

The provisions of this Article are based on the principles described above and the following premises:

- in the absence of *disease* and vaccination, the farmed and wild animal *populations* would become susceptible over a period of time;
- the *disease* agents to which these provisions apply are likely to produce identifiable clinical signs in observable susceptible animals;
- to increase the probability of detecting the specific disease agent, the susceptibility of the aquatic animal and the timing of sampling must be under appropriate conditions;
- the *Competent Authority* will be able to investigate, diagnose and report *disease*, if present;
- the appropriate diagnostic method as described in the *Aquatic Manual* be used
- any claim for the absence of *disease* over a long period of time in a susceptible *population* can be substantiated by effective *disease* investigation and reporting by a Member.

## 1. Objectives

The objective of this kind of surveillance system is to contribute on an on-going basis evidence to demonstrate freedom from disease in a particular country, *zone* or *compartment* with a known confidence and reference to a predetermined design prevalence and diagnostic test characteristics. The level of confidence and the design prevalence will depend on the testing situation, disease and host *population* characteristics and on the resources available.

A single such survey can contribute evidence adding to an on-going collection of health data (see also Section 5. Specific requirements for complex non-survey data sources). However, single surveys in isolation rarely, if ever, provide sufficient evidence that an *aquatic animal* disease is absent and must be augmented with on-going targeted evidence collection (e.g. ongoing disease sampling or passive detection capabilities) to substantiate claims of freedom from disease.

## 2. Population

The *population* of *epidemiological units* must be clearly defined. The *target population* consists of all individuals of all *susceptible species* to the disease in a country, *zone* or *compartment* to which the surveillance results apply. Sometimes components of the *target population* are at higher risk of being the point of introduction for an exotic disease. In these cases, it is advisable to focus surveillance efforts on this part of the *population*, such as farms on a geographical border.

The design of the survey will depend on the size and structure of the *population* being studied. If the *population* is relatively small and can be considered to be homogenous with regards to risk of infection, a single-stage survey can be used. If different *subpopulations* of the same *aquaculture* establishment do not share water, they may be considered as epidemiologically separate *populations*.

In larger *populations* where a sampling frame is not available, or when there is a likelihood of clustering of disease, multi-stage sampling is required. In two-stage sampling, at the first stage of sampling, groups of animals (e.g. ponds, farms or villages) are selected. At the second stage, animals are selected for testing from each of the selected groups.

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

## 3. Sources of evidence

Surveillance data may originate from a number of different sources, including:

- a) ~~structured~~, *population*-based surveys using one or more tests to detect the aetiological agent or evidence of infection;
- b) other ~~structured~~ non-random sources of data, such as:
  - i) sentinel sites;
  - ii) disease notifications and laboratory investigation records;
  - iii) academic and other scientific studies;
- c) a knowledge of the biology of the agent, including environmental, host *population* distribution, known geographical distribution, vector distribution and climatic information;

Annex XVI (contd)

- d) history of imports of potentially infected material;
- e) biosecurity measures in place;
- f) any other sources of information that provide contributory evidence regarding disease in the country, *zone* or *compartment*.

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

#### 4. Statistical methodology

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

- a) The survey design
- b) The *sensitivity* and *specificity* of the test, or test system
- c) The design prevalence (or prevalences where a multi-stage design is used)
- d) The results of the survey.

Analysis of data for evidence of freedom from infection involves estimating the probability (a) that the evidence observed (the results of surveillance) could have been produced under the null hypothesis that infection is present in the *population* at a specified prevalence(s) (the design prevalences). The confidence in (or, equivalently, the *sensitivity* of) the surveillance system that produced the evidence is equal to 1-a. If the confidence level exceeds a pre-set threshold, the evidence is deemed adequate to demonstrate freedom from infection.

The required level of confidence in the surveillance system (probability that the system would detect infection if infection were present at the specified level) must be greater than or equal to 95%.

The power (probability that the system would report that no infection is present if infection is truly not present) may be set to any value. By convention, this is often set to 80%, but may be adjusted according to the country's or *zone's* requirements.

Different statistical methodologies for the calculation of the probability a, including both quantitative and qualitative approaches, are acceptable as long as they are based on accepted scientific principles.

The methodology used to calculate the confidence in the surveillance system must be scientifically based and clearly documented, including references to published work describing the methodology.

Statistical analysis of surveillance data often requires assumptions about *population* parameters or test characteristics. These are usually based on expert opinion, previous studies on the same or different *populations*, expected biology of the agent, and so on. The uncertainty around these assumptions must be quantified and considered in the analysis (e.g. in the form of prior probability distributions in a Bayesian setting).

For surveillance systems used to demonstrate freedom from specific diseases, calculation of the confidence of a surveillance system is based on the null hypothesis that infection is present in the *population*. The level of infection is specified by the design prevalence. In the simplest case, this is the prevalence of infection in a homogenous *population*. More commonly, in the presence of a complex (e.g. multi-level) *population* structure more than one design prevalence value is required, for instance, the animal-level prevalence (proportion of infected animals in an infected farm) and the group-level prevalence (proportion of infected farms in the country, *zone* or *compartment*). Further levels of clustering may be considered, requiring further design prevalence values.

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

- At the individual animal level, the design prevalence is based on the biology of the infection in the *population*. It is equal to the minimum expected prevalence of infection in the *study population*, if the infection had become established in that *population*. It is dependent on the dynamics of infection in the *population* and the definition of the *study population* (which may be defined to maximise the expected prevalence in the presence of infection).
- A suitable design prevalence value at the animal level (e.g. prevalence of infected animals in a cage) may be:
  - between 1% and 5% for infections that are present in a small part of the *population* e.g. are transmitted slowly or are at the early stages of an outbreak, etc.;
  - over 5% for highly transmissible infections.

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

- At higher levels (e.g. cage, pond, farm, village, etc.) the design prevalence usually reflects the prevalence of infection that is practically and reasonably able to be detected by a *surveillance* system. Detection of infection at the lowest limit (a single infected *unit* in the *population*) is rarely feasible in large *populations*. The expected behaviour of the infection may also play a role. Infections that have the ability to spread rapidly between farms may have a higher farm-level design prevalence than slow-moving infections.

A suitable design prevalence value for the first level of clustering, (e.g. proportion of infected farms in a *zone*) ~~may be up to~~ is normally not greater than 2%. If a higher design prevalence is selected, it must be justified.

When surveillance data are used to estimate incidence and prevalence measures for the purpose of describing disease occurrence in terms of animal *unit*, time and place, these measures can be calculated for an entire *population* and specific time period, or for subsets defined by host characteristics (e.g. age-specific incidence). Incidence estimation requires on-going surveillance to detect new cases while prevalence is the estimated proportion of infected individuals in a *population* at a given time point. The estimation process must consider test *sensitivity* and *specificity*.

Annex XVI (contd)5. Clustering of infection

Infection in a country, *zone* or *compartment* usually clusters rather than being uniformly distributed through a *population*. Clustering may occur at a number of different levels (e.g. a cluster of moribund fish in a pond, a cluster of ponds in a farm, or a cluster of farms in a *zone*). Except when dealing with demonstrably homogenous *populations*, surveillance must take this clustering into account in the design and the statistical analysis of the data, at least at what is judged to be the most significant level of clustering for the particular animal *population* and infection.

6. Test characteristics

All surveillance involves performing one or more tests for evidence of the presence of current or past infection, ranging from detailed laboratory examinations to farmer observations. The performance level of a test at the *population* level is described in terms of its *sensitivity* and *specificity*. ~~These probabilities of the correct test result refer to the entire sampling process, including sample selection, collection, handling and processing (which if not conducted in the optimal way for the disease in question, as described in the disease chapters of the *Aquatic Manual*, will reduce the *sensitivity* of the method), and the actual laboratory test performance.~~ Imperfect *sensitivity* and/or *specificity* impact on the interpretation of surveillance results and must be taken into account in the analysis of surveillance data. For example, in the case of a test with imperfect *specificity*, if the *population* is free of disease or has a very low prevalence of infection, all or a large proportion of positive tests will be false. Subsequently, samples that test positive can be confirmed or refuted using a highly specific test. Where more than one test is used in a *surveillance* system (sometimes called using tests in series or parallel), the *sensitivity* and *specificity* of the test combination must be calculated.

All calculations must take the performance level (*sensitivity* and *specificity*) of any tests used into account. The values of *sensitivity* and *specificity* used for calculations must be specified, and the method used to determine or estimate these values must be documented. Test *sensitivity* and *specificity* can be different when applied to different *populations* and testing scenarios. For example, test *sensitivity* may be lower when testing carrier animals with low level infections compared to moribund animals with clinical disease. Alternatively, *specificity* depends on the presence of cross-reacting agents, the distribution of which may be different under different conditions or regions. Ideally, test performance should be assessed under the conditions of use otherwise increased uncertainty exists regarding their performance. In the absence of local assessment of tests, values for *sensitivity* and/or *specificity* for a particular test that are specified in the *Aquatic Manual* may be used but the increased uncertainty associated with these estimates should be incorporated into the analysis of results.

Pooled testing involves the pooling of specimens from multiple individuals and performing a single test on the pool. Pooled testing is an acceptable approach in many situations. Where pooled testing is used, the results of testing must be interpreted using *sensitivity* and *specificity* values that have been determined or estimated for that particular pooled testing procedure and for the applicable pool sizes being used. Analysis of the results of pooled testing must, where possible, be performed using accepted, statistically based methodologies, which must be fully documented, including published references.

When applied to a *surveillance* system, the probabilities of correct assessment of the health status of the *epidemiological unit* is affected by the entire sampling process, including sample selection, collection, handling and processing, as well as the actual laboratory test performance.

## 7. Multiple sources of information

Where multiple different data sources providing evidence of freedom from infection exist, each of these data sources may be analysed accordingly. The resulting estimates of the confidence in each data source may be combined to provide an overall level of confidence for the combined data sources.

The methodology used to combine the estimates from multiple data sources:

- a) must be scientifically valid, and fully documented, including references to published material; and
- b) should, where possible, take into account any lack of statistical independence between different data sources.

Surveillance information gathered from the same country, *zone* or *compartment* at different times (e.g. repeated annual surveys) may provide cumulative evidence of animal health status. Such evidence gathered over time may be combined to provide an overall level of confidence. However, a single larger survey, or the combination of data collected during the same time period from multiple random or non-random sources, may be able to achieve the same level of confidence in a shorter period of time.

Analysis of surveillance information gathered intermittently or continuously over time should, where possible, incorporate the time of collection of the information to take into account the decreased value of older information. The *sensitivity*, *specificity* and completeness of data from each source should also be taken into account for the final overall confidence level estimation.

## 8. Sampling

The objective of sampling from a *population* is to select a subset of *units* from the *population* that is representative of the *population* with respect to the characteristic of interest (in this case, the presence or absence of infection). The survey design may involve sampling at several levels. For sampling at the level of the *epidemiological units* or higher *units*, a formal *probability sampling* (e.g. simple random sampling) method must be used. Sampling should be carried out in such a way as to provide the best likelihood that the sample will be representative of the *population*, within the practical constraints imposed by different environments and production systems.

When sampling below the level of the *epidemiological unit* (e.g. individual animal), the sampling method used should provide the best practical chance of generating a sample that is representative of the *population* of the chosen *epidemiological unit*. Collecting a truly representative sample of individual animals (whether from a pond, cage or fishery) is often very difficult. To maximise the chance of finding infection, the aim should be to *bias* the sampling towards infected animals, e.g. selecting moribund animals, life stages with a greater chance of active infection, etc.

Biased or targeted sampling in this context involves sampling from a defined *study population* that has a different probability of infection than the *target population* of which it is a *subpopulation*. Once the *study population* has been identified, the objective is still to select a representative sample from this *subpopulation*.

The sampling method used at all levels must be fully documented and justified.

Annex XVI (contd)9. Sample size

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

- The *sensitivity* and *specificity* of the diagnostic test, or test system;
- The design prevalence (or prevalences where a multi-stage design is used);
- The level of confidence that is desired of the survey results.

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

- The size of the *population* (but it is acceptable to assume that the *population* is infinitely large);
- The desired power of the survey;
- Uncertainty about *sensitivity* and *specificity*.

The specific sampling requirements will need to be tailor-made for each individual disease, taking into account its characteristics and the *specificity* and *sensitivity* of the accepted testing methods for detecting the disease agent in host *populations*.

FreeCalc<sup>5</sup> is a suitable software for the calculation of sample sizes at varying parameter values. The table below provides examples of sample sizes generated by the software for a type I and type II error of 5% (i.e. 95% confidence and 95% statistical power). However, this does not mean that a type 1 and type 2 error of 0.05 should always be used. For example, using a test with *sensitivity* and *specificity* of 99%, 528 *units* should be sampled. If 9 or less of those *units* test positive, the *population* can still be considered free of the disease at a design prevalence of 2% provided that all effort is made to ensure that all presumed false positives are indeed false. This means that there is a 95% confidence that the prevalence is 2% or lower.

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

10. Quality assurance

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

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<sup>5</sup> FreeCalc – Cameron, AR. Software for the calculation of sample size and analysis of surveys to demonstrate freedom from disease. Available for free download from <http://www.ausvet.com.au>.

Annex XVI (contd)

Design prevalence	Sensitivity (%)	Specificity (%)	Sample size	Maximum number of false +ve if the population is free
2	100	100	149	0
2	100	99	524	9
2	100	95	1671	98
2	99	100	150	0
2	99	99	528	9
2	99	95	1707	100
2	95	100	157	0
2	95	99	542	9
2	95	95	1854	108
2	90	100	165	0
2	90	99	607	10
2	90	95	2059	119
2	80	100	186	0
2	80	99	750	12
2	80	95	2599	148
5	100	100	59	0
5	100	99	128	3
5	100	95	330	23
5	99	100	59	0
5	99	99	129	3
5	99	95	331	23
5	95	100	62	0
5	95	99	134	3
5	95	95	351	24
5	90	100	66	0
5	90	99	166	4
5	90	95	398	27
5	80	100	74	0
5	80	99	183	4
5	80	95	486	32
10	100	100	29	0
10	100	99	56	2
10	100	95	105	9
10	99	100	29	0
10	99	99	57	2
10	99	95	106	9
10	95	100	30	0
10	95	99	59	2
10	95	95	109	9
10	90	100	32	0
10	90	99	62	2
10	90	95	123	10
10	80	100	36	0
10	80	99	69	2
10	80	95	152	12

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Article x.x.x.9.

**Specific requirements for complex non-survey data sources for freedom from disease**

Data sources that provide evidence of freedom from infection, but are not based on ~~structured~~ *population*-based surveys may also be used to demonstrate freedom, either alone or in combination with other data sources. Different methodologies may be used for the analysis of such data sources, but the methodology must comply with the provisions of Section B.3. The approach used should, where possible, also take into account any lack of statistical independence between observations.

Analytical methodologies based on the use of step-wise probability estimates to describe the surveillance system may determine the probability of each step either by:

1. the analysis of available data, using a scientifically valid methodology; or where no data are available,
2. the use of estimates based on expert opinion, gathered and combined using a formal, documented and scientifically valid methodology.

Where there is significant uncertainty and/or variability in estimates used in the analysis, stochastic modelling or other equivalent techniques should be used to assess the impact of this uncertainty and/or variability on the final estimate of confidence.

Article x.x.x.10.

**Surveillance for distribution and occurrence of disease**

Surveillance to determine distribution and occurrence of *disease* or of other relevant health related events is widely used to assess the prevalence and incidence of selected *disease* as an aid to decision making, for example implementation of control and eradication programmes. It also has relevance for the international movement of animals and products when movement occurs among infected countries.

In contrast to surveillance to demonstrate freedom from *disease*, surveillance for the distribution and occurrence of *disease* is usually designed to collect data about a number of variables of animal health relevance, for example:

- prevalence or incidence of *disease* in wild or cultured animals;
- morbidity and mortality rates;
- frequency of *disease* risk factors and their quantification;
- frequency distribution of variables in *epidemiological units*
- frequency distribution of the number of days elapsing between suspicion of *disease* and laboratory confirmation of the diagnosis and/or to the adoption of control measures;
- farm production records, etc.

This section describes surveillance to estimate parameters of disease occurrence.

1. Objectives

The objective of this kind of surveillance system is to contribute on an on-going basis evidence to assess the occurrence and distribution of disease or infection in a particular country, *zone* or *compartment*. This will provide information for domestic disease control programmes and relevant disease occurrence information to be used by trading partners for qualitative and quantitative risk assessment.

A single such survey can contribute evidence adding to an on-going collection of health data (see also Section 5. Specific requirements for complex non-survey data sources).

## 2. Population

The *population of epidemiological units* must be clearly defined. The *target population* consists of all individuals of all species susceptible to the disease in a country, *zone* or *compartment* to which the surveillance results apply. Some local areas within a region may be known to be free of the disease of concern, allowing resources to be concentrated on known positive areas for greater precision of prevalence estimates and only verification of expected 0 prevalence areas.

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

In larger *populations* where a sampling frame is not available, or when there is a likelihood of clustering of disease, multi-stage sampling is required. ~~In two stage sampling, at the first stage of sampling, groups of animals (e.g. ponds, farms or villages) are selected. At the second stage, animals are selected for testing from each of the selected groups. For example, a multi-stage sampling process may involve sampling of farms or villages followed by sampling of fish from selected ponds within the sampled farms/villages.~~

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

## 3. Sources of evidence

Surveillance data may originate from a number of different sources, including:

- a) ~~structured~~, *population*-based surveys using one or more tests to detect the agent;
- b) other ~~structured~~ non-random sources of data, such as:
  - i) sentinel sites;
  - ii) disease notifications and laboratory investigation records;
  - iii) academic and other scientific studies;
- c) a knowledge of the biology of the agent, including environmental, host *population* distribution, known geographical distribution, vector distribution and climatic information;
- d) history of imports of potentially infected material;
- e) biosecurity measures in place;
- f) any other sources of information that provide contributory evidence regarding disease or infection in the country, *zone* or *compartment*.

The sources of evidence must be fully described. ~~In the case of a structured survey, this~~ must include a description of the sampling strategy used for the selection of *units* for testing. For complex surveillance systems, a full description of the system is required including consideration of any *biases* that may be inherent in the system. Evidence to support changes in prevalence/incidence of endemic disease must be based on valid, reliable methods to generate precise estimates with known error.

Annex XVI (contd)4. Statistical methodology

Analysis of survey data should be in accordance with the provisions of this chapter and should consider the following factors:

- a) The survey design;
- b) The *sensitivity* and *specificity* of the test, or test system;
- c) The results of the survey.

For surveillance systems used to describe disease patterns, the purpose is to estimate prevalence or incidence with confidence intervals or probability intervals. The magnitude of these intervals expresses the precision of the estimates and is related to sample size. Narrow intervals are desirable but will require larger sample sizes and more dedication of resources. The precision of the estimates and the power to detect differences in prevalence between *populations* or between time points depends not only on sample size, but also on the actual value of the prevalence in the *population* or the actual difference. For this reason, when designing the surveillance system, a prior estimate/assumption of expected prevalence or expected difference in prevalence must be made.

For the purpose of describing disease occurrence, measures of animal *unit*, time and place can be calculated for an entire *population* and specific time period, or for subsets defined by host characteristics (e.g. age-specific incidence). Incidence estimation requires on-going surveillance to detect new cases in a specified time period while prevalence is the estimated proportion of infected individuals in a *population* at a given time point. The estimation process must consider test *sensitivity* and *specificity*.

Statistical analysis of surveillance data often requires assumptions about *population* parameters or test characteristics. These are usually based on expert opinion, previous studies on the same or different *populations*, expected biology of the agent, information contained in the specific disease chapter of the *Aquatic Manual*, and so on. The uncertainty around these assumptions must be quantified and considered in the analysis (e.g. in the form of prior probability distributions in a Bayesian setting).

When surveillance objectives are to estimate prevalence/incidence or changes in disease patterns, statistical analysis must account for sampling error. Analytic methods should be thoroughly considered and consultation with biostatistician/quantitative epidemiologist consulted beginning in the planning stages and continued throughout the programme.

5. Clustering of infection

Infection in a country, *zone* or *compartment* usually clusters rather than being uniformly distributed through a *population*. Clustering may occur at a number of different levels (e.g. a cluster of moribund fish in a pond, a cluster of ponds in a farm, or a cluster of farms in a *zone*). Except when dealing with demonstrably homogenous *populations*, surveillance must take this clustering into account in the design and the statistical analysis of the data, at least at what is judged to be the most significant level of clustering for the particular animal *population* and infection. For endemic diseases, it is important to identify characteristics of the *population* which contribute to clustering and thus provide efficiency in disease investigation and control.

## 6. Test characteristics

All surveillance involves performing one or more tests for evidence of the presence of current or past infection, ranging from detailed laboratory examinations to farmer observations. The performance level of a test at the *population* level is described in terms of its *sensitivity* and *specificity*. Imperfect *sensitivity* and/or *specificity* impact on the interpretation of surveillance results and must be taken into account in the analysis of surveillance data. For example, in *populations* with low prevalence of infection, a large proportion of positive tests may be false unless the tests used have perfect *specificity*. To ensure detection in such instances, a highly sensitive test is frequently used for initial screening and then confirmed with highly specific tests.

All calculations must take the performance level (*sensitivity* and *specificity*) of any tests used into account. The values of *sensitivity* and *specificity* used for calculations must be specified, and the method used to determine or estimate these values must be documented. Test *sensitivity* and *specificity* can be different when applied to different *populations* and testing scenarios. For example, test *sensitivity* may be lower when testing carrier animals with low level infections compared to moribund animals with clinical disease. Alternatively, *specificity* depends on the presence of cross-reacting agents, the distribution of which may be different under different conditions or regions. Ideally, test performance should be assessed under the conditions of use otherwise increased uncertainty exists regarding their performance. In the absence of local assessment of tests, values for *sensitivity* and/or *specificity* for a particular test that are specified in the *Aquatic Manual* may be used but the increased uncertainty associated with these estimates should be incorporated into the analysis of results.

Pooled testing involves the pooling of specimens from multiple individuals and performing a single test on the pool. Pooled testing is an acceptable approach in many situations. Where pooled testing is used, the results of testing must be interpreted using *sensitivity* and *specificity* values that have been determined or estimated for that particular pooled testing procedure and for the applicable pool sizes being used. Analysis of the results of pooled testing must, where possible, be performed using accepted, statistically based methodologies, which must be fully documented, including published references.

Test results from surveillance for endemic disease will provide estimates of apparent prevalence (AP). Using diagnostic *sensitivity* (DSe) and diagnostic *specificity* (DSp) as described in chapter 1.1.2 of this *Aquatic Manual*, true prevalence (TP) should be calculated with the following formula:

$$TP = (AP + DSp - 1) / (DSe + DSp - 1)$$

In addition, it should be remembered that different laboratories may obtain conflicting results for various test, host, or procedure-related reasons. Therefore, *sensitivity* and *specificity* parameters should be validated for the particular laboratory and process.

## 7. Multiple sources of information

Where multiple different data sources providing information on infection or disease are generated, each of these data sources may be analysed and presented separately.

Surveillance information gathered from the same country, *zone* or *compartment* at different times and similar methodology (e.g. repeated annual surveys) may provide cumulative evidence of animal health status and changes. Such evidence gathered over time may be combined (e.g. using Bayesian methodology) to provide more precise estimates and details of disease distribution within a *population*.

Apparent changes in disease occurrence of endemic diseases may be real or due to other factors influencing detection proficiency.

Annex XVI (contd)8. Sampling

The objective of sampling from a *population* is to select a subset of *units* from the *population* that is representative of the *population* with respect to the characteristic of interest (in this case, the presence or absence of infection). The survey design may involve sampling at several levels. For sampling at the level of the *epidemiological units* or higher *units*, a formal *probability sampling* (e.g. simple random sampling) method must be used. Sampling should be carried out in such a way as to provide the best likelihood that the sample will be representative of the *population*, within the practical constraints imposed by different environments and production systems.

When sampling below the level of the *epidemiological unit* (e.g. individual animal), the method used should be probability-based sampling. Collecting a true probability-based sample is often very difficult and care should therefore be taken in the analysis and interpretation of results obtained using any other method, the danger being that inferences could not be made about the sampled *population*.

The sampling method used at all levels must be fully documented and justified.

9. Sample size

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

- The *sensitivity* and *specificity* of the diagnostic test (single or in combination);
- Expected prevalence or incidence in the *population* (or prevalences/incidences where a multi-stage design is used);
- The level of confidence that is desired of the survey results.
- The precision desired (i.e. the width of the confidence or probability intervals).

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

- The size of the *population* (but it is acceptable to assume that the *population* is infinitely large);
- Uncertainty about *sensitivity* and *specificity*.

The specific sampling requirements will need to be tailor-made for each individual disease, taking into account its characteristics and the *specificity* and *sensitivity* of the accepted testing methods for detecting the disease agent in host *populations*.

A number of software packages, e.g. Survey Tool Box ([www.aciar.gov.au](http://www.aciar.gov.au); [www.ausvet.com.au](http://www.ausvet.com.au)), WinPEPI ([www.sagebrushpress.com/pepibook.html](http://www.sagebrushpress.com/pepibook.html)) can be used for the calculation of sample sizes.

In the case in which the values of  $Se$  and  $Sp$  are not known (e.g. no information is available in the specific disease chapter in the *Aquatic Manual*), they should not automatically be assumed to be 100%. Assumed values should be produced in consultation with subject-matter experts.

10. Quality assurance

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

Article x.x.x.11.

### Examples of surveillance programmes

The following examples describe surveillance systems and approaches to the analysis of evidence for demonstrating freedom from *disease*. The purpose of these examples is:

- to illustrate the range of approaches that may be acceptable;
- to provide practical guidance and models that may be used for the design of specific surveillance systems; and
- to provide references to available resources that are useful in the development and analysis of surveillance systems.

While these examples demonstrate ways in which freedom from *disease* may be successfully demonstrated, they are not intended to be prescriptive. Countries are free to use different approaches, as long as they meet the requirements of this chapter.

The examples deal with the use of ~~structured~~ surveys and are designed to illustrate different survey designs, sampling schemes, the calculation of sample size, and analysis of results. It is important to note that alternative approaches to demonstrating freedom using complex non-survey-based data sources are also currently being developed and may soon be published<sup>6</sup>.

#### 1. Example 1. – one-stage ~~structured~~ survey (farm certification )

##### a) Context

A freshwater aquaculture industry raising fish in tanks has established a farm certification scheme. This involves demonstrating farm-level freedom from a particular (hypothetical) disease (Disease X). The disease does not spread very quickly, and is most common during the winter months, with adult fish at the end of the production cycle being most severely affected. Farms consist of a number of grow-out tanks, ranging from 2 to 20, and each tank holds between 1000 and 5000 fish.

##### b) Objective

The objective is to implement surveillance that is capable of providing evidence that an individual farm is free from Disease X. (The issue of national or *zone* freedom, as opposed to farm freedom, is considered in the next example.)

##### c) Approach

The accreditation scheme establishes a set of standard operating procedures and requirements for declaration of freedom, based on the guidelines given in this chapter. These require farms to undertake a ~~structured~~ survey capable of producing 95% confidence that the disease would be detected if it were present. Once farms have been surveyed without detecting disease, they are recognised as free, as long as they maintain a set of minimum biosecurity standards. These standards are designed to prevent the introduction of Disease X into the farm (through the implementation of controls specific to the method of spread of that disease) and to ensure that the disease would be detected rapidly if it were to enter the farm (based on evidence of adequate health record keeping and the prompt investigation of unusual disease events). The effective implementation of these biosecurity measures is evaluated with annual on-farm audits conducted by independent auditors.

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<sup>6</sup> International EpiLab, Denmark, Research Theme 1: Freedom from disease.  
[http://www.vetinst.dk/high\\_uk.asp?page\\_id=196](http://www.vetinst.dk/high_uk.asp?page_id=196)

Annex XVI (contd)

## d) Survey standards

Based on the guidelines given in this chapter, a set of standards are established for the conduct of surveys to demonstrate freedom from infection with causative agent of Disease X. These standards include:

- i) The level of confidence required of the survey is 95% (i.e. Type I error = 5%).
- ii) The power of the survey is arbitrarily set at 95% (i.e. Type II error = 5%, which means that there is a 5% chance of concluding that a non-diseased farm is infected).
- iii) The *target population* is all the fish on the farm. Due to the patterns of disease in this production system, in which only fish in the final stages of grow-out, and only in winter are affected, the *study population* is defined as grow-out fish during the winter months.
- iv) The issue of clustering is considered. As fish are grouped into tanks, this is the logical level at which to consider clustering. However, when a farm is infected, the disease often occurs in multiple tanks, so there is little evidence of strong clustering. Also, the small number of tanks on a single farm means that it is difficult to define a design prevalence at the tank level (i.e. the proportion of infected tanks that the survey should be able to detect on the farm). For these reasons, it is decided to treat the entire grow-out *population* of each farm as a single homogenous *population*.
- v) Stratification is also considered. In order to ensure full representation, it is decided to stratify the sample size by tank, proportional to the *population* of each tank.
- vi) The design prevalence at the animal level is determined based on the epidemiology of the disease. The disease does not spread quickly, however, in the defined *target population*, it has been reported to affect at least 10% of fish, if the *population* is infected. In order to take the most conservative approach, an arbitrarily low design prevalence of 2% is used. A prevalence of 10% may have been used (and would result in a much smaller sample size), but the authorities were not convinced by the thought that the *population* could still be infected at a level of say 5%, and disease still not be detected.
- vii) The test used involves destructive sampling of the fish, and is based on an antigen-detection enzyme-linked immunosorbent assay (ELISA). Disease X is present in some parts of the country (hence the need for a farm-level accreditation programme). This has provided the opportunity for the *sensitivity* and the *specificity* of the ELISA to be evaluated in similar *populations* to those on farms. A recent study (using a combination of histology and culture as a gold standard) estimated the *sensitivity* of the ELISA to be 98% (95% confidence interval 96.7–99.2%), and the *specificity* to be 99.4% (99.2–99.6%). Due to the relatively narrow confidence intervals, it was decided to use the point estimates of the *sensitivity* and *specificity* rather than complicate calculations by taking the uncertainty in those estimates into account.

## e) Sample size

The sample size required to meet the objectives of the survey is calculated to take the *population* size, the test performance, the confidence required and the design prevalence into account. As the *population* of each farm is relatively large, differences in the total *population* of each farm have little effect on the calculated sample size. The other parameters for sample size calculation are fixed across all farms. Therefore, a standard sample size (based on the use of this particular ELISA, in this *population*) is calculated. The sample size calculations are performed using the *FreeCalc* software<sup>7</sup>. Based on the parameters listed above, the sample size required is calculated to be 410 fish per farm. In addition, the program calculates that, given the imperfect *specificity*, it is still possible for the test to produce up to five false-positive reactors from an uninfected *population* using this sample size. The authorities are not comfortable with dealing with false-positive reactors, so it is decided to change the test system to include a confirmatory test for any positive reactors. Culture is selected as the most appropriate test, as it has a *specificity* that is considered to be 100%. However, its *sensitivity* is only 90% due to the difficulty of growing the organism.

As two tests are now being used, the performance of the test system must be calculated, and the sample size recalculated based on the test system performance.

Using this combination of tests (in which a sample is considered positive only if it tests positive to both tests), the *specificity* of the combined two tests can be calculated by the formula:

$$Sp_{Combined} = Sp_1 + Sp_2 - (Sp_1 \times Sp_2)$$

which produces a combined *specificity* of  $1 + 0.994 - (1 \times 0.994) = 100\%$

The *sensitivity* may be calculated by the formula:

$$Se_{Combined} = Se_1 \times Se_2$$

which produces a combined *sensitivity* of  $0.9 \times 0.98 = 88.2\%$

These new values are used to calculate the survey sample size yielding a result of 169 fish. It is worth noting that attempts to improve the performance of a test (in this case increase *specificity*) generally result in a decrease in the performance of the other aspect of the test performance (*sensitivity* in this example). However, in this case, the loss of *sensitivity* is more than compensated for by the decreased sample size due to the improved *specificity*.

It is also worth noting that, when using a test system with 100% *specificity*, the effective power of the survey will always be 100%, regardless of the figure used in the design. This is because it is not possible to make a Type II error, and conclude that the farm is infected when it is not.

A check of the impact of *population* size on the calculated sample size is worthwhile. The calculated sample size is based on an infinitely large *population*. If the *population* size is smaller, the impact on sample size is shown in the following table:

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<sup>7</sup> FreeCalc – Cameron, AR. Software for the calculation of sample size and analysis of surveys to demonstrate freedom from disease. Available for free download from <http://www.ausvet.com.au>.

Annex XVI (contd)

Population size	Sample size
1000	157
2000	163
5000	166
10,000	169

Based on these calculations, it is clear that, for the *population* sizes under consideration, there is little effect on the sample size. For the sake of simplicity, a standard sample size of 169 is used, regardless of the number of grow-out fish on the farm.

## f) Sampling

The selection of individual fish to include in the sample should be done in such a manner as to give the best chance of the sample being representative of the *study population*. A fuller description of how this may be achieved under different circumstances is provided in *Survey Toolbox*<sup>8</sup>. An example of a single farm will be used to illustrate some of the issues.

One farm has a total of eight tanks, four of which are used for grow-out. At the time of the survey (during winter), the four grow-out tanks have 1850, 4250, 4270 and 4880 fish, respectively, giving a total *population* of 15,250 grow-out fish.

Simple random sampling from this entire *population* is likely to produce sample sizes from each tank roughly in proportion to the number of fish in each tank. However, proportional stratified sampling will guarantee that each tank is represented in proportion. This simply involves dividing the sample size between tanks in proportion to their *population*. The first tank has 1850 fish out of a total of 15,250, representing 12.13%. Therefore 12.13% of the sample (21 fish) should be taken from the first tank. Using a similar approach the sample size for the other three tanks is 47, 47 and 54 fish, respectively.

Once the sample for each tank is determined, the problem remains as to how to select 21 fish from a tank of 1850 so that they are representative of the *population*. Several options exist.

- i) If the fish can be handled individually, random systematic sampling may be used. ~~This is likely to be the case if, for example:~~ For example, samples can be collected at harvest or during routine management activities involving handling the fish (such as grading or vaccination).

- ~~fish are harvested during winter and samples can be collected at harvest; or~~
- ~~routine management activities involving handling the fish (such as grading or vaccination) are conducted during the winter.~~

If fish are handled, systematic sampling simply involves selecting a fish at regular intervals. For instance, to select 21 from 1850, the sampling interval should be  $1850/21 = 88$ . This means that every 88<sup>th</sup> fish from the tank should be sampled. To ensure randomness, it is good practice to use a random number between 1 and 88 (in this case) to select the first fish (e.g. using a random number table), and then select every 88<sup>th</sup> fish after that.

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<sup>8</sup> Survey Toolbox for Aquatic Animal Diseases – A Practical Manual and Software Package. Cameron A.R. (2002). Australian Centre for International Agricultural Research (ACIAR), Monograph No. 94, 375 pp. ISBN 1 86320 350 8. Printed version available from ACIAR <http://www.aciar.gov.au> Electronic version available for free download from <http://www.ausvet.com.au>.

- ii) If fish cannot be handled individually (by far the most common, and more difficult, circumstance) then the fish to be sampled must be captured from the tanks. Fish should be captured in the most efficient and practical way possible, however every effort should be made to try to ensure that the sample is representative. In this example, a dip net is the normal method used for capturing fish. Using a dip net, convenience sampling would involve capturing 21 fish by repeatedly dipping at one spot and capturing the easiest fish (perhaps the smaller ones). This approach is strongly discouraged. One method of increasing the representativeness is to sample at different locations in the tank – some at one end, some at either side, some at the other end, some in the middle, some close to the edge. Additionally, if there are differences among the fish, an attempt should be made to capture fish in such a way as to give different groups of fish a chance of being caught (i.e. do not just try to catch the small ones, but include big ones as well).

This method of collecting a sample is far from the ideal of random sampling, but due to the practical difficulties of implementing random sampling of individual fish, this approach is acceptable, as long as the efforts made to increase the representativeness of the sample are both genuine and fully documented.

g) Testing

Specimens are collected, processed and tested according to standardised procedures developed under the certification programme and designed to meet the requirements of the *Aquatic Manual*. The testing protocol dictates that any specimens that test positive to ELISA be submitted for culture, and that any positive culture results indicate a true positive specimen (i.e. that the farm is not free from disease). It is important that this protocol be adhered to exactly. If a positive culture is found, then it is not acceptable to retest it, unless further testing is specified in the original testing protocol, and the impact of such testing accounted for in the test system *sensitivity* and *specificity* estimates (and therefore the sample size).

h) Analysis

If the calculated sample size of 169 is used, and no positive reactors are found, then the survey will have a confidence of 95%. This can be confirmed by analysing the results using the *FreeCalc* software mentioned above (which reports a confidence level of 95.06%).

It may happen in some cases that the survey is not conducted exactly as planned, and the actual sample size is less than the target sample size. However, the size of the farm may also be smaller. In these cases, it is advisable to analyse the farm data on a farm-by-farm basis. For example, if only 165 specimens were collected from a farm with only 2520 fish, the resulting confidence would still be 95%. If only 160 fish were collected, the confidence is only 94.5%. If a rigid target of 95% confidence is used, then this survey would fail to meet that target and more evidence would be required.

2. Example 2 – two-stage structured survey (national freedom)

a) Context

A country aims to declare freedom from Disease Y of crustaceans. The industry in this country is based largely on small-holder ponds, grouped closely together in and around villages. The disease is reasonably highly contagious, and causes mass mortality mid to late in the production cycle, with affected animals becoming moribund and dying in a matter of days. Affected animals

Annex XVI (contd)

how few characteristic signs, but an infected pond will almost invariably break down with mass mortality unless harvested beforehand. It is more common in late summer, but can occur at any time of year. It also occurs occasionally early in the production cycle. In this country, there are some limitations to the availability of laboratory facilities and the transport infrastructure. However, there is a relatively large government structure, and a comprehensive network of fisheries officers.

## b) Objective

The objective is to establish national freedom from Disease Y. The surveillance system must meet the requirements of this chapter, but must also be able to be practically implemented in this small-holder production system.

## c) Approach

The aquaculture authorities decide to use a survey to gather evidence of freedom, using a two-stage survey design (sampling villages at the first level, and ponds at the second). Laboratory testing of specimens from a large number of farms is not considered feasible, so a combined test system is developed to minimise the need for expensive laboratory tests.

The *unit* of observation and analysis is, in this case, the pond, rather than the individual animal. This means that the diagnosis is being made at the pond level (an infected pond or a non-infected pond) rather than at the animal level.

The survey is therefore a survey to demonstrate that no villages are infected (using a random sample of villages and making a village-level diagnosis). The test used to make a village-level diagnosis is, in fact, another survey, this time to demonstrate that no ponds in the village are affected. A test is then performed at the pond level (farmer observation followed, if necessary, by further laboratory testing).

## d) Survey standards

- i) The confidence to be achieved by the survey is 95%. The power is set at 95% (but is likely to be virtually 100% if the test system used achieves nearly 100% *specificity*, as demonstrated in the previous example).
- ii) The *target population* is all ponds stocked with shrimp in the country during the study period. The *study population* is the same, except that those remote areas to which access is not possible are excluded. As outbreaks can occur at any time of year, and at any stage of the production cycle, it is decided not to further refine the definition of the *population* to target a particular time or age.
- iii) Three tests are used. The first is farmer observation, to determine if mass mortality is occurring in a particular pond. If a pond is positive to the first test (i.e. mass mortality is detected), a second test is applied. The second test used is polymerase chain reaction (PCR). Cases positive to PCR are further tested using transmission experiments.
- iv) Farmer observation can be treated as a test just like any other. In this case, the observation of mass mortality is being used as a test for the presence of Disease Y. As there are a variety of other diseases that are capable of causing mass mortality, the test is not very specific. On the other hand, it is quite unusual for Disease Y to be present, and not result in mass mortality, so the test is quite sensitive. A standard *case definition* is established for

'mass mortality' (for instance, greater than 20% of the pond's *population* of shrimp observed dead in the space of less than 1 week). Based on this definition, farmers are able to 'diagnose' each pond as having mass mortality. Some farmers may be over-sensitive and decide that mass mortality is occurring when only a small proportion of shrimp are found dead (false positives, leading to a decrease in *specificity*) while a small number of others fail to recognise the mortalities, decreasing *sensitivity*.

In order to quantify the *sensitivity* and *specificity* of farmer observation of mass mortalities, as a test for Disease Y, a separate study is carried out. This involves both a retrospective study of the number of mass mortality events in a *population* that is thought to be free from disease, as well as a study of farmers presented with a series of mortality scenarios, to assess their ability to accurately identify a pond with mass mortality. By combining these results, it is estimated that the *sensitivity* of farmer-reported mass mortalities as a test for Disease Y is 87% while the *specificity* is 68%.

- v) When a farmer detects a pond with mass mortality, specimens are collected from moribund shrimp following a prescribed protocol. Tissue samples from 20 shrimp are collected, and pooled for PCR testing. In the laboratory, the ability of pooled PCR to identify a single infected animal in a pool of 20 has been studied, and the *sensitivity* of the procedure is 98.6%. A similar study of negative specimens has shown that positive results have occasionally occurred, probably due to laboratory contamination, but maybe also because of the presence of non-viable genetic material from another source (shrimp-based feed stuffs are suspected). The *specificity* is therefore estimated at 99%.
- vi) Published studies in other countries have shown that the *sensitivity* of transmission tests, the third type of test to be used, is 95%, partly due to variability in the load of the agent in inoculated material. The *specificity* is agreed to be 100%.
- vii) Based on these figures, the combined test system *sensitivity* and *specificity* are calculated using the formulae presented in Example 1, first with the first two tests, and then with the combined effect of the first two tests and the third test. The result is a *sensitivity* of 81.5% and a *specificity* of 100%.
- viii) The design prevalence must be calculated at two levels. First, the pond-level design prevalence (the proportion of ponds in a village that would be infected if disease were present) is determined. In neighbouring infected countries, experience has shown that ponds in close contact with each other are quickly infected. It is unusual to observe an infected village with fewer than 20% of ponds infected. Conservatively, a design prevalence of 5% is used. The second value for design prevalence applies at the village level, or the proportion of infected villages that could be identified by the survey. As it is conceivable that the infection may persist in a local area without rapid spread to other parts of the country, a value of 1% is used. This is considered to be the lowest design prevalence value for which a survey can be practically designed.
- ix) The population of villages in the country is 65,302, according to official government records. Those with shrimp ponds number 12,890, based on records maintained by the aquaculture authorities. These are generated through a five-yearly agricultural census, and updated annually based on reports of fisheries officers. There are no records available of the number of ponds in each of these villages.

Annex XVI (contd)

## e) Sample size

Sample size is calculated for the two levels of sampling, first the number of villages to be sampled and then the number of ponds to be sampled. The number of villages to be sampled depends on the *sensitivity* and the *specificity* of the test used to classify villages as infected or not infected. As the 'test' used in each village is really just another survey, the *sensitivity* is equal to the confidence and the *specificity* is equal to the power of the village-level survey. It is possible to adjust both confidence and power by changing the sample size in the village survey (number of ponds examined), which means that we can determine, within certain limits, what *sensitivity* and *specificity* we achieve.

This allows a flexible approach to sample size calculation. If a smaller first-stage sample size is desired (a small number of villages), a high *sensitivity* and *specificity* are needed, which means that the number of ponds in each village that need to be examined is larger. A smaller number of ponds will result in lower *sensitivity* and *specificity*, requiring a larger number of villages. The approach to determining the optimal (least cost) combination of first- and second-stage sample sizes is described in *Survey Toolbox*.

A further complication is presented by the fact that each village has a different number of ponds. In order to achieve the same (or similar) confidence and power (*sensitivity* and *specificity*) for each village, a different sample size may be required. The authorities choose to produce a table of sample sizes for the number of ponds to sample in each village, based on the total ponds in each village.

An example of one possible approach to determining the sample size follows:

The target *sensitivity* (confidence) achieved by each village-level survey is 95%. The target *specificity* is 100%. Using the *FreeCalc* software, with a design prevalence of 1% (the survey is able to detect disease if 1% or more villages are infected), the first-stage sample size is calculated as 314 villages. Within each village, the test used is the combined test system described above with a *sensitivity* of 81.5% and a *specificity* of 100%. Based on these figures the following table is developed, listing the number of ponds that need to be sampled in order to achieve 95% *sensitivity*.

## f) Sampling

First-stage sampling (selection of villages) is done using random numbers and a sampling frame based on the fisheries authorities list of villages with shrimp ponds. The villages are listed on a spreadsheet with each village numbered from 1 to 12,890. A random number table (such as that included in *Survey Toolbox*) or software designed for the generation of random numbers (such as EpiCalc<sup>9</sup>) is used.

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<sup>9</sup> <http://www.myatt.demon.co.uk/epicalc.htm>

Population	Sample size
30	29
40	39
60	47
80	52
100	55
120	57
140	59
160	61
180	62
200	63
220	64
240	64
260	65
280	65
300	66
320	66
340	67
360	67
380	67
400	67
420	68
440	68
460	68
480	68
500	68
1000	70

The second stage of sampling involves random selection of ponds within each village. This requires a sampling frame, or list of each pond in the village. The fisheries authorities use trained local fisheries officers to coordinate the survey. For each selected village, the officer visits the village and convenes a meeting of all shrimp farmers. At the meeting, they are asked how many ponds they have and a list of farmers' names and the number of ponds is compiled. A simple random sample of the appropriate number of ponds (between 29 and 70, from the table above, depending on the number of ponds in the village) is selected from this list. This is done either using software (such as *Survey Toolbox's Random Animal program*), or manually with a random number table or decimal dice for random number selection. Details of this process are described in *Survey Toolbox*. This selection process identifies a particular pond in terms of the name of the owner, and the sequence number amongst the ponds owned (e.g. Mr Smith's 3<sup>rd</sup> pond). Identification of the actual pond is based on the owners own numbering system for the ponds.

#### g) Testing

Once ponds have been identified, the actual survey consists of 'testing those ponds'. In practice, this involves the farmers observing the ponds during one complete production cycle. The local fisheries officer makes weekly visits to each farmer to check if any of the selected ponds have suffered mass mortality. If any are observed (i.e. the first test is positive), 20 moribund shrimp are collected for laboratory examination (first PCR, and then, if positive, transmission experiments).

Annex XVI (contd)

## h) Analysis

Analysis is performed in two stages. First, the results from each village are analysed to ensure that they meet the required level of confidence. If the target sample size is achieved (and only negative results obtained), the confidence should be 95% or greater in each village. At the second stage, the results from each village are analysed to provide a country level of confidence. Again, if the target sample size (number of villages) is achieved, this should exceed 95%.

3. Example 3. – spatial sampling and the use of tests with imperfect specificity

## a) Context

A country has an oyster culture industry, based primarily on rack culture of oysters in 23 estuaries distributed along the coastline. In similar regions in other countries, Disease Z causes mortalities in late summer/early autumn. During an outbreak a high proportion of oysters are affected, however, it is suspected that the agent may be present at relatively low prevalence in the absence of disease outbreaks.

## b) Objective

The national authorities wish to demonstrate national freedom from Disease Z. If the disease should be detected, a secondary objective of the survey is to collect adequate evidence to support zoning at the estuary level.

## c) Approach

The authorities conclude that clinical surveillance for disease outbreaks is inadequate because of the possibility of low level subclinical infections. It is therefore decided to base surveillance on a ~~structured~~ two-stage survey, in which sampled oysters are subjected to laboratory testing. The first stage of the survey is the selection of estuaries. However, due to the objective of providing evidence for zoning (should disease be found in any of the estuaries), it is decided to use a census approach and sample every estuary. In essence this means that there will be 23 separate surveys, one for each estuary. A range of options for sampling oysters are considered, including sampling at harvest or marketing, or using farms (oyster leases) as a level of sampling or stratification. However the peak time of activity of the agent does not correspond to the harvest period, and the use of farms would exclude the significant numbers of wild oysters present in the estuaries. It is therefore decided to attempt to simulate simple random sampling from the entire oyster *population* in the estuary, using a spatial sampling approach.

## d) Survey standards

- i) The *target population* is all of the oysters in each of the estuaries. The *study population* is the oysters present during the peak disease-risk period in late summer early autumn. Wild and cultured oysters are both susceptible to disease, and may have associated with them different (but unknown) risks of infection. They are therefore both included in the *study population*. As will be described below, sampling is based on mapping. Therefore the *study population* can more accurately be described as that *population* falling within those mapped areas identified as oyster habitats.
- ii) A design prevalence value is only required at the oyster level (as a census is being used at the estuary level). While the disease is often recognised with very high prevalence during outbreaks, a low value is used to account for the possibility of persistence of the agent in the absence of clinical signs. A value of 2% is selected.

- iii) The test used is histopathology with immuno-staining techniques. This test is known to produce occasional false-positive results due to nonspecific staining, but is very sensitive. Published studies indicate values of 99.1% for *sensitivity* and 98.2% for *specificity*. No other practical tests are available. This means that it is not possible to definitively differentiate false positives from true positives, and that in a survey of any size, a few false positives are expected (i.e. 1.8%).
- iv) The confidence is set at 95% and the power at 80%. In the previous examples, due to the assumed 100% *specificity* achieved by use of multiple tests, the effective power was 100%. In this case, with imperfect *specificity*, there will be a risk of falsely concluding that a healthy estuary is infected, so the power is not 100%. The choice of a relatively low figure (80%) means that there is a 1 in 5 chance of falsely calling an estuary infected when it is not infected, but it also dramatically decreases the survey costs, through a lower sample size.

e) Sample size

Based on the assumption that the sampling procedure will mimic simple random sampling, the sample size (number of oysters to sample per estuary) can be calculated with *FreeCalc*. The *population* size (number of oysters per estuary) is assumed to be very large. The calculated sample size, using the *sensitivity*, *specificity* and design prevalence figures given above, is 450. *FreeCalc* also reports that, based on this sample size and the *specificity* of the test, it is possible to get 10 or fewer false-positive test results, and still conclude that the *population* is free from disease. This is because, if the *population* were infected at 2% or greater, the anticipated number of positive reactors from a sample of 450 would be greater than 10. In fact, we would expect 9 true positives ( $450 \times 2\% \times 99.1\%$ ) and 8 false positives ( $450 \times 98\% \times 1.8\%$ ) or a total of 17 positives if the *population* were infected at a prevalence of 2%.

This illustrates how probability theory and adequate sample size can help differentiate between true- and false-positive results when there is no alternative but to use a test with imperfect *specificity*.

f) Sampling

The aim is to collect a sample of 450 oysters that represent an entire estuary. Simple random sampling depends on creating a sampling frame listing every oyster (not possible) and systematic sampling depends on being able to (at least conceptually) line up all the oysters (again, not possible). The authorities decide to use spatial sampling to approximate simple random sampling. Spatial sampling involves selecting random points (defined by coordinates), and then selecting oysters near the selected points. In order to avoid selecting many points with no oysters nearby, the estuary is first mapped (the fisheries authorities already have digital maps defining oyster leases available). To these maps areas with significant concentrations of wild oysters are also added, based on local expertise. Pairs of random numbers are generated such that the defined point falls within the defined oyster areas. Other schemes are considered (including using a rope marked at regular intervals, laid out on a lease to define a transect, and collecting an oyster adjacent to each mark on the rope) but the random coordinate approach is adopted.

Annex XVI (contd)

Survey teams then visit each point by boat (using a GPS Global Positioning System unit to pinpoint the location). A range of approaches is available for selecting which oyster to select from a densely populated area, but it should involve some effort at randomness. Survey staff opt for a simple approach: when the GPS receiver indicates that the site has been reached, a pebble is tossed in the air and the oyster closest to the point where it lands is selected. Where oysters are arranged vertically (e.g. wild oysters growing up a post), a systematic approach is used to determine the depth of the oyster to select. First, an oyster at the surface, next, an oyster halfway down, and thirdly, an oyster as deep as can be reached from the boat.

This approach runs the risk of *bias* towards lightly populated areas, so an estimate of the relative density of oysters at each sampling point is used to weight the results (see *Survey Toolbox* for more details).

## g) Testing

Specimens are collected, processed, and analysed following a standardised procedure. The results are classified as definitively positive (showing strong staining in a highly characteristic pattern, possibly with associated signs of tissue damage), probably positive (on the balance of probabilities, but less characteristic staining), and negative.

## h) Analysis

The interpretation of the results when using a test with imperfect *specificity* is based on the assumption that, in order to conclude that the *population* is free from infection, any positive result identified is really a false positive. With a sample size of 450, up to 10 false positives may be expected while still concluding that the *population* is free from disease. However, if there is reasonable evidence that there is even a single true positive, then the *population* cannot be considered free. This is the reason for the classification of positive results into definitive and probable positives. If there are any definitive positives at all, the *population* in that estuary must be considered infected. The probable positives are consistent with false positives, and therefore up to 10 may be accepted. Using *FreeCalc* the actual confidence achieved based on the number of (presumed) false positives detected can be calculated. For instance, if 8 'probably positive' results were detected from an estuary, the confidence level for the survey would be 98.76%. On the other hand, if 15 'probably positive' results were detected, the confidence is only 61.9%, indicating that the estuary is likely to be infected.

## i) Discussion

Normally, it may be safely assumed that a surveillance system aimed at demonstrating freedom from disease is 100% specific. This is because any suspected occurrence of disease is investigated until a definitive decision can be made. If the conclusion is that the case is truly a case of disease, then there is no issue of declaring freedom – the disease is known to be present. This example presents a different situation where, due to lack of suitable tests, it is not possible for the surveillance system to be 100% specific. This may represent an unusual situation in practice, but illustrates that methods exist for dealing with this sort of problem. In practice, a conclusion that a country (or estuary) is free from infection, in the face of a small (but statistically acceptable) number of positive results, will usually be backed up by further evidence (such as the absence of clinical disease).

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Original: English  
January 2008

## **REPORT OF THE MEETING OF THE OIE *AD HOC* GROUP ON THE OIE LIST OF AQUATIC ANIMAL DISEASES - MOLLUSC TEAM - FOR THE OIE *AQUATIC ANIMAL HEALTH CODE***

**Paris, 25-27 January 2008**

The OIE *ad hoc* Group on the OIE List of Aquatic Animal Diseases - Mollusc Team (hereinafter referred to as the *ad hoc* Group) for the OIE *Aquatic Animal Health Code (Aquatic Code)* held its meeting at the OIE Headquarters from 25 to 27 January 2008.

On behalf of Dr Bernard Vallat, Director General of the OIE, Dr Sarah Kahn, Head of the International Trade Department, welcomed the members of the *ad hoc* Group and thanked them for their willingness to be involved in addressing this mandate of the OIE.

The members of the *ad hoc* Group are listed in [Annex I](#). The agenda adopted is given in [Annex II](#) and the terms of reference are listed in [Annex III](#).

### **1. Infestation with *Terebrasabella heterouncinata***

The *ad hoc* Group addressed the request from the Aquatic Animal Health Standards Commission (Aquatic Animals Commission) on the sabellid worm (*Terebrasabella heterouncinata*) by developing a full assessment of the disease against the OIE criteria for listing aquatic animal disease provided in Chapter 1.2.2. of the *Aquatic Code* (refer to Annex IV).

Since this sabellid worm is limited to the shell and does not penetrate into live tissues, it cannot be referred to as an infection. Based on the definitions in the *Aquatic Code* for *infestation* and *disease*, the *ad hoc* Group recommended that the disease is referred to as *infestation with Terebrasabella heterouncinata*.

The *ad hoc* Group reviewed the preliminary assessment previously developed by the *ad hoc* Group on the OIE List of Aquatic Animal Diseases – Mollusc Team, and reviewed available published and grey literature.

Annex XVII (contd)

The *ad hoc* Group provided additional information on criteria 1A, 2A, 6B and 8C as follows: 1A - the impact of the disease on farmed abalone; 2A - what little is known about its potential in the wild; 6B – biological characteristics increasing the potential for spread; 8C – diagnostic methods currently available. The assessment highlighted the highly transmissible nature of this hermaphroditic organism, its significant economic impact on abalone farms, the history of its translocation with infested abalone, and its current limited known geographic distribution. In addition, the *ad hoc* Group recognised that little is known about polychaete pests infesting aquatic species.

The overall assessment of this disease against the OIE criteria for listing aquatic animal disease confirmed the previous assessment. Based on this assessment using current knowledge, the *ad hoc* Group recommended that infestation with *Terebrasabella heterouncinata* be considered for addition to the diseases listed by the OIE in the *Aquatic Code*.

The *ad hoc* Group will draft chapters for the *Aquatic Code* and *Aquatic Manual* pending the final decision on disease listing of infestation with *Terebrasabella heterouncinata*.

## 2. Abalone viral mortality complex

As part of the review and scientific assessment of abalone viral ganglioneuritis (AVG) and abalone viral mortality (AVM), the *ad hoc* Group considered the Member comments previously received. The *ad hoc* Group also reviewed a comprehensive collection of available published literature on abalone viral mortalities, peer reviewed and grey literature including the information provided by Australia. Key information extracted from these references is summarised in the Table presented in Annex V.

In a first approach, published literature allowed grouping of reports into five different clinical entities (refer to Annex V).

Differing methodologies of examination hindered direct comparison of pathology and etiological agents. Based largely on clinical and epidemiological data, and virus description, the *ad hoc* Group, grouped the syndromes into two groups, those with a sub-acute to chronic course (including effects on growth and shell formation) and those with an acute course (heavy mortality within a few days). Homology of the viruses involved (both within and between these groups) cannot currently be excluded or confirmed.

The first reports were of a slowly progressing disease, described as amyotrophia, typically occurring in spring to early summer, as temperatures are increasing, with a course of 40 days or longer and a marked impact on growth and shell formation prior to death, was first reported in Western areas of Japan in the late 1980s (Nakatsugawa *et al.*, 1988). *Haliotis discus discus* is the main affected species, with later reports of this disease in *H. discus hannai* and *H. madaka* in this area (Momoyama *et al.*, 1999). Clinical signs in *H. discus discus* include poor growth, reduction in muscle tissue, and abnormalities of shell growth, sometimes including a split in the anterior margin (Momoyama *et al.*, 1999).

A clinically similar disease, known as crack shell disease, was seen in *H. discus hannai* in northern China in 1993, and has persisted in Liaoning and Shandong Provinces. It is important to note that Nie and Wang 2004 reported importations in 1986 of *H. discus discus* from Japan. Severe losses occurred in *H. discus hannai* especially in the years following the initial outbreak. The disease now occurs, although with reduced severity, with hybrids of this species. The disease shows similar gross lesions and time frame, with cracked shells being a common feature. Viruses have been implicated in both of these syndromes.

In contrast, an acute disease with a rapid onset and high mortality within a few days was first detected in *H. diversicolor aquatilis* in the Dongshan district of Fujian Province in the spring of 1999 (Huang *et al.*, 1999). It subsequently spread southwards to Guangdong Province (Nie and Wang, 2004), and later to Hainan and Guangxi Provinces (Zhang *et al.*, 2004). Most outbreaks occurred in *H. diversicolor aquatilis* and were associated with a spherical virus (with icosahedral core) of ~100 nm, with a smooth envelope. However, there is one report in *H. diversicolor supertexta* in which two other viral morphologies were observed in addition to the smooth enveloped virus particles as described above (Zhang *et al.*, 2001). The *ad hoc* Group considered it likely the latter particles are unrelated to the major mortalities.

Annex XVII (contd)

Epidemiological data suggest that this acute disease spread to *H. divericolor supertexta* in Taiwan, where a disease with a similar clinical appearance was first observed in January 2003. Subsequent studies of the disease in Taiwan revealed neurological lesions as the major pathology, in association with a herpes-like virus. The disease has therefore been termed ganglioneuritis.

The origin of the outbreak of a similar disease in *H. rubra*, *H. laevigata* and their hybrids in Victoria, Australia in late 2005 is not known.

The major lesions in amyotrophia, and in both Taiwan and Australia, are nerve related, with acute inflammation associated with a herpes-like virus in Taiwan and Australia, and more chronic lesions (possibly gliomas) in amyotrophia.

Whether nerve lesions and similar neurotropism occur in crack shell disease and acute abalone mortality in China is uncertain as different methods of examination were used. Chinese researchers reported on electron microscopy carried out on a selection of visceral tissues; the use of light microscopy was not reported. Electron microscopic examination suggests systemic infection in both crack shell disease and acute viral mortality; nervous tissues were seldom examined.

Electron microscopic examination of animals with amyotrophia and ganglioneuritis has concentrated on the nervous tissue lesions detected by light microscopy. Examination for systemic infection in other tissues has not yet been undertaken for the ganglioneuritis cases in Taiwan or Australia. Electron microscopic examinations of amyotrophia have concentrated on the clinical stage of the disease rather than the early post-infection period where systemic infection is more likely to be detected.

By reviewing the available scientific literature, the group came to the following conclusions:

- It is recognised that descriptions of spherical virus associated with abalone mortality outbreaks made by Huang *et al.* (1999), Song *et al.* (2000), Zhang *et al.* (2001), Fang *et al.* (2002) and reviewed by Zhang *et al.* in 2004 are consistent. They constitute an acute syndrome of abalone viral mortality.
- The spiked icosahedral enveloped virus described by Zhang *et al.* (2001) ranging in size between 135 and 150 nm is considered as different from other spherical viruses descriptions of acute abalone mortality outbreaks. They also reported a smaller particle size of ~ 40 nm. In the absence of other corroborating reports, and given the sparse availability of scientific data, the significance of these findings is difficult to interpret.
- Description of crack shell disease (Wang *et al.*, 1997; Li *et al.*, 1998; Nie and Wang, 2004) and viral amyotrophia (Nakatsugawa *et al.*, 1988; Nakatsugawa 1990; Otsu and Sasaki, 1997; Nakatsugawa *et al.*, 1999; Nakatsugawa *et al.*, 2000; Muroga 2001) are consistently described; they constitute a sub-acute to chronic syndrome within the abalone viral mortality complex.
- The suggestion of a retroviral nature of amyotrophia (Nakatsugawa *et al.*, 1999) is not well supported by the published scientific data, nor has it been corroborated by further studies.
- Descriptions of small icosahedral particles (~35-55nm) by Harada *et al.* (1993) and Yu *et al.* (2007) are inconsistent with other studies in which particles of >100nm have been found and transmission trials (Momoyama, 2000).
- Herpes-like virus ganglioneuritis described in Taiwan (Chang *et al.*, 2005) and in Australia (Hooper *et al.*, 2007) are a consistent group of acute viral syndrome.
- There are similarities in virus characteristics and clinical expression of infection between the spherical virus acute mortality and herpes-like virus ganglioneuritis. These diseases may be caused by similar, related or the same virus. A lack of histopathology descriptions precludes differentiation of these viral diseases (Huang *et al.*, 1999; Song *et al.*, 2000; Zhang *et al.*, 2001 and 2004; Fang *et al.*, 2002; Nie and Wang, 2004).

Annex XVII (contd)

- Information available shows that movements of live animals and contaminated equipment within the geographical range of these diseases have happened and may have contributed to the spread of this disease complex.
- Recent genomic characterisation of the Australian herpes-like virus (Wong *et al.*, 2007) provides a baseline for comparative studies.
- Currently, specific diagnostic methods are on the brink of being released for herpes-like virus ganglioneuritis (Dr Chang, personal communication; Crane *et al.*, 2007).
- There is a need for further coordinated research using standardized methods to reduce the current fragmentation of the scientific information. Studies should mainly aim to provide thorough pathological descriptions of chronic and acute syndromes, and molecular characterization of viral isolates. A more detailed list of research objectives is given in Annex VI.

Conclusions

The *ad hoc* Group concluded that:

1. Abalone viral ganglioneuritis should be listed because it meets the criteria for listing of an emerging aquatic animal disease.
2. The lack of comparable data precluded drawing conclusions on the relationships between abalone viral ganglioneuritis and abalone viral mortality. A single viral etiology for this complex cannot be excluded. Abalone viral ganglioneuritis should therefore be listed as part of the abalone viral mortality complex.

Recommendations

In consequence, the *ad hoc* Group recommended that:

1. A complex of abalone viral mortality remains on the diseases listed by the OIE (Chapter 1.2.3. of the *Aquatic Code*) under listing according to Article 1.2.2.2.;
2. Within the abalone viral mortality complex, two syndromes are recognized;
3. These syndromes are referred to as: (i) abalone herpes-like virus disease (including ganglioneuritis diseases seen in Taiwan and Australia and the acute disease seen in southern China) and (ii) cracked-shell-amyotrophobia-virus disease (including amyotrophobia from Japan and cracked-shell disease from northern China), as described in the case definition (Annex VII).

The *ad hoc* Group will review the disease card information and prepare chapters for the *Aquatic Code* and the *Aquatic Manual* pending decisions on these recommendations.

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.../Annexes

**REPORT OF THE MEETING OF THE OIE AD HOC GROUP ON THE OIE LIST OF AQUATIC ANIMAL DISEASES - MOLLUSC TEAM - FOR THE OIE AQUATIC ANIMAL HEALTH CODE**

**Paris, 25-27 January 2008**

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**REPORT OF THE MEETING OF THE OIE AD HOC GROUP ON THE OIE LIST OF AQUATIC ANIMAL DISEASES - MOLLUSC TEAM - FOR THE OIE AQUATIC ANIMAL HEALTH CODE**

**Paris, 25-27 January 2008**

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**Adopted agenda**

**Welcome from the Director General**

**Adoption of the Agenda**

**1. Terms of Reference**

**2. Abalone viral ganglioneuritis (AVG) and abalone viral mortality (AVM)**

- 2.1. Consider comments made by Members and review currently available scientific information on abalone viral ganglioneuritis (AVG) and abalone viral mortality (AVM) to make recommendations on whether AVG should be listed, and if so, whether separately or as part of the AVM complex;
- 2.2. Draft chapters on abalone viral mortality to be considered for inclusion in the *Aquatic Code* and *Aquatic Manual*;
- 2.3. Update scientific information provided in the current disease card for abalone viral mortality, and, if need be, develop disease card for abalone viral ganglioneuritis;

**3. Sabellid worm (*Terebrasabella heterouncinata*)**

- 3.1. Review the preliminary assessment on the sabellid worm (*Terebrasabella heterouncinata*) and develop a full assessment providing documented, scientific justification for listing;
- 3.2. Pending the outcomes of the assessment, to draft chapters to be considered for inclusion in the *Aquatic Code* and *Aquatic Manual*;

**4. Any other business**



**REPORT OF THE MEETING OF THE OIE *AD HOC* GROUP ON THE OIE LIST OF AQUATIC ANIMAL DISEASES - MOLLUSC TEAM - FOR THE OIE *AQUATIC ANIMAL HEALTH CODE***

**Paris, 25-27 January 2008**

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**Terms of Reference**

1. Review the preliminary assessment on the sabellid worm (*Terebrasabella heterouncinata*) (provided in the attached 2006 report of the ad hoc Group on Abalone Diseases) and develop a full assessment providing documented, scientific justification for listing
2. Pending on the outcomes of the assessment, to draft chapters to be considered for inclusion in the OIE Aquatic Animal Health Code and Manual of Diagnostic Tests for Aquatic Animals
3. Consider comments made by Members and review currently available scientific information on abalone viral ganglioneuritis (AVG) and abalone viral mortality (AVM) to make recommendations on whether AVG should be listed, and if so, whether separately or as part of the AVM complex
4. Update scientific information provided in the current disease card for abalone viral mortality, and, if need be, develop disease card for abalone viral ganglioneuritis
5. Draft chapters on abalone viral mortality to be considered for inclusion in the OIE Aquatic Animal Health Code and Manual of Diagnostic Tests for Aquatic Animals
6. Produce a draft report and draft chapters by 1 March 2008, i.e. in time for the March 2008 meeting of the OIE Aquatic Animal Health Standards Commission.



**Full assessment of infestation with *Terebrasabella heterouncinata* against the OIE criteria for listing aquatic animal disease**

No.	Criteria	Parameters that support a listing	Listing	Explanatory notes
1	A	Loss of production due to slower growth rates and shell deformities that resulted in decreased marketability and value of product. In general a slight increase in mortalities associated with handling has been observed; elevated losses have been predicted under conditions of poor water quality. (8, 13, 3, 11).	+	
	Or			
2	A	Lack of quantitative data on impact in the wild. Eradication from the one site in California where the sabellid worm was established in wild gastropod populations was successful (1, 2, 9). Population surveys have not found the sabellid worm at any other site in California examined including those adjacent to known infected farms (6; 9). No significant impacts have been reported in wild invertebrate populations in South Africa where the sabellid worm is now known to be endemic. The sabellid worm was unknown prior to its initial observation in farmed California abalone (7, 5, 12, 11). There is a wide range of potential hosts, however host susceptibility varies among species with patello- and veti-gastropods being the preferred over many caeno-gastropods (12).	-	Because of its endemic nature in South Africa absence of noted impact may be related to absence of baseline data for comparison. No abalone are endemic to Chile where this sabellid worm has also been observed in farmed abalone.
	Or			
3	A	Not harmful to human health	-	
	And			
4	B	<i>T. heterouncinata</i> is the aetiological agent of the disease (5, 11, 3).	+	Genus and species were created after the outbreaks in California (5) and whether or not other species in this genus are defined is currently unknown.
	Or			
5	B	The aetiology is known (see B4).	NA	NA
	And			
6	B	Origin of the parasite: South Africa (5; 12) Now spread to: Chile (10), Mexico (Baja California) (8) and USA (California) (7; 5).	+	

Annex XVII (contd)Annex IV (contd)**Full assessment of infestation with *Terebrasabella heterouncinata* against the OIE criteria for listing aquatic animal disease (contd)**

No.	Criteria	Parameters that support a listing	Listing	Explanatory notes
6 (cont d)	B	It was demonstrated that this sabellid worm is a functional, simultaneous hermaphrodite which indicates that isolated individuals can produce reproductively viable offspring (4). Therefore the risk of spreading from infested populations is high. Sabellid worm reproduction is directly temperature dependent with reproduction observed at all experimental temperatures examined (between 11.2C and 20.9C).		
	And			
7	B	There are no published reports of infestations with this sabellid worm in gastropods from Europe, the Mediterranean and Australasia.	+	
	And			
8	C	Presence of macroscopic signs (e.g. presence of worm tubes on the growing edge of the abalone shell; heavy infestations result in visibly abnormal shell deposition, cessation of horizontal growth and, in some species, shell doming and lack of respiratory pore development.) can be considered a presumptive diagnosis. Shell radiography can assist in detecting the presence of worm tubes.  Microscopic observations of excised or intact worms can be used as a confirmatory diagnosis within the known geographic range of this sabellid worm.  Sentinel abalone or other accepted host species may be used in bioassays in conjunction with the above signs for monitoring purposes. Diagnosis is easier using smaller individuals with new lesions.  Scanning electron microscopy is necessary for confirmation of the species when suggestive worms or lesions are found in new locations or new host species.	+	3; 5; 12
			list	

Listing here:-

1	2	3	4	5	6	7	8	Add to the OIE list?
+	-	-	+	N/A	+	+	+	list

Annex XVII (contd)Annex IV (contd)**References**

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Table. Synoptic table of abalone viral infection reports

Type	Name	Short name	Year of occurrence	Geographic origin	Type of mortality	Host species	Particles	Virus location	Nucleic acid	Transmission
1	Amyotrophia	RLV		Japan since the early 1980s (Otsu & Sasaki, 1997; Nakatsugawa 1990; Nakatsugawa <i>et al.</i> , 1999; Nakatsugawa <i>et al.</i> , 2000; Muroga 2001)	<p>Chronic, mantle retraction, growth cessation, tumor like cell masses, and muscle atrophy, gliomas, impaired shell growth in <i>H.d.h.</i>, <i>H.d.d.</i> and <i>H.m.</i> but not in <i>H.g.</i> (Momoyama <i>et al.</i>, 1999)</p> <p>The development of glioma-like masses was temperature dependent. Masses were observed in nerve trunk and peripheral nerves of the foot of juvenile abalone 40 days after water-borne transmission at 18C. Lesions occurred earlier at 24C, but tended to heal by 40 days in survivors, and only slight lesions seen by 60 days at 12C. (Momoyama, 2000)</p> <p>Impaired shell growth including some incisions in front margin of shell in <i>H.d.d.</i> (Momoyama <i>et al.</i>, 1999)</p> <p>Survival of juveniles (year class) following exposure varied between families of <i>H. discus discus</i> from 0-93%. (Hara <i>et al.</i>, 2004)</p>	<p><i>Haliotis discus hannai</i>, <i>H. discus discus</i>, <i>H. madaka</i> (Mamoyama <i>et al.</i>, 1999)</p> <p>0-2 yr olds of <i>H.d.d</i> susceptible with susceptibility decreasing with increasing age; 2 yr old (Nakatsugawa &amp; Momoyama 1999). asymptomatic survivors acted as carriers (Nakatsugawa <i>et al.</i>, 2000)</p>	<p>55 nm, icosahedral with 35 nm core (Harada <i>et al.</i> 1993);</p> <p>120 nm icosahedral (Nakatsugawa <i>et al.</i> 1999)</p> <p>Experiment shows agent passed through 220nm filter but not 100nm filter (Momoyama 2000).</p>	Detected in cells near the nerve, and macrophages (Otsu and Sasaki 1997; Harada <i>et al.</i> 1993)		<p>Yes, immersion and IM injection, filtrates 0.22microns from infected abs (Nakatsugawa <i>et al.</i>, 1999)</p> <p>18C bath exposure 40+ days nerve lesions but at 12C only observed slight changes by the end of the study (60 days), at 24C cell masses formed earlier but recovered by 40 days post inoculation. The agent passed through 220nm filter but not 100nm filter Momoyama (2000).</p> <p>Horizontal transmission via infective waters shown by Nakatsugawa <i>et al.</i>, (200).</p>

Annex XVII (contd)Annex V (contd)

Table. Synoptic table of abalone viral infection reports (contd)

Type	Name	Short name	Year of occurrence	Geographic origin	Type of mortality	Host species	Particles	Virus location	Nucleic acid	Transmission
1	Amyotrophia				<p>Disease outbreaks occur in Spring and early summer, when temperatures range 16-25C (Muroga 2001). Disease is suppressed at &gt;23C (Nakatsugawa, 1990)</p> <p>Transmission period is late winter to early spring (Tadamitsu <i>et al.</i>, 2005)</p>					
2	Crack shell disease	CSD	1992-3	<p>Northern China (Wang <i>et al.</i>, 1997)</p> <p>First observation in 1993 (Review by Nie &amp; Wang, 2004, Zhang <i>et al.</i>, 2004.)</p>	<p>Chronic, low activity, lethargic, anorexic, thin shell, decrease growth rate, 50% mortality in 20 days; young animals more susceptible –(Wang <i>et al.</i>, 1997) . up to 90% mortality reported in larvae and young juveniles (Zhang <i>et al.</i>, 2004)</p>	<i>Haliotis discus hannai</i>	<p>90-140 nm, spherical, enveloped (Wang <i>et al.</i> 1997; Li <i>et al.</i>, 1998), 60-120nm nucleocapside (Wang <i>et al.</i>, 1997)</p> <p>Authors suggest retro virus like, however morphology contradicts this. Needs more study (Wang <i>et al.</i>, 1997)</p>	Detected in the cytoplasm of haemocytes, connective tissue of a variety of organs (Wang <i>et al.</i> , 1997; Li <i>et al.</i> , 1998)	unknown	Oral inoculation once per day to 15mm animals, 50% mortality within 20 days (Wang <i>et al.</i> , 1997).

## Annex XVII (contd)

## Annex V (contd)

Table. Synoptic table of abalone viral infection reports (contd)

3 (general)	Abalone spherical virus	ASV	1999	Southern China. Initial outbreak in 1999 in Dongshan, Fujian Province (Zhang <i>et al.</i> , 2001). It caused 100% mortality in 22 farms within 43 days. (Huang <i>et al.</i> , 1999; Nie and Wang, 2004)	All abalone sizes are affected (Wang <i>et al.</i> , 2004). Acute, high mortality within few days, copious mucus production, contracted feet and mantle, stiff muscle.	<i>Haliotis diversicolor</i>				Temperature dependant 17-20C 100%. Nb mortality at 23-26C (Wang <i>et al.</i> , 2004)
2a		ASVa		Dongshan, Fujian Province (Fang <i>et al.</i> , 2002; Song <i>et al.</i> , 2000)	Mass mortality in farmed abalone (Song <i>et al.</i> , 2000); Fang <i>et al.</i> (2002) report up to 100%	<i>Haliotis diversicolor aquatilis</i>	100nm, icosahedral capsid, enveloped (Fang <i>et al.</i> , 2002; Song <i>et al.</i> , 2000)	In the cytoplasm of digestive gland, kidney and intestine (Fang <i>et al.</i> , 2002)	DNA virus (Fang <i>et al.</i> , 2002)	Cohabitation (40% after 15 days), injection (100% mortality in 4-6 days) and bath (no mortality) are reported in Song <i>et al.</i> , (2000)
2a continued				Huang <i>et al.</i> , 1999			Negative staining circular to oval 35-75nm; by TEM spherical 5(0?)-80 x 120-150nm (Huang <i>et al.</i> , 1999)		unknown	
				Between 1999 and 2002, during the early winter, at around 21C, the disease reappeared in Dongshan and spread to Guangdong Province (Nie and Wang, 2004), later to Hainan and Guangxi Provinces (Zhang <i>et al.</i> , 2004)	Acute with short course, 100% mortality 22 farms within 43 days (Huang <i>et al.</i> , 1999; Nie and Wang, 2004); clinical signs contracted foot, animals on bottom, pond water turbid and frothy with suspended vomit; after mortality, dark foot muscle still adhered to tank surfaces. No change in feeding behavior prior to outbreak (Huang <i>et al.</i> , 1999).			Assemble in vesical of digestive gland, suspected a nucleo replicating virus (Huang <i>et al.</i> , 1999)		Positive transmission to all age classes by infected water, equipment and humans (Huang <i>et al.</i> , 1999)

Annex XVII (contd)Annex V (contd)

Table. Synoptic table of abalone viral infection reports (contd)

2b		ASVb		Dongshan, Fujian Province (Zhang <i>et al.</i> , 2001)		<i>Haliotis diversicolor supertexta</i>	135-150nm, spiked envelope, icosahedra nucleocapsid 100-110 nm (Zhang <i>et al.</i> 2001). Note that poor TEM makes confirmation of this morphology difficult	Assembled in cytoplasm of digestive gland and intestine epithelium & connective tissue cells (Zhang <i>et al.</i> 2001).	DNA	none
2c		ASVc		Dongshan, Fujian Province (Zhang <i>et al.</i> , 2001)		<i>Haliotis diversicolor supertexta</i>	95 – 110 nm icosahedral, smooth envelop – authors also reported 40-45 nm particles (Zhang <i>et al.</i> 2001)..  Also reported 40-45 nm particles in same cells.	Assembled in cytoplasm of digestive gland and intestine epithelium & connective tissue cells		

Annex XVII (contd)Annex V (contd)**Table. Synoptic table of abalone viral infection reports (contd)**

3	Abalone viral ganglioneuritis	HLV		<p>NE Taiwan in farmed abalone when temperatures drop to 16-19C (Chang <i>et al.</i> 2005); anecdotal information (talking with farmers) suggests wild abalone also affected (Chang pers. comm.).</p> <p>Note: In NE Taiwan water temperatures range from &lt;16 to &gt;30C, thus losses typically occur in winter when water temperatures are lowest.</p> <p>Spread of disease between farms was rapid (within 9-53d covering 60 km of coastline) but not linear geographically. Suspected spread via equipment, workers, and abalone movements (Chang, unpubl. data).</p> <p>Disease has remained limited to the NE region of Taiwan (Chang, unpubl. data)</p>	<p>Acute mortalities began 3d after the onset of clinical signs (anorexia and water changes noted below) and reached usually 100% within 10d of onset of clinical signs; Chang <i>et al.</i>, 2005)</p> <p>During epidemic water is turbid and frothy (occasionally appeared greasy).</p>	<i>Haliotis diversicolor supertexta</i> (Chang <i>et al.</i> , 2005)	90-100nm, enveloped virus with single layer containing hexagonal capsid – herpes-like virus (Chang <i>et al.</i> , 2005)	Cerebral ganglion with nucleocapsid in nucleus and enveloped virions in cytoplasm (Chang <i>et al.</i> 2005)	DNA (Chang <i>et al.</i> 2005)	Experimental via IM injection and bath resulted in 100% mortality within 2d and 3d, resp. On farm observations with <i>H. discus</i> suggested no transmission to this species during epidemic (based on survivorship and histology data collected during epidemic) (Chang <i>et al.</i> 2005)
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Annex XVII (contd)Annex V (contd)**Table. Synoptic table of abalone viral infection reports (contd)**

4	Ganglioneuritis	GNV	Victoria Australia (Pt Fairy and Portland) (OIE notification 2006-2007).  Initial observations in farmed abalone and subsequently in wild stocks (OIE notification 2006-2007).  Suspect initial outbreaks may be related to abalone broodstock transfers (Hooper <i>et al.</i> , 2007)	Acute mortalities – initial farm outbreak >50 in most tanks with 90% losses within 14d in one tank (Hooper <i>et al.</i> , 2007).  Some affected abalone with swollen, flaccid and protruding mouth parts, reduced pedal adhesion, curled mantle edge, many with elevated shells, reduced righting reflex, and reduced foot movements. No cessation of feeding except with affected mouth; many dead animals lacked any clinical signs (Hooper <i>et al.</i> , 2007).  To date, no clear seasonal pattern has emerged. Temp range – around 13-15 in winter, maximum of 22 in summer.	<i>Haliotis laevigata</i> , <i>H. rubra</i> and hybrids of these two species	Herpes-like virus, capsids averaging 104nm enveloped with icosahedral capsid and dense core.	Most particles intranuclear, occasionally in cytoplasm	DNA	Horizontal via conhabitation and bath exposure with 100% losses within 36d and 38d (1-100% dilutions of infected tank water), resp. (Crane <i>et al.</i> 2006). IM injection resulted in 100% mortality within 25d (Crane <i>et al.</i> 2006).
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**Table. Synoptic table of abalone viral infection reports (contd)**

4	Ganglioneuritis	GNV		<p>Disease spread rapidly within farms and has progressed more slowly in wild stocks. Spread not linear. Observed spread pattern has not been linear, though it is uncertain to what extent this is due to variable observation intensity (interruptions due to rough weather) and the discontinuous nature of the population. It is suspected that the virus is spread more readily in periods of calm water, possibly due to less immediate dilution. (pers com S McGlashen, Victorian DPI)</p>						
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Annex XVII (contd)Annex V (contd)**References**

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## RESEARCH OBJECTIVES

Key knowledge gaps required to be addressed to define the relationships between these syndromes are:

- The range of lesions (at the light microscope level) in cracked shell disease and acute abalone mortality in China.
  - Distribution of virus in tissues other than nervous system in ganglioneuritis
  - Define the early lesions, and virus distribution early in the infection period in amyotrophia.
  - Clarification of virus type in amyotrophia
  - Sequence comparisons. Note: sequence data is being obtained for herpes-like virus involved from ganglioneuritis in both Taiwan and Australia, and a PCR test based on sequences from the Australian virus is expected to be available shortly.
  - Application of molecular detection tools in all cases of abalone viral mortality syndrome
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## CASE DEFINITION FOR ABALONE VIRAL MORTALITY (AVM) COMPLEX

### General Description

Within the AVM complex, two syndromes have emerged over the past ~15 years: one has an acute course (herpes-like virus disease, HLVD) and the other has a more sub-acute to chronic course (crack-shell-amyotrophia-virus disease, CSAVD). Both syndromes impact multiple abalone species in Australasia (China, Japan, Taiwan, and Australia) with significant losses. However, different clinical courses and presentations lead to currently require different case definitions. Upon comparison of nucleic acid sequences and development of molecular tests, case definitions may change.

### Abalone herpes-like virus (AHLV) disease

Known affected species - to date, primarily observed in both subspecies of *Haliotis diversicolor* (*aquaticilis* and *supertexta*) and in *Haliotis laevegata*, *H. rubra* and hybrids of *H. laevegata* x *H. rubra*.

Gross observations - rapid onset of mortality in tanks or ponds with no visible change in abalone feeding habits prior to onset. During outbreaks, tank water is typically turbid and frothy with several reports of suspended, presumably regurgitated, food particles and mucus in water. Affected abalone with clinical signs varying from none to having a stiff pedal muscle with darkened lateral mantle, increased mucus production reported in many cases and may present swollen, prolapsed mouth with everted radula in some cases (noted in Australian abalone species). Mortalities typically observed within 3 days of onset of clinical signs, and dead abalone may remain adhered to substrata. Losses often complete within 9-14d. Losses typically occur when water temperatures are <22C and often range from 16-19C.

Microscopic observations - when used light microscopic observations have suggested that the main pathological change is ganglioneuritis with lesions prominent in cerebral and pedal ganglia<sup>10</sup>. Lesions characterized by nerve tissue necrosis accompanied by hemocytosis in the parenchyma and extend into neurolemma. These lesions can also be observed in nerves under mucosa of esophagus and intestine. No Cowdry type A inclusions were observed; however neuronal cells may contain marginated chromatin.

Transmission electron microscopic (TEM) observations illustrate spherical, enveloped virus (~100nm) with icosahedral (hexagonal) nucleocapsid and dense core. Naked virions observed in nucleus and particles with smooth envelop in cytoplasm. Negative-contrast electron microscopy also reveals hexagonal particles with single, smooth envelope (~100nm).

Presumptive diagnosis – a combination of clinical signs and microscopic features as described above.

Confirmatory diagnosis – presumptive diagnosis in conjunction with the presence of spherical virus containing an icosahedral nucleocapsid and dense core using TEM<sup>11</sup>. Occasionally only empty capsids are visible in nucleus of infected cells.

### Crack-shell-amyotrophia-virus (CSAV) disease

Known affected species - to date, primarily observed in *Haliotis discus discus* and *H. discus hannai*, and, to a lesser extent, *Haliotis madaka*.

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<sup>10</sup> To date descriptions of the AHLV from China have not included histopathology.

<sup>11</sup> Molecular tests for AHLV are currently under development.

Annex XVII (contd)Annex VII (contd)

Gross observations – reduced growth and/or abnormal shell deposition, sub-acute or slow losses with up to 50% mortality in 20 days. Affected abalone lethargic with retracted mantle, abnormal shell deposition often poses a thin, cracked shell. Anorexia reported in many cases. Juveniles typically more susceptible than older animals. Water temperature modulates disease with losses often at 18-20C.

Microscopic observations - light microscopic observations suggest the main pathological change in symptomatic animals includes the presence of tumor-like masses presented as whorls or spheres of lightly basophilic cells within nerve trunks of pedal ganglia and transverse commissures ('gliomas'). Nuclei of affected cells may be contracted and tumor centers necrotic<sup>12</sup>.

Transmission electron microscopic (TEM) observation may reveal 90-140nm spherical, enveloped virions with an icosahedral nucleocapsid in cells near nerves and in the cytoplasm of hemocytes and connective tissue cells of a variety of organs.

Presumptive diagnosis – a combination of clinical signs and microscopic features as described above.

Confirmatory diagnosis – presumptive diagnosis in conjunction with the presence of 90-140nm spherical, enveloped virions with an icosahedral nucleocapsid in infected cells.

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<sup>12</sup> To date descriptions of the CSAV from China have not included histopathology



Original: English  
February 2008

## REPORT OF THE MEETING OF THE OIE *AD HOC* GROUP ON AQUATIC ANIMAL HEALTH SURVEILLANCE

Paris (France), 28 January – 1 February 2008

The OIE *ad hoc* Group on Aquatic Animal Health Surveillance (hereinafter referred to as the *ad hoc* Group) met at the OIE Headquarters in Paris from 28 January to 1 February 2008.

The members of the *ad hoc* Group and other participants are listed at [Annex I](#). The Agenda adopted is given at [Annex II](#).

On behalf of the Director General of the OIE, Dr Sarah Kahn, Head of the International Trade Department, welcomed all members and thanked them for their work on this important topic. She discussed the development of a stand-alone OIE Handbook on Aquatic Animal Health Surveillance and the value of such a publication for OIE Members.

Dr Barry Hill then took over as Chair of the meeting and presented the draft agenda and terms of reference ([Annex III](#)). He acknowledged the importance of the work of the *ad hoc* Group and reminded members of the extensive work programme for the meeting.

### 1. **Appendix of the OIE *Aquatic Animal Health Code* on Guidelines for Aquatic Animal Health Surveillance**

At the time of the *ad hoc* Group meeting, comments on the draft Guidelines for aquatic animal health surveillance had been received from the Australia, Belize, Japan, New Zealand, EU, and the United States of America (USA).

The *ad hoc* Group discussed these comments, agreed with most of them and amended the text accordingly. The *ad hoc* Group's responses to all the comments and proposed amendments were submitted to the OIE Aquatic Animal Health Standards Commission (hereinafter referred to as the "Aquatic Animals Commission") for consideration at their next meeting in March 2008. The amended draft Guidelines are presented at [Annex IV](#).

Annex XVIII (contd)**2. Disease specific surveillance chapters for the OIE Aquatic Animal Health Code**

The *ad hoc* Group was tasked with drafting disease specific surveillance chapters for the OIE *Aquatic Animal Health Code* (hereinafter referred to as the “*Aquatic Code*”), taking into account the approach taken in the OIE *Terrestrial Animal Health Code* (hereinafter referred to as the “*Terrestrial Code*”). The *ad hoc* Group reviewed the example chapters from the *Terrestrial Code* in an attempt to identify areas of similarity on which the disease specific surveillance chapters of the *Aquatic Code* could be harmonised. The *ad hoc* Group noted a lack of harmonisation among chapters of different diseases of the *Terrestrial Code*. Given differences in clinical expression of diseases in aquatic versus terrestrial animals and the recent direction taken by the *Aquatic Code* in respect to aquatic animal surveillance, the *ad hoc* Group found it difficult to see obvious ways to harmonise the style and content with those of the *Terrestrial Code*.

The *ad hoc* Group explored many avenues towards the development of a template and, given the fact that the proposed draft Guidelines for aquatic animal health surveillance in the *Aquatic Code* will require significant revision to be more appropriate to the style of the *Aquatic Code*, concluded that at this stage it is not feasible to produce a definitive template to be used by chapter authors in the development of disease specific surveillance chapters. Instead the *ad hoc* Group drafted a rough outline listing information for a possible template which is presented at Annex V. The *ad hoc* Group requested comments from the Aquatic Commission on this approach and is willing to develop the template further based on the Aquatic Commission feedback. The *ad hoc* Group recognised that drafting the disease specific surveillance chapters will require enlisting an expert/s with knowledge of both surveillance and the specific disease.

Noting that the *Terrestrial Code* contains only seven disease specific surveillance guidelines, four of which are diseases for which the OIE provides official statements of country/zone status at the request of Members, the *ad hoc* Group recommended that the Aquatic Commission take a similar approach and, if disease specific surveillance guidelines are to be developed, select diseases that should be the subject of such chapters.

**3. OIE Handbook on Aquatic Animal Health Surveillance**

The *ad hoc* Group met with Dr Kahn to establish the objectives and time frame for the proposed publication. It was agreed that the objectives would be to provide practical guidance in the form of a reference document for Members wishing to develop or refine their aquatic animal health surveillance programmes. The publication should address the needs for surveillance in a range of environments, reflecting the diverse circumstances of OIE Members. In recognising that such a resource is not currently available and high demand is anticipated, it was agreed that the goal for publication of this Handbook be the end of 2008.

The *ad hoc* Group developed an extensive outline that incorporated material drafted in previous *ad hoc* Group meetings and chapters of the *Aquatic Code* and the OIE *Manual of Diagnostic Test for Aquatic Animals*. This outline was then rearranged to ensure a practical approach for the users of the Handbook. The *ad hoc* Group initiated drafting additional text and noted that further substantial work will be required to finalise the manuscript for the handbook.

The *ad hoc* Group developed a work plan with the goal of completing the draft manuscript by August 2008 before submission to the OIE Central Bureau for review and preparation for publishing. The *ad hoc* Group plans to work on this task as much as possible by electronic exchange but concluded that additional physical meetings will be required to complete the task.

Dr Kahn presented closing remarks on behalf of Dr Vallat, who was unable to join the *ad hoc* Group due to mission travel. Dr Kahn congratulated the *ad hoc* Group on its hard work and noted that the results were testimony to the excellent contributions of all members throughout the discussions.

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.../Annexes

**REPORT OF THE MEETING OF THE OIE *AD HOC* GROUP ON  
AQUATIC ANIMAL HEALTH SURVEILLANCE**

**Paris (France), 28 January – 1 February 2008**

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**REPORT OF THE MEETING OF THE OIE *AD HOC* GROUP ON  
AQUATIC ANIMAL HEALTH SURVEILLANCE  
Paris (France), 28 January – 1 February 2008**

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**Adopted agenda**

**Welcome from the Director General**

**Adoption of the agenda**

- 1. Terms of Reference**
  - 2. Review of progress to date with preparation of the surveillance guidelines**
  - 3. OIE *Aquatic Animal Health Code* chapter on guidelines for aquatic animal health surveillance**
    - 3.1. Review comments made by Members
    - 3.2. Revise the chapter
  - 4. Disease-specific surveillance chapters for the OIE *Aquatic Animal Health Code***

Draft example disease-specific surveillance chapters for the OIE *Aquatic Animal Health Code*, taking into account as appropriate the approach in the OIE *Terrestrial Animal Health Code*.
  - 5. OIE Handbook on Aquatic Animal Health Surveillance**
    - 5.1. Decide the content and layout of the OIE Handbook
    - 5.2. Prepare the text for OIE Handbook on Aquatic Animal Health Surveillance
  - 6. New template for the specific disease chapters in the OIE *Manual of Diagnostic Tests for Aquatic Animals***
  - 7. Any other business**
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**REPORT OF THE MEETING OF THE OIE *AD HOC* GROUP ON  
AQUATIC ANIMAL HEALTH SURVEILLANCE**

**Paris (France), 28 January – 1 February 2008**

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**Terms of Reference**

1. Consider comments made by Members' on the proposed Guidelines for aquatic animal health surveillance for the OIE *Aquatic Animal Health Code* and make amendments to the Guidelines as necessary.
  2. Draft example disease-specific surveillance chapters for the OIE *Aquatic Animal Health Code*, taking into account the approach taken in the OIE *Terrestrial Animal Health Code*.
  3. Prepare text for a stand-alone OIE Handbook for Aquatic Animal Health surveillance and contribute to the layout of the publication.
  4. Submit a report to the OIE Aquatic Animal Health Standards Commission by 1st March 2008, i.e. in time for their March meeting.
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## APPENDIX X.X.X.

## GUIDELINES FOR AQUATIC ANIMAL HEALTH SURVEILLANCE

Article x.x.x.1.

### Introduction and objectives

1. Surveillance activities may be performed to achieve any of the following objectives:

- demonstrating the absence of *disease*,
- identifying events requiring notification as listed in Article 1.2.1.3. of the *Aquatic Code*.
- determining the occurrence or distribution of endemic *disease*, including changes to their incidence or prevalence (or its contributing factors), in order to:
  - provide information for domestic *disease* control programmes,
  - provide relevant *disease* occurrence information to be used by trading partners for qualitative and quantitative risk assessment.

The type of surveillance applied depends on the desired outputs needed to support decision-making. Surveillance data determine the quality of *disease* status reports and should satisfy information requirements for accurate risk analysis both for *international trade* as well as for national decision-making. Surveillance of endemic diseases provides valuable information for day-to-day health management and can act as the foundation for detecting outbreaks of exotic disease and demonstrating specific disease freedom.

Surveillance systems described in this chapter should also be used to generate information for decisions on prescribed disease prevention and control programmes. However, the actual strategies for prevention and control are beyond the scope of this chapter on surveillance guidelines.

Having a suitable management strategy to respond to surveillance data is of utmost importance for the successful implementation of surveillance systems.

2. Essential prerequisites to enable a Member to provide information for the evaluation of its animal health status are:
- a) that the particular Member complies with the provisions of Chapter 1.4.3. of the *Aquatic Code* on the quality and evaluation of the *Competent Authorities*;
  - b) that, where possible, surveillance data be complemented by other sources of information (e.g. scientific publications, research data, documented field observations and other non-survey data);
  - c) that transparency in the planning and execution of surveillance activities and the analysis and availability of data and information, be maintained at all times, in accordance with Chapter 1.2.1. of the *Aquatic Code*.

Annex XVIII (contd)Annex IV (contd)

3. The following guidelines may be applied to all *diseases*, their agents and susceptible species as listed in the *Aquatic Manual*, and are designed to assist with the development of surveillance methodologies. Where possible, the development of surveillance systems using these guidelines should be based on the relevant information in the individual *disease* chapters in the *Aquatic Manual*. These guidelines are also applicable to other diseases that are not included in the *Aquatic Code* but which may be of importance to a country or region, such as new or emerging diseases. There is sometimes a perception that surveillance can only be conducted using sophisticated methodologies. However, an effective surveillance system can also be developed by making use of gross observations and already available resources.
4. It would be impractical to try to develop a surveillance system for all the known aquatic animal diseases for which a country has susceptible species. Therefore prioritising the diseases to be included in a surveillance system should be conducted considering:
  - the needs to provide assurance of disease status for trade purposes
  - the resources of the country
  - the financial impact or threat posed by the different diseases
  - the importance of an industry-wide disease control programme within a country or region
5. More detailed information in each disease chapter (where it exists) of the *Aquatic Manual* may be used to further refine the general approaches described in this chapter. Where detailed *disease* specific information is not available, surveillance can also be conducted following the guidelines in this chapter. Access to epidemiological expertise would be invaluable for the design, implementation of the system and interpretation of results derived from a surveillance system.

Article x.x.x.2.

### **Principles of surveillance**

1. Surveillance may be based on many different data sources and can be classified in a number of ways, including:
  - a) the means by which data are collected (targeted versus non-targeted);
  - b) the *disease* focus (pathogen-specific versus general surveillance); and
  - c) the way in which units for observation are selected (~~structured~~ surveys versus non-random data sources).
2. Surveillance activities include:
  - a) ~~structured~~ population-based surveys, such as:
    - i) systematic sampling at slaughter;
    - ii) random surveys;

Annex XVIII (contd)Annex IV (contd)

- b) ~~structured~~ non-random surveillance activities, such as:
- i) *disease* reporting or notifications;
  - ii) control programmes/health schemes;
  - iii) targeted testing/screening;
  - iv) ante-mortem and post-mortem inspections;
  - v) laboratory investigation records;
  - vi) biological specimen banks;
  - vii) sentinel units;
  - viii) field observations;
  - ix) farm production records.
3. In addition, surveillance data should be supported by related information, such as:
- a) data on the epidemiology of the *disease*, including environmental, and host and wild reservoir population distributions;
  - b) data on farmed and wild animal movements and trading patterns for aquatic animals and aquatic animal products, including potential for exposure to wild aquatic animal populations, water sources or other contacts;
  - c) national animal health regulations, including information on compliance with them and their effectiveness;
  - d) history of imports of potentially infected material; and
  - e) biosecurity measures in place.
4. The sources of evidence should be fully described. ~~In the case of a structured~~ A survey, ~~this~~ should include a description of the sampling strategy used for the selection of units for testing. For ~~structured~~ non-random data sources, a full description of the system is required including the source(s) of the data, when the data were collected, and a consideration of any *biases* that may be inherent in the system.

Article x.x.x.3.

### **Critical elements of surveillance**

In assessing the quality of a surveillance system, the following critical elements need to be addressed in conjunction with an evaluation of the *Competent Authority* (Chapter 1.4.3.).

#### **1. Populations**

Ideally, surveillance should be carried out in such a way as to take into account all animal species susceptible to the *disease* in a country, *zone* or *compartment*. The surveillance activity may cover all individuals in the population or part of them. Estimates of total population at risk for each species are required. When surveillance is conducted only on a *subpopulation*, care should be taken regarding the inferences made from the results.

Definitions of appropriate populations should be based on the specific recommendations of the *disease* chapters of the *Aquatic Manual*.

Annex XVIII (contd)Annex IV (contd)2. Epidemiological unit

The relevant *epidemiological unit* for the surveillance system should be defined and documented to ensure that it is representative of the population or targeted *subpopulations* that would generate the most useful inferences about *disease* patterns. Therefore, it should be chosen taking into account factors such as carriers, reservoirs, vectors, immune status, genetic resistance and age, sex, and other host criteria.

3. Clustering

*Disease* in a country, *zone* or *compartment* usually clusters rather than being uniformly or randomly distributed through a population. Clustering of *disease* may occur in space (e.g. tank, pond, farm, or *compartment*), time (e.g. season), or animal subgroups (e.g. age, physiological condition). Clustering should be taken into account in the design of surveillance activities and interpretation of surveillance data.

4. Case and outbreak definitions

Clear and unambiguous case and outbreak definitions should be developed and documented for each *disease* under surveillance, using, where they exist, the standards in this Appendix and the *Aquatic Manual*.

5. Analytical methodologies

Surveillance data should be analysed using appropriate methodologies, and at the appropriate organisational levels to facilitate effective decision making, whether it be planning interventions or demonstrating status.

Methodologies for the analysis of surveillance data should be flexible to deal with the complexity of real life situations. No single method is applicable in all cases. Different methodologies may be needed to accommodate the relevant pathogens, varying production and surveillance systems, and types and amounts of data and information available.

The methodology used should be based on the best available information that is in accord with current scientific thinking. The methodology should be in accordance with this Appendix and fully documented, and supported by reference to the scientific literature and other sources, including expert opinion. Sophisticated mathematical or statistical analyses should only be carried out when justified by the proper amount and quality of field data.

Consistency in the application of different methodologies should be encouraged and transparency is essential in order to ensure fairness and rationality, consistency in decision making and ease of understanding. The uncertainties, assumptions made, and the effect of these on the final conclusions should be documented.

6. Testing

Surveillance involves the detection of *disease* by the use of appropriate case definitions based on the results of one or more tests for evidence of *disease* status. In this context, a test may range from detailed laboratory examinations to field observations and the analysis of production records. The performance of a test at the population level (including field observations) may be described in terms of its *sensitivity* and *specificity* and predictive values. Imperfect *sensitivity* and/or *specificity* will have an impact on the conclusions from surveillance. Therefore, these parameters should be taken into account in the design of surveillance systems and analysis of surveillance data as described in this Appendix.

Annex XVIII (contd)

Annex IV (contd)

Although not determined for many aquatic *diseases*, *sensitivity* and *specificity* should be estimated as best as possible for a specific testing situation. Alternatively, where values for *sensitivity* and/or *specificity* for a particular test and testing situation are estimated in the disease chapter in the *Aquatic Manual*, these values may be used as a guide.

Samples from a number of animals or units may be pooled and subjected to a testing protocol. The results should be interpreted using *sensitivity* and *specificity* values that have been determined or estimated for that particular pool size and testing procedure.

#### 7. Quality assurance

Surveillance systems should incorporate the principles of quality assurance and be subjected to periodic auditing to ensure that all components of the system function and provide verifiable documentation of procedures and basic checks to detect significant deviations of procedures from those documented in the design.

#### 8. Validation

Results from animal health surveillance systems are subject to one or more potential *biases*. When assessing the results, care should be taken to identify potential *biases* that can inadvertently lead to an over-estimate or an under-estimate of the parameters of interest.

#### 9. Data collection and management

The success of a surveillance system is dependent on a reliable process for data collection and management. The process may be based on paper records or computerised. Even where data are collected for non-survey purposes (e.g. during *disease* control interventions, inspections for movement control or during *disease* eradication schemes), the consistency and quality of data collection and event reporting in a format that facilitates analysis, is critical. Factors influencing the quality of collected data include:

- a) the distribution of, and communication between, those involved in generating and transferring data from the field to a centralised location;
- b) motivation of the people involved in the surveillance system;
- c) the ability of the data processing system to detect missing, inconsistent or inaccurate data, and to address these problems;
- d) maintenance of disaggregated data rather than the compilation of summary data;
- e) minimisation of transcription errors during data processing and communication.

Article x.x.x.4.

### ~~Structured p~~ **Population-based surveys**

In addition to the principles for surveillance discussed in article 6, the following guidelines should be used when planning, implementing and analysing surveys.

Annex XVIII (contd)Annex IV (contd)1. Types of surveys

Surveys may be conducted on the entire *target population* (i.e. a census) or on a sample. Periodic or repeated surveys conducted in order to document *disease* freedom should be done using probability based sampling methods (simple random selection, cluster sampling, stratified sampling, systematic sampling) so that data from the *study population* can be extrapolated to the *target population* in a statistically valid manner. Non-probability based sampling methods (convenience, expert choice, quota) can also be used. Recognising the inherent impracticalities in sampling from some aquatic populations, non-probability based sampling could be used when *biases* are recognised and used to optimise detection.

The sources of information should be fully described and should include a detailed description of the sampling strategy used for the selection of units for testing. Also, consideration should be made of any *biases* that may be inherent in the survey design.

2. Survey design

The population of *epidemiological units* should first be clearly defined; hereafter sampling units appropriate for each stage, depending on the design of the survey, should be defined.

The design of the survey will depend on the size and structure of the population being studied, the epidemiology of the *disease* and the resources available.

3. Sampling

The objective of sampling from a population is to select a subset of units from the population that is representative of the population with respect to the object of the study such as the presence or absence of *disease*. Sampling should be carried out in such a way as to provide the best likelihood that the sample will be representative of the population, within the practical constraints imposed by different environments and production systems. In order to detect the presence of a *disease* in a population of unknown *disease* status, ~~targeted~~ sampling methods that optimise the detection of *disease* can be used. In such cases, care should be taken regarding the inferences made from the results.

4. Sampling methods

When selecting *epidemiological units* from within a population the objectives of the surveillance system should be considered. In general, *probability sampling* (e.g. simple random selection) is preferable. When this is not possible, sampling should provide the best practical chance of generating optimal inferences about *disease* patterns in the *target population*.

In any case, the sampling method used at all stages should be fully documented and justified.

5. Sample size

In general, surveys are conducted either to demonstrate the presence or absence of a factor (e.g. *disease*) or to estimate a parameter (e.g. the prevalence of *disease*). The method used to calculate sample size for surveys depends on the purpose of the survey, the expected prevalence, the level of confidence desired of the survey results and the performance of the tests used.

Article x.x.x.5.

**Structured-a Non-random data sources used in surveillance**

Surveillance systems routinely use ~~structured~~ non-random data, either alone or in combination with surveys.

1. Common non-random surveillance data sources

A wide variety of non-random surveillance data sources may be available. These vary in their primary purpose and the type of surveillance information they are able to provide. Some surveillance systems are primarily established as *early detection systems*, but may also provide valuable information to demonstrate freedom from *disease*. Other systems provide cross-sectional information suitable for prevalence estimation, either once or repeatedly, while yet others provide continuous information, suitable for the estimate of incidence data (e.g. *disease* reporting systems, sentinel sites, testing schemes).

## a) Disease reporting or notification systems

Data derived from *disease* reporting systems can be used in combination with other data sources to substantiate claims of animal health status, to generate data for risk analysis, or for early detection. The first step of a *disease* reporting or notification system is often based on the observation of abnormalities (e.g. clinical signs, reduced growth, elevated mortality rates, behavioural changes, etc.), which can provide important information about the occurrence of endemic, exotic or new *diseases*. Effective laboratory support is however, an important component of most reporting systems. Reporting systems relying on laboratory confirmation of suspect clinical cases should use tests that have a high *specificity*. Reports should be released by the laboratory in a timely manner, with the amount of time from *disease* detection to report generation minimised.

## b) Control programmes/health schemes

Animal *disease* control programmes or health schemes, while focusing on the control or eradication of specific *diseases*, should be planned and structured in such a manner as to generate data that are scientifically verifiable and contribute to ~~structured~~ surveillance.

## c) Targeted testing/screening

This may involve testing targeted to selected sections of the population (subpopulations), in which *disease* is more likely to be introduced or found. Examples include testing culled and dead animals, animals exhibiting clinical signs, animals located in a defined geographical area and specific age or commodity group.

## d) Post-harvest inspections

Inspections of aquatic animal slaughter premises or processing plants may provide valuable surveillance data provided diseased aquatic animals survive to slaughter. Post-harvest inspections are likely to provide good coverage only for particular age groups and geographical areas. Post-harvest surveillance data are subject to obvious *biases* in relation to target and *study populations* (e.g. only animals of a particular class and age may be slaughtered for human consumption in significant numbers). Such *biases* need to be recognised when analysing surveillance data.

Annex XVIII (contd)Annex IV (contd)

Both for traceback in the event of detection of *disease* and for analysis of spatial and population-level coverage, there should be, if possible, an effective identification system that relates each animal in the slaughter premises/processing plant to its locality of origin.

## e) Laboratory investigation records

Analysis of laboratory investigation records may provide useful surveillance information. The coverage of the system will be increased if analysis is able to incorporate records from national, accredited, university and private sector laboratories. Valid analysis of data from different laboratories depends on the existence of standardised diagnostic procedures and standardised methods for interpretation and data recording. If available, the method listed in the *Aquatic Manual* in relation to the purpose of testing should be used. As with post-harvest inspections, there needs to be a mechanism to relate specimens to the farm of origin. It must be recognised that laboratory submissions may not accurately reflect the *disease* situation on the farm.

## f) Biological specimen banks

Specimen banks consist of stored specimens, gathered either through representative sampling or opportunistic collection or both. Specimen banks may contribute to retrospective studies, including providing support for claims of historical freedom from *disease*, and may allow certain studies to be conducted more quickly and at lower cost than alternative approaches.

## g) Sentinel units

Sentinel units/sites involve the identification and regular testing of one or more of animals of known health/exposure status in a specified geographical location to detect the occurrence of *disease*. They are particularly useful for surveillance of *diseases* with a strong spatial component, such as vector-borne *diseases*. Sentinel units provide the opportunity to target surveillance depending on the likelihood of *disease* (related to vector habitats and host population distribution), cost and other practical constraints. Sentinel units may provide evidence of freedom from *disease*, or provide data on prevalence and incidence as well as the distribution of *disease*. Cohabitation of sentinel units (preferably of the most susceptible species and life stage) with a susceptible population should be considered for testing *disease* in populations of valuable animals, the lethal sampling of which may be unacceptable (e.g. ornamental fish) or in animal subpopulations where sampling techniques are incapable of detecting the presence of disease or infection (e.g. where vaccination means that serological tests are inapplicable).

## h) Field observations

Clinical observations of *epidemiological units* in the field are an important source of surveillance data. The *sensitivity* and/or *specificity* of field observations may be relatively low, but these can be more easily determined and controlled if a clear, unambiguous and easy to apply standardised case definition is applied. Education of potential field observers in application of the case definition and reporting is an important component. Ideally, both the number of positive observations and the total number of observations should be recorded.

## i) Farm production records

Systematic analysis of farm production records may be used as an indicator of the presence or absence of *disease* at the population level. If production records are accurate and consistently maintained, the *sensitivity* of this approach may be quite high (depending on the *disease*), but the *specificity* is often quite low.

## 2. Critical elements for ~~structured~~ non-random data used in surveillance

There ~~is~~<sup>are</sup> a number of critical factors that should be taken into account when using ~~structured~~ non-random surveillance data such as coverage of the population, duplication of data, and *sensitivity* and *specificity* of tests that may give rise to difficulties in the interpretation of data. Surveillance data from non-random data sources may increase the level of confidence or be able to detect a lower level of prevalence with the same level of confidence compared to ~~structured~~ surveys.

## 3. Analytical methodologies

Different scientifically valid methodologies may be used for the analysis of non-random surveillance data. This most often requires information on parameters of importance to the surveillance system, such as *sensitivity* and *specificity* and prior probabilities of infection (e.g. for negative predictive value calculations). Where no such data are available, estimates based on expert opinions, gathered and combined using a formal, documented and scientifically valid methodology may be used.

## 4. Combination of multiple sources of data

The methodology used to combine the evidence from multiple or recurrent (e.g. time series) data sources should be scientifically valid, and fully documented including references to published material.

Surveillance information gathered from the same country, *zone* or *compartment* at different times (e.g. repeated annual surveys) may provide cumulative evidence of animal health status. Such evidence gathered over time may be combined to provide an overall level of confidence. However, a single larger survey, or the combination of data collected during the same time period from multiple random or non-random sources, may be able to achieve the same level of confidence in a shorter period of time.

Analysis of surveillance information gathered intermittently or continuously over time should, where possible, incorporate the time of collection of the information to take into account the decreased value of older information. The *sensitivity*, *specificity* and completeness of data from each source should also be taken into account for the final overall confidence level estimation.

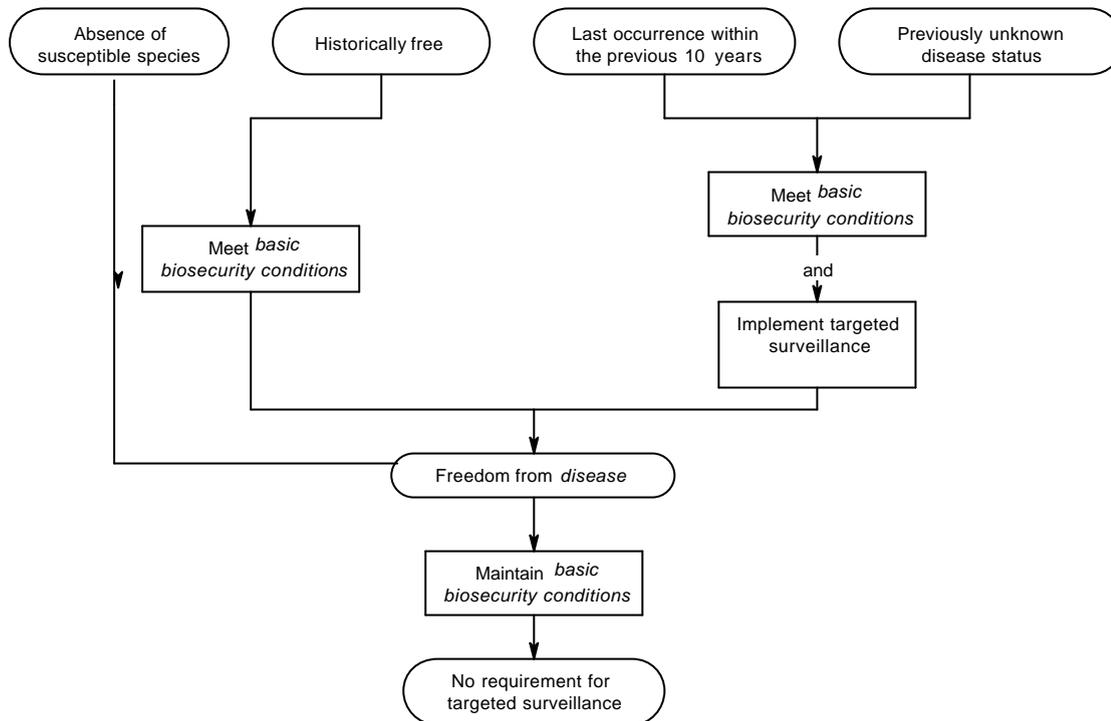
Article x.x.x.6.

## **Pathways to demonstrate freedom from disease**

The different paths to declaration of freedom from *disease* are summarised in the diagram below.

### 1. Absence of susceptible species

Unless otherwise specified in the relevant *disease* chapter, a country, *zone* or *compartment* may be recognised as being free from *disease* without applying *targeted surveillance* if there are no *susceptible species* (as listed in the relevant chapter of this *Aquatic Manual*, or in the scientific literature) present in that country, *zone* or *compartment*.

Annex XVIII (contd)Annex IV (contd)2. Historically free

Unless otherwise specified in the relevant *disease* chapter, a country, *zone* or *compartment* may be declared free from *disease* without formally applying a pathogen-specific surveillance programme when:

- a) there has never been a substantiated occurrence of *disease* reported officially or in the scientific literature (peer reviewed), or
- b) *disease* has not occurred for at least 10 years, provided that the *disease* agents are likely to produce identifiable clinical signs in observable susceptible animals.

and for at least the past 10 years:

- c) the *basic biosecurity conditions* are in place and effectively enforced;
- d) no vaccination against the *disease* has been carried out unless otherwise allowed for in the *Aquatic Code*;
- e) *disease* is not known to be established in wild aquatic animals within the country or *zone* intended to be declared free. (A country or *zone* cannot apply for historical freedom if there is any evidence of *disease* in wild aquatic animals. However, specific surveillance in wild aquatic animals is not necessary.)

Annex XVIII (contd)

Annex IV (contd)

A country, *zone* or *compartment* that was *self-declared* free on the basis of the absence of susceptible species, but subsequently introduces any of the susceptible species as listed in the *Aquatic Manual*, may be considered historically free from the *disease* provided that:

- f) the country, *zone* or *compartment* of origin was declared free of the *disease* at the time of introduction;
- g) *basic biosecurity conditions* were introduced prior to the introduction;
- h) no vaccination against the *disease* has been carried out unless otherwise allowed for in the *disease* specific chapter of this *Aquatic Code*.

### 3. Last occurrence within the previous 10 years/previously unknown status

Countries, *zones* or *compartments* that have achieved eradication (or in which the *disease* has ceased to occur) within the previous 10 years or where the *disease* status is unknown, should follow the pathogen-specific surveillance requirements in the *Aquatic Manual* if they exist. In the absence of *disease* specific information to aid the development of a surveillance system, declaration of *disease* freedom should follow at least 2 surveys per year (for at least 2 consecutive years) to be conducted 3 or more months apart, at the appropriate life stage and at times of the year when temperature and season offer the best opportunity to detect the pathogen. Surveys should be designed to provide an overall 95% confidence and with a design prevalence at the animal and higher (i.e. pond, farm, village, etc.) levels being 2% or lower (this value may be different for different *diseases* and may be provided in the specific *disease* chapter in the *Aquatic Manual*). Such surveys should not be based on voluntary submission and should be developed following the guidelines provided in the *Aquatic Manual*. Survey results will provide sufficient evidence of *disease* freedom provided that for at least the past 10 years these additional criteria are met:

- a) the *basic biosecurity conditions* are in place and effectively enforced;
- b) no vaccination against the *disease* has been carried out unless otherwise provided in the *Aquatic Code*;
- c) *disease* is not known to be established in wild aquatic animals within the country or *zone* intended to be declared free. (A country or *zone* cannot apply for freedom if there is any evidence of *disease* in wild aquatic animals. Specific surveillance in wild aquatic animals of susceptible species is necessary to confirm absence.)

Article x.x.x.7.

### **Maintenance of disease free status**

A country or *zone* that has been declared free from *disease* following the provisions of the *Aquatic Code* may discontinue pathogen-specific surveillance while maintaining the *disease* free status provided that:

1. if present, the pathogen is likely to produce identifiable clinical signs in observable *susceptible species*;
2. the *basic biosecurity conditions* are in place and effectively enforced;
3. no vaccination against the *disease* has been carried out unless otherwise provided in the *Aquatic Code*;
4. surveillance has demonstrated that *disease* is not present in wild aquatic animal populations of *susceptible species*.

Annex XVIII (contd)Annex IV (contd)

A special case can be made for a disease free compartment located in a country or zone that is not declared disease free, ~~proven to be free from disease~~ if surveillance should be is maintained at a level commensurate with the degree of risk and exposure to potential sources of *disease* is prevented.

Article x.x.x.8.

### **Design of surveillance programmes to demonstrate freedom from disease**

A surveillance programme to demonstrate freedom from *disease* should meet the following requirements in addition to the general requirements for surveillance outlined in this Appendix.

Freedom from *disease* implies the absence of the pathogenic agent in the country, *zone* or *compartment*. Scientific methods cannot provide absolute certainty of the absence of *disease*. Demonstrating freedom from *disease* involves providing sufficient evidence to demonstrate (to a level of confidence acceptable to Members) that *disease* with a specified pathogen is not present in a population. In practice, it is not possible to prove (i.e. be 100% confident) that a population is free from *disease*. Instead, the aim is to provide adequate evidence (to an acceptable level of confidence), that *disease*, if present, is present in less than a specified proportion of the population.

However, apparent *disease* at any level in the target population automatically invalidates any freedom from *disease* claim unless the positive test results are accepted as false positives based on *specificity* values described in the relevant *disease* chapter.

The provisions of this Article are based on the principles described above and the following premises:

- in the absence of *disease* and vaccination, the farmed and wild animal populations would become susceptible over a period of time;
- the *disease* agents to which these provisions apply are likely to produce identifiable clinical signs in observable susceptible animals;
- to increase the probability of detecting the specific disease agent, the susceptibility of the aquatic animal and the timing of sampling must be under appropriate conditions;
- the *Competent Authority* will be able to investigate, diagnose and report *disease*, if present;
- the appropriate diagnostic method as described in the OIE Aquatic Manual be used
- any claim for the absence of *disease* over a long period of time in a susceptible population can be substantiated by effective *disease* investigation and reporting by a Member.

#### 1. Objectives

The objective of this kind of surveillance system is to contribute on an on-going basis evidence to demonstrate freedom from *disease* in a particular country, *zone* or *compartment* with a known confidence and reference to a predetermined design prevalence and diagnostic test characteristics. The level of confidence and the design prevalence will depend on the testing situation, *disease* and host population characteristics and on the resources available.

A single such survey can contribute evidence adding to an on-going collection of health data (see also Section 5. Specific requirements for complex non-survey data sources). However, single surveys in isolation rarely, if ever, provide sufficient evidence that an aquatic animal *disease* is absent and must be augmented with on-going targeted evidence collection (e.g. ongoing *disease* sampling or passive detection capabilities) to substantiate claims of freedom from *disease*.

## 2. Population

The *population* of *epidemiological units* must be clearly defined. The target population consists of all individuals of all *species susceptible* to the disease in a country, *zone* or *compartment* to which the surveillance results apply. Sometimes components of the target population are at higher risk of being the point of introduction for an exotic disease. In these cases, it is advisable to focus surveillance efforts on this part of the population, such as farms on a geographical border.

The design of the survey will depend on the size and structure of the *population* being studied. If the *population* is relatively small and can be considered to be homogenous with regards to risk of infection, a single-stage survey can be used. If different subpopulations of the same *aquaculture establishment* do not share water, they may be considered as epidemiologically separate populations.

In larger *populations* where a sampling frame is not available, or when there is a likelihood of clustering of disease, multi-stage sampling is required. In two-stage sampling, at the first stage of sampling, groups of animals (e.g. ponds, farms or villages) are selected. At the second stage, animals are selected for testing from each of the selected groups.

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

## 3. Sources of evidence

Surveillance data may originate from a number of different sources, including:

- a) ~~structured~~, population-based surveys using one or more tests to detect the aetiological agent or evidence of infection;
- b) other ~~structured~~ non-random sources of data, such as:
  - i) sentinel sites;
  - ii) disease notifications and laboratory investigation records;
  - iii) academic and other scientific studies;
- c) a knowledge of the biology of the agent, including environmental, host *population* distribution, known geographical distribution, vector distribution and climatic information;
- d) history of imports of potentially infected material;
- e) biosecurity measures in place;
- f) any other sources of information that provide contributory evidence regarding disease in the country, *zone* or *compartment*.

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

Annex XVIII (contd)Annex IV (contd)4. Statistical methodology

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

- a) The survey design
- b) The *sensitivity* and *specificity* of the test, or test system
- c) The design prevalence (or prevalences where a multi-stage design is used)
- d) The results of the survey.

Analysis of data for evidence of freedom from infection involves estimating the probability (a) that the evidence observed (the results of surveillance) could have been produced under the null hypothesis that infection is present in the *population* at a specified prevalence(s) (the design prevalences). The *confidence* in (or, equivalently, the *sensitivity* of) the surveillance system that produced the evidence is equal to  $1-a$ . If the *confidence* level exceeds a pre-set threshold, the evidence is deemed adequate to demonstrate freedom from infection.

The required level of *confidence* in the surveillance system (probability that the system would detect infection if infection were present at the specified level) must be greater than or equal to 95%.

The power (probability that the system would report that no infection is present if infection is truly not present) may be set to any value. By convention, this is often set to 80%, but may be adjusted according to the country's or *zone's* requirements.

Different statistical methodologies for the calculation of the probability a, including both quantitative and qualitative approaches, are acceptable as long as they are based on accepted scientific principles.

The methodology used to calculate the *confidence* in the surveillance system must be scientifically based and clearly documented, including references to published work describing the methodology.

Statistical analysis of surveillance data often requires assumptions about population parameters or test characteristics. These are usually based on expert opinion, previous studies on the same or different populations, expected biology of the agent, and so on. The uncertainty around these assumptions must be quantified and considered in the analysis (e.g. in the form of prior probability distributions in a Bayesian setting).

For surveillance systems used to demonstrate freedom from specific diseases, calculation of the *confidence* of a surveillance system is based on the null hypothesis that infection is present in the *population*. The level of infection is specified by the design prevalence. In the simplest case, this is the prevalence of infection in a homogenous *population*. More commonly, in the presence of a complex (e.g. multi-level) population structure more than one design prevalence value is required, for instance, the animal-level prevalence (proportion of infected animals in an infected farm) and the group-level prevalence (proportion of infected farms in the country, *zone* or *compartment* ). Further levels of clustering may be considered, requiring further design prevalence values.

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

- At the individual animal level, the design prevalence is based on the biology of the infection in the *population*. It is equal to the minimum expected prevalence of infection in the *study population*, if the infection had become established in that *population*. It is dependent on the dynamics of infection in the *population* and the definition of the *study population* (which may be defined to maximise the expected prevalence in the presence of infection).
- A suitable design prevalence value at the animal level (e.g. prevalence of infected animals in a cage) may be:
  - between 1% and 5% for infections that are present in a small part of the population e.g. are transmitted slowly or are at the early stages of an outbreak, etc.;
  - over 5% for highly transmissible infections.

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

- At higher levels (e.g. cage, pond, farm, village, etc.) the design prevalence usually reflects the prevalence of infection that is practically and reasonably able to be detected by a *surveillance system*. Detection of infection at the lowest limit (a single infected *unit* in the *population*) is rarely feasible in large *populations*. The expected behaviour of the infection may also play a role. Infections that have the ability to spread rapidly between farms may have a higher farm-level design prevalence than slow-moving infections.

A suitable design prevalence value for the first level of clustering, (e.g. proportion of infected farms in a *zone*) ~~may be up to~~ normally not greater than 2%. If a higher design prevalence is selected, it must be justified.

When surveillance data are used to estimate incidence and prevalence measures for the purpose of describing disease occurrence in terms of animal unit, time and place, these measures can be calculated for an entire population and specific time period, or for subsets defined by host characteristics (e.g. age-specific incidence). Incidence estimation requires on-going surveillance to detect new cases while prevalence is the estimated proportion of infected individuals in a population at a given time point. The estimation process must consider test *sensitivity* and *specificity*.

## 5. Clustering of infection

Infection in a country, *zone* or *compartment* usually clusters rather than being uniformly distributed through a *population*. Clustering may occur at a number of different levels (e.g. a cluster of moribund fish in a pond, a cluster of ponds in a farm, or a cluster of farms in a *zone*). Except when dealing with demonstrably homogenous *populations*, surveillance must take this clustering into account in the design and the statistical analysis of the data, at least at what is judged to be the most significant level of clustering for the particular animal *population* and infection.

Annex XVIII (contd)Annex IV (contd)6. Test characteristics

All surveillance involves performing one or more *tests* for evidence of the presence of current or past infection, ranging from detailed laboratory examinations to farmer observations. The performance level of a *test* at the *population* level is described in terms of its *sensitivity* and *specificity*. ~~These probabilities of the correct test result refer to the entire sampling process, including sample selection, collection, handling and processing (which if not conducted in the optimal way for the disease in question, as described in the disease chapters of the *Aquatic Manual*, will reduce the sensitivity of the method), and the actual laboratory test performance.~~ Imperfect *sensitivity* and/or *specificity* impact on the interpretation of surveillance results and must be taken into account in the analysis of surveillance data. For example, in the case of a test with imperfect *specificity*, if the population is free of disease or has a very low prevalence of infection, all or a large proportion of positive tests will be false. Subsequently, samples that test positive can be confirmed or refuted using a highly specific test. Where more than one test is used in a *surveillance system* (sometimes called using tests in series or parallel), the *sensitivity* and *specificity* of the test combination must be calculated.

All calculations must take the performance level (*sensitivity* and *specificity*) of any *tests* used into account. The values of *sensitivity* and *specificity* used for calculations must be specified, and the method used to determine or estimate these values must be documented. Test *sensitivity* and *specificity* can be different when applied to different populations and testing scenarios. For example, test sensitivity may be lower when testing carrier animals with low level infections compared to moribund animals with clinical disease. Alternatively, *specificity* depends on the presence of cross-reacting agents, the distribution of which may be different under different conditions or regions. Ideally, test performance should be assessed under the conditions of use otherwise increased uncertainty exists regarding their performance. In the absence of local assessment of tests, values for *sensitivity* and/or *specificity* for a particular *test* that are specified in this *Aquatic Manual* may be used but the increased uncertainty associated with these estimates should be incorporated into the analysis of results.

Pooled testing involves the pooling of specimens from multiple individuals and performing a single *test* on the pool. Pooled testing is an acceptable approach in many situations. Where pooled testing is used, the results of testing must be interpreted using *sensitivity* and *specificity* values that have been determined or estimated for that particular pooled testing procedure and for the applicable pool sizes being used. Analysis of the results of pooled testing must, where possible, be performed using accepted, statistically based methodologies, which must be fully documented, including published references.

When applied to a *surveillance system*, the probabilities of correct assessment of the health status of the *epidemiological unit* is affected by the entire sampling process, including sample selection, collection, handling and processing, as well as the actual laboratory test performance.

7. Multiple sources of information

Where multiple different data sources providing evidence of freedom from infection exist, each of these data sources may be analysed accordingly. The resulting estimates of the *confidence* in each data source may be combined to provide an overall level of *confidence* for the combined data sources.

The methodology used to combine the estimates from multiple data sources:

- a) must be scientifically valid, and fully documented, including references to published material; and
- b) should, where possible, take into account any lack of statistical independence between different data sources.

Annex XVIII (contd)

Annex IV (contd)

Surveillance information gathered from the same country, *zone* or *compartment* at different times (e.g. repeated annual surveys) may provide cumulative evidence of animal health status. Such evidence gathered over time may be combined to provide an overall level of confidence. However, a single larger survey, or the combination of data collected during the same time period from multiple random or non-random sources, may be able to achieve the same level of confidence in a shorter period of time.

Analysis of surveillance information gathered intermittently or continuously over time should, where possible, incorporate the time of collection of the information to take into account the decreased value of older information. The *sensitivity*, *specificity* and completeness of data from each source should also be taken into account for the final overall confidence level estimation.

## 8. Sampling

The objective of sampling from a population is to select a subset of units from the population that is representative of the *population* with respect to the characteristic of interest (in this case, the presence or absence of infection). The survey design may involve sampling at several levels. For sampling at the level of the *epidemiological units* or higher *units*, a formal *probability sampling* (e.g. simple random sampling) method must be used. Sampling should be carried out in such a way as to provide the best likelihood that the sample will be representative of the *population*, within the practical constraints imposed by different environments and production systems.

When sampling below the level of the *epidemiological unit* (e.g. individual animal), the sampling method used should provide the best practical chance of generating a sample that is representative of the *population* of the chosen *epidemiological unit*. Collecting a truly representative sample of individual animals (whether from a pond, cage or fishery) is often very difficult. To maximise the chance of finding infection, the aim should be to *bias* the sampling towards infected animals, e.g. selecting moribund animals, life stages with a greater chance of active infection, etc.

Biased or ~~targeted~~ sampling in this context involves sampling from a defined *study population* that has a different probability of infection than the *target population* of which it is a subpopulation. Once the *study population* has been identified, the objective is still to select a representative sample from this subpopulation.

The sampling method used at all levels must be fully documented and justified.

## 9. Sample size

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

- The *sensitivity* and *specificity* of the diagnostic test, or test system;
- The design prevalence (or prevalences where a multi-stage design is used);
- The level of *confidence* that is desired of the survey results.

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

- The size of the *population* (but it is acceptable to assume that the *population* is infinitely large);
- The desired power of the survey;
- Uncertainty about *sensitivity* and *specificity*.

Annex XVIII (contd)Annex IV (contd)

The specific sampling requirements will need to be tailor-made for each individual disease, taking into account its characteristics and the *specificity* and *sensitivity* of the accepted testing methods for detecting the disease agent in host populations.

FreeCalc<sup>13</sup> is a suitable software for the calculation of sample sizes at varying parameter values. The table below provides examples of sample sizes generated by the software for a type I and type II error of 5% (i.e. 95% confidence and 95% statistical power). However, this does not mean that a type 1 and type 2 error of 0.05 should always be used. For example, using a test with *sensitivity* and *specificity* of 99%, 528 units should be sampled. If 9 or less of those units test positive, the population can still be considered free of the disease at a design prevalence of 2% provided that all effort is made to ensure that all presumed false positives are indeed false. This means that there is a 95% confidence that the prevalence is 2% or lower.

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

Design prevalence	Sensitivity (%)	Specificity (%)	Sample size	Maximum number of false +ve if the population is free
2	100	100	149	0
2	100	99	524	9
2	100	95	1671	98
2	99	100	150	0
2	99	99	528	9
2	99	95	1707	100
2	95	100	157	0
2	95	99	542	9
2	95	95	1854	108
2	90	100	165	0
2	90	99	607	10
2	90	95	2059	119
2	80	100	186	0
2	80	99	750	12
2	80	95	2599	148
5	100	100	59	0
5	100	99	128	3
5	100	95	330	23
5	99	100	59	0
5	99	99	129	3
5	99	95	331	23
5	95	100	62	0
5	95	99	134	3
5	95	95	351	24
5	90	100	66	0
5	90	99	166	4
5	90	95	398	27
5	80	100	74	0
5	80	99	183	4

<sup>13</sup> FreeCalc – Cameron, AR. Software for the calculation of sample size and analysis of surveys to demonstrate freedom from disease. Available for free download from <http://www.ausvet.com.au>.

Annex XVIII (contd)Annex IV (contd)

Design prevalence	Sensitivity (%)	Specificity (%)	Sample size	Maximum number of false +ve if the population is free
5	80	95	486	32
10	100	100	29	0
10	100	99	56	2
10	100	95	105	9
10	99	100	29	0
10	99	99	57	2
10	99	95	106	9
10	95	100	30	0
10	95	99	59	2
10	95	95	109	9
10	90	100	32	0
10	90	99	62	2
10	90	95	123	10
10	80	100	36	0
10	80	99	69	2
10	80	95	152	12

#### 10. Quality assurance

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

Article x.x.x.9.

#### **Specific requirements for complex non-survey data sources for freedom from disease**

Data sources that provide evidence of freedom from infection, but are not based on ~~structured~~ population-based surveys may also be used to demonstrate freedom, either alone or in combination with other data sources. Different methodologies may be used for the analysis of such data sources, but the methodology must comply with the provisions of Section B.3. The approach used should, where possible, also take into account any lack of statistical independence between observations.

Analytical methodologies based on the use of step-wise probability estimates to describe the surveillance system may determine the probability of each step either by:

1. the analysis of available data, using a scientifically valid methodology; or where no data are available,
2. the use of estimates based on expert opinion, gathered and combined using a formal, documented and scientifically valid methodology.

Where there is significant uncertainty and/or variability in estimates used in the analysis, stochastic modelling or other equivalent techniques should be used to assess the impact of this uncertainty and/or variability on the final estimate of *confidence*.

Annex XVIII (contd)Annex IV (contd)

Article x.x.x.10.

**Surveillance for distribution and occurrence of disease**

Surveillance to determine distribution and occurrence of *disease* or of other relevant health related events is widely used to assess the prevalence and incidence of selected *disease* as an aid to decision making, for example implementation of control and eradication programmes. It also has relevance for the international movement of animals and products when movement occurs among infected countries.

In contrast to surveillance to demonstrate freedom from *disease*, surveillance for the distribution and occurrence of *disease* is usually designed to collect data about a number of variables of animal health relevance, for example:

- prevalence or incidence of *disease* in wild or cultured animals;
- morbidity and mortality rates;
- frequency of *disease* risk factors and their quantification;
- frequency distribution of variables in *epidemiological units*;
- frequency distribution of the number of days elapsing between suspicion of *disease* and laboratory confirmation of the diagnosis and/or to the adoption of control measures;
- farm production records, etc.

This section describes surveillance to estimate parameters of disease occurrence.

1. Objectives

The objective of this kind of surveillance system is to contribute on an on-going basis evidence to assess the occurrence and distribution of disease or infection in a particular country, *zone* or *compartment*. This will provide information for domestic disease control programmes and relevant disease occurrence information to be used by trading partners for qualitative and quantitative risk assessment.

A single such survey can contribute evidence adding to an on-going collection of health data (see also Section 5. Specific requirements for complex non-survey data sources).

2. Population

The *population* of *epidemiological units* must be clearly defined. The target population consists of all individuals of all species susceptible to the disease in a country, *zone* or *compartment* to which the surveillance results apply. Some local areas within a region may be known to be free of the disease of concern, allowing resources to be concentrated on known positive areas for greater precision of prevalence estimates and only verification of expected 0 prevalence areas.

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

Annex XVIII (contd)

Annex IV (contd)

In larger *populations* where a sampling frame is not available, or when there is a likelihood of clustering of disease, multi-stage sampling is required. ~~In two stage sampling, at the first stage of sampling, groups of animals (e.g. ponds, farms or villages) are selected. At the second stage, animals are selected for testing from each of the selected groups. For example, a multi-stage sampling process may involve sampling of farms or villages followed by sampling of fish from selected ponds within the sampled farms/villages.~~

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

### 3. Sources of evidence

Surveillance data may originate from a number of different sources, including:

- a) ~~structured~~, population-based surveys using one or more *tests* to detect the agent;
- b) other ~~structured~~ non-random sources of data, such as:
  - i) sentinel sites;
  - ii) disease notifications and laboratory investigation records;
  - iii) academic and other scientific studies;
- c) a knowledge of the biology of the agent, including environmental, host *population* distribution, known geographical distribution, vector distribution and climatic information;
- d) history of imports of potentially infected material;
- e) biosecurity measures in place;
- f) any other sources of information that provide contributory evidence regarding disease or infection in the country, *zone* or *compartment*.

The sources of evidence must be fully described. ~~In the case of a  $\Delta$  structured survey, this~~ must include a description of the sampling strategy used for the selection of *units* for testing. For complex surveillance systems, a full description of the system is required including consideration of any *biases* that may be inherent in the system. Evidence to support changes in prevalence/incidence of endemic disease must be based on valid, reliable methods to generate precise estimates with known error.

### 4. Statistical methodology

Analysis of survey data should be in accordance with the provisions of this chapter and should consider the following factors:

- a) The survey design;
- b) The *sensitivity* and *specificity* of the test, or test system;
- c) The results of the survey.

Annex XVIII (contd)Annex IV (contd)

For surveillance systems used to describe disease patterns, the purpose is to estimate prevalence or incidence with confidence intervals or probability intervals. The magnitude of these intervals expresses the precision of the estimates and is related to sample size. Narrow intervals are desirable but will require larger sample sizes and more dedication of resources. The precision of the estimates and the power to detect differences in prevalence between populations or between time points depends not only on sample size, but also on the actual value of the prevalence in the population or the actual difference. For this reason, when designing the surveillance system, a prior estimate/assumption of expected prevalence or expected difference in prevalence must be made.

For the purpose of describing disease occurrence, measures of animal unit, time and place can be calculated for an entire population and specific time period, or for subsets defined by host characteristics (e.g. age-specific incidence). Incidence estimation requires on-going surveillance to detect new cases in a specified time period while prevalence is the estimated proportion of infected individuals in a population at a given time point. The estimation process must consider test *sensitivity* and *specificity*.

Statistical analysis of surveillance data often requires assumptions about population parameters or test characteristics. These are usually based on expert opinion, previous studies on the same or different populations, expected biology of the agent, information contained in the specific disease chapter of the *Aquatic Manual*, and so on. The uncertainty around these assumptions must be quantified and considered in the analysis (e.g. in the form of prior probability distributions in a Bayesian setting).

When surveillance objectives are to estimate prevalence/incidence or changes in disease patterns, statistical analysis must account for sampling error. Analytic methods should be thoroughly considered and consultation with biostatistician/quantitative epidemiologist consulted beginning in the planning stages and continued throughout the programme.

#### 5. Clustering of infection

Infection in a country, *zone* or *compartment* usually clusters rather than being uniformly distributed through a *population*. Clustering may occur at a number of different levels (e.g. a cluster of moribund fish in a pond, a cluster of ponds in a farm, or a cluster of farms in a *zone*). Except when dealing with demonstrably homogenous *populations*, surveillance must take this clustering into account in the design and the statistical analysis of the data, at least at what is judged to be the most significant level of clustering for the particular animal *population* and infection. For endemic diseases, it is important to identify characteristics of the population which contribute to clustering and thus provide efficiency in disease investigation and control.

#### 6. Test characteristics

All surveillance involves performing one or more *tests* for evidence of the presence of current or past infection, ranging from detailed laboratory examinations to farmer observations. The performance level of a *test* at the *population* level is described in terms of its *sensitivity* and *specificity*. Imperfect *sensitivity* and/or *specificity* impact on the interpretation of surveillance results and must be taken into account in the analysis of surveillance data. For example, in populations with low prevalence of infection, a large proportion of positive tests may be false unless the tests used have perfect *specificity*. To ensure detection in such instances, a highly sensitive test is frequently used for initial screening and then confirmed with highly specific tests.

Annex XVIII (contd)

Annex IV (contd)

All calculations must take the performance level (*sensitivity* and *specificity*) of any *tests* used into account. The values of *sensitivity* and *specificity* used for calculations must be specified, and the method used to determine or estimate these values must be documented. Test *sensitivity* and *specificity* can be different when applied to different populations and testing scenarios. For example, test *sensitivity* may be lower when testing carrier animals with low level infections compared to moribund animals with clinical disease. Alternatively, *specificity* depends on the presence of cross-reacting agents, the distribution of which may be different under different conditions or regions. Ideally, test performance should be assessed under the conditions of use otherwise increased uncertainty exists regarding their performance. In the absence of local assessment of tests, values for *sensitivity* and/or *specificity* for a particular *test* that are specified in this *Aquatic Manual* may be used but the increased uncertainty associated with these estimates should be incorporated into the analysis of results.

Pooled testing involves the pooling of specimens from multiple individuals and performing a single *test* on the pool. Pooled testing is an acceptable approach in many situations. Where pooled testing is used, the results of testing must be interpreted using *sensitivity* and *specificity* values that have been determined or estimated for that particular pooled testing procedure and for the applicable pool sizes being used. Analysis of the results of pooled testing must, where possible, be performed using accepted, statistically based methodologies, which must be fully documented, including published references.

Test results from surveillance for endemic disease will provide estimates of apparent prevalence (AP). Using diagnostic *sensitivity* (DSe) and diagnostic *specificity* (DSp) as described in chapter 1.1.2 of this *Aquatic Manual*, true prevalence (TP) should be calculated with the following formula:

$$TP = (AP + DSp - 1) / (DSe + DSp - 1)$$

In addition, it should be remembered that different laboratories may obtain conflicting results for various test, host, or procedure-related reasons. Therefore, *sensitivity* and *specificity* parameters should be validated for the particular laboratory and process.

## 7. Multiple sources of information

Where multiple different data sources providing information on infection or disease are generated, each of these data sources may be analysed and presented separately.

Surveillance information gathered from the same country, *zone* or *compartment* at different times and similar methodology (e.g. repeated annual surveys) may provide cumulative evidence of animal health status and changes. Such evidence gathered over time may be combined (e.g. using Bayesian methodology) to provide more precise estimates and details of disease distribution within a population.

Apparent changes in disease occurrence of endemic diseases may be real or due to other factors influencing detection proficiency.

## 8. Sampling

The objective of sampling from a *population* is to select a subset of *units* from the *population* that is representative of the *population* with respect to the characteristic of interest (in this case, the presence or absence of infection). The survey design may involve sampling at several levels. For sampling at the level of the *epidemiological units* or higher *units*, a formal *probability sampling* (e.g. simple random sampling) method must be used. Sampling should be carried out in such a way as to provide the best likelihood that the sample will be representative of the *population*, within the practical constraints imposed by different environments and production systems.

Annex XVIII (contd)Annex IV (contd)

When sampling below the level of the *epidemiological unit* (e.g. individual animal), the method used should be probability-based sampling. Collecting a true probability-based sample is often very difficult and care should therefore be taken in the analysis and interpretation of results obtained using any other method, the danger being that inferences could not be made about the sampled *population*.

The sampling method used at all levels must be fully documented and justified.

9. Sample size

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

- The *sensitivity* and *specificity* of the *diagnostic test* (single or in combination);
- Expected prevalence or incidence in the *population* (or prevalences/incidences where a multi-stage design is used);
- The level of *confidence* that is desired of the survey results.
- The *precision* desired (i.e. the width of the *confidence* or *probability intervals*).

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

- The size of the *population* (but it is acceptable to assume that the *population* is infinitely large);
- Uncertainty about *sensitivity* and *specificity*.

The specific sampling requirements will need to be tailor-made for each individual disease, taking into account its characteristics and the *specificity* and *sensitivity* of the accepted testing methods for detecting the disease agent in host populations.

A number of software packages, e.g. Survey Tool Box ([www.aciar.gov.au](http://www.aciar.gov.au); [www.ausvet.com.au](http://www.ausvet.com.au)), WinPEPI ([www.sagebrushpress.com/pepibook.html](http://www.sagebrushpress.com/pepibook.html)) can be used for the calculation of sample sizes.

In the case in which the values of Se and Sp are not known (e.g. no information is available in the specific disease chapter in the *Aquatic Manual*), they should not automatically be assumed to be 100%. Assumed values should be produced in consultation with subject-matter experts.

10. Quality assurance

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

Article x.x.x.11.

**Examples of surveillance programmes**

The following examples describe surveillance systems and approaches to the analysis of evidence for demonstrating freedom from *disease*. The purpose of these examples is:

- to illustrate the range of approaches that may be acceptable;

- to provide practical guidance and models that may be used for the design of specific surveillance systems; and
- to provide references to available resources that are useful in the development and analysis of surveillance systems.

While these examples demonstrate ways in which freedom from *disease* may be successfully demonstrated, they are not intended to be prescriptive. Countries are free to use different approaches, as long as they meet the requirements of this chapter.

The examples deal with the use of ~~structured~~ surveys and are designed to illustrate different survey designs, sampling schemes, the calculation of sample size, and analysis of results. It is important to note that alternative approaches to demonstrating freedom using complex non-survey-based data sources are also currently being developed and may soon be published<sup>14</sup>.

#### 1. Example 1. – one-stage ~~structured~~ survey (farm certification)

##### a) Context

A freshwater aquaculture industry raising fish in tanks has established a farm certification scheme. This involves demonstrating farm-level freedom from a particular (hypothetical) disease (Disease X). The disease does not spread very quickly, and is most common during the winter months, with adult fish at the end of the production cycle being most severely affected. Farms consist of a number of grow-out tanks, ranging from 2 to 20, and each tank holds between 1000 and 5000 fish.

##### b) Objective

The objective is to implement surveillance that is capable of providing evidence that an individual farm is free from Disease X. (The issue of national or *zone* freedom, as opposed to farm freedom, is considered in the next example.)

##### c) Approach

The accreditation scheme establishes a set of standard operating procedures and requirements for declaration of freedom, based on the guidelines given in this chapter. These require farms to undertake a ~~structured~~ survey capable of producing 95% confidence that the disease would be detected if it were present. Once farms have been surveyed without detecting disease, they are recognised as free, as long as they maintain a set of minimum biosecurity standards. These standards are designed to prevent the introduction of Disease X into the farm (through the implementation of controls specific to the method of spread of that disease) and to ensure that the disease would be detected rapidly if it were to enter the farm (based on evidence of adequate health record keeping and the prompt investigation of unusual disease events). The effective implementation of these biosecurity measures is evaluated with annual on-farm audits conducted by independent auditors.

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<sup>14</sup> International EpiLab, Denmark, Research Theme 1: Freedom from disease.  
[http://www.vetinst.dk/high\\_uk.asp?page\\_id=196](http://www.vetinst.dk/high_uk.asp?page_id=196)

Annex XVIII (contd)Annex IV (contd)

## d) Survey standards

Based on the guidelines given in this chapter, a set of standards are established for the conduct of surveys to demonstrate freedom from infection with causative agent of Disease X. These standards include:

- i) The level of confidence required of the survey is 95% (i.e. Type I error = 5%).
- ii) The power of the survey is arbitrarily set at 95% (i.e. Type II error = 5%, which means that there is a 5% chance of concluding that a non-diseased farm is infected).
- iii) The target population is all the fish on the farm. Due to the patterns of disease in this production system, in which only fish in the final stages of grow-out, and only in winter are affected, the *study population* is defined as grow-out fish during the winter months.
- iv) The issue of clustering is considered. As fish are grouped into tanks, this is the logical level at which to consider clustering. However, when a farm is infected, the disease often occurs in multiple tanks, so there is little evidence of strong clustering. Also, the small number of tanks on a single farm means that it is difficult to define a design prevalence at the tank level (i.e. the proportion of infected tanks that the survey should be able to detect on the farm). For these reasons, it is decided to treat the entire grow-out population of each farm as a single homogenous population.
- v) Stratification is also considered. In order to ensure full representation, it is decided to stratify the sample size by tank, proportional to the population of each tank.
- vi) The design prevalence at the animal level is determined based on the epidemiology of the disease. The disease does not spread quickly, however, in the defined target population, it has been reported to affect at least 10% of fish, if the population is infected. In order to take the most conservative approach, an arbitrarily low design prevalence of 2% is used. A prevalence of 10% may have been used (and would result in a much smaller sample size), but the authorities were not convinced by the thought that the population could still be infected at a level of say 5%, and disease still not be detected.
- vii) The test used involves destructive sampling of the fish, and is based on an antigen-detection enzyme-linked immunosorbent assay (ELISA). Disease X is present in some parts of the country (hence the need for a farm-level accreditation programme). This has provided the opportunity for the *sensitivity* and the *specificity* of the ELISA to be evaluated in similar populations to those on farms. A recent study (using a combination of histology and culture as a gold standard) estimated the *sensitivity* of the ELISA to be 98% (95% confidence interval 96.7–99.2%), and the *specificity* to be 99.4% (99.2–99.6%). Due to the relatively narrow confidence intervals, it was decided to use the point estimates of the *sensitivity* and *specificity* rather than complicate calculations by taking the uncertainty in those estimates into account.

## e) Sample size

The sample size required to meet the objectives of the survey is calculated to take the population size, the test performance, the confidence required and the design prevalence into account. As the population of each farm is relatively large, differences in the total population of each farm have little effect on the calculated sample size. The other parameters for sample size calculation are fixed across all farms. Therefore, a standard sample size (based on the use of this particular ELISA, in this population) is calculated. The sample size calculations are performed using the *FreeCalc* software<sup>15</sup>. Based on the parameters listed above, the sample size required is calculated to be 410 fish per farm. In addition, the program calculates that, given the imperfect *specificity*, it is still possible for the test to produce up to five false-positive reactors from an uninfected population using this sample size. The authorities are not comfortable with dealing with false-positive reactors, so it is decided to change the test system to include a confirmatory test for any positive reactors. Culture is selected as the most appropriate test, as it has a *specificity* that is considered to be 100%. However, its *sensitivity* is only 90% due to the difficulty of growing the organism.

As two tests are now being used, the performance of the test system must be calculated, and the sample size recalculated based on the test system performance.

Using this combination of tests (in which a sample is considered positive only if it tests positive to both tests), the *specificity* of the combined two tests can be calculated by the formula:

$$Sp_{Combined} = Sp_1 + Sp_2 - (Sp_1 \times Sp_2)$$

which produces a combined *specificity* of  $1 + 0.994 - (1 \times 0.994) = 100\%$

The *sensitivity* may be calculated by the formula:

$$Se_{Combined} = Se_1 \times Se_2$$

which produces a combined *sensitivity* of  $0.9 \times 0.98 = 88.2\%$

These new values are used to calculate the survey sample size yielding a result of 169 fish. It is worth noting that attempts to improve the performance of a test (in this case increase *specificity*) generally result in a decrease in the performance of the other aspect of the test performance (*sensitivity* in this example). However, in this case, the loss of *sensitivity* is more than compensated for by the decreased sample size due to the improved *specificity*.

It is also worth noting that, when using a test system with 100% *specificity*, the effective power of the survey will always be 100%, regardless of the figure used in the design. This is because it is not possible to make a Type II error, and conclude that the farm is infected when it is not.

A check of the impact of population size on the calculated sample size is worthwhile. The calculated sample size is based on an infinitely large population. If the population size is smaller, the impact on sample size is shown in the following table:

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<sup>15</sup> FreeCalc – Cameron, AR. Software for the calculation of sample size and analysis of surveys to demonstrate freedom from disease. Available for free download from <http://www.ausvet.com.au>.

Annex XVIII (contd)Annex IV (contd)

Population size	Sample size
1000	157
2000	163
5000	166
10,000	169

Based on these calculations, it is clear that, for the population sizes under consideration, there is little effect on the sample size. For the sake of simplicity, a standard sample size of 169 is used, regardless of the number of grow-out fish on the farm.

## f) Sampling

The selection of individual fish to include in the sample should be done in such a manner as to give the best chance of the sample being representative of the *study population*. A fuller description of how this may be achieved under different circumstances is provided in *Survey Toolbox*<sup>16</sup>. An example of a single farm will be used to illustrate some of the issues.

One farm has a total of eight tanks, four of which are used for grow-out. At the time of the survey (during winter), the four grow-out tanks have 1850, 4250, 4270 and 4880 fish, respectively, giving a total population of 15,250 grow-out fish.

Simple random sampling from this entire population is likely to produce sample sizes from each tank roughly in proportion to the number of fish in each tank. However, proportional stratified sampling will guarantee that each tank is represented in proportion. This simply involves dividing the sample size between tanks in proportion to their population. The first tank has 1850 fish out of a total of 15,250, representing 12.13%. Therefore 12.13% of the sample (21 fish) should be taken from the first tank. Using a similar approach the sample size for the other three tanks is 47, 47 and 54 fish, respectively.

Once the sample for each tank is determined, the problem remains as to how to select 21 fish from a tank of 1850 so that they are representative of the population. Several options exist.

- i) If the fish can be handled individually, random systematic sampling may be used. ~~This is likely to be the case if, for example:~~ For example, samples can be collected at harvest or during routine management activities involving handling the fish (such as grading or vaccination).

- ~~fish are harvested during winter and samples can be collected at harvest; or~~
- ~~routine management activities involving handling the fish (such as grading or vaccination) are conducted during the winter.~~

If fish are handled, systematic sampling simply involves selecting a fish at regular intervals. For instance, to select 21 from 1850, the sampling interval should be  $1850/21 = 88$ . This means that every 88<sup>th</sup> fish from the tank should be sampled. To ensure randomness, it is good practice to use a random number between 1 and 88 (in this case) to select the *first* fish (e.g. using a random number table), and then select every 88<sup>th</sup> fish after that.

<sup>16</sup> Survey Toolbox for Aquatic Animal Diseases – A Practical Manual and Software Package. Cameron A.R. (2002). Australian Centre for International Agricultural Research (ACIAR), Monograph No. 94, 375 pp. ISBN 1 86320 350 8. Printed version available from ACIAR <http://www.aciar.gov.au> Electronic version available for free download from <http://www.ausvet.com.au>.

Annex XVIII (contd)

Annex IV (contd)

- ii) If fish cannot be handled individually (by far the most common, and more difficult, circumstance) then the fish to be sampled must be captured from the tanks. Fish should be captured in the most efficient and practical way possible, however every effort should be made to try to ensure that the sample is representative. In this example, a dip net is the normal method used for capturing fish. Using a dip net, convenience sampling would involve capturing 21 fish by repeatedly dipping at one spot and capturing the easiest fish (perhaps the smaller ones). This approach is strongly discouraged. One method of increasing the representativeness is to sample at different locations in the tank – some at one end, some at either side, some at the other end, some in the middle, some close to the edge. Additionally, if there are differences among the fish, an attempt should be made to capture fish in such a way as to give different groups of fish a chance of being caught (i.e. do not just try to catch the small ones, but include big ones as well).

This method of collecting a sample is far from the ideal of random sampling, but due to the practical difficulties of implementing random sampling of individual fish, this approach is acceptable, as long as the efforts made to increase the representativeness of the sample are both genuine and fully documented.

g) Testing

Specimens are collected, processed and tested according to standardised procedures developed under the certification programme and designed to meet the requirements of this *Aquatic Manual*. The testing protocol dictates that any specimens that test positive to ELISA be submitted for culture, and that any positive culture results indicate a true positive specimen (i.e. that the farm is not free from disease). It is important that this protocol be adhered to exactly. If a positive culture is found, then it is not acceptable to retest it, unless further testing is specified in the original testing protocol, and the impact of such testing accounted for in the test system *sensitivity* and *specificity* estimates (and therefore the sample size).

h) Analysis

If the calculated sample size of 169 is used, and no positive reactors are found, then the survey will have a confidence of 95%. This can be confirmed by analysing the results using the *FreeCalc* software mentioned above (which reports a confidence level of 95.06%).

It may happen in some cases that the survey is not conducted exactly as planned, and the actual sample size is less than the target sample size. However, the size of the farm may also be smaller. In these cases, it is advisable to analyse the farm data on a farm-by-farm basis. For example, if only 165 specimens were collected from a farm with only 2520 fish, the resulting confidence would still be 95%. If only 160 fish were collected, the confidence is only 94.5%. If a rigid target of 95% confidence is used, then this survey would fail to meet that target and more evidence would be required.

2. Example 2 – two-stage structured survey (national freedom)

a) Context

A country aims to declare freedom from Disease Y of crustaceans. The industry in this country is based largely on small-holder ponds, grouped closely together in and around villages. The disease is reasonably highly contagious, and causes mass mortality mid to late in the production cycle, with affected animals becoming moribund and dying in a matter of days. Affected animals show few characteristic signs, but an infected pond will almost invariably break down with mass mortality unless harvested beforehand. It is more common in late summer, but can occur at any time of year. It also occurs occasionally early in the production cycle. In this country, there are some limitations to the availability of laboratory facilities and the transport infrastructure. However, there is a relatively large government structure, and a comprehensive network of fisheries officers.

Annex XVIII (contd)Annex IV (contd)

## b) Objective

The objective is to establish national freedom from Disease Y. The surveillance system must meet the requirements of this chapter, but must also be able to be practically implemented in this small-holder production system.

## c) Approach

The aquaculture authorities decide to use a survey to gather evidence of freedom, using a two-stage survey design (sampling villages at the first level, and ponds at the second). Laboratory testing of specimens from a large number of farms is not considered feasible, so a combined test system is developed to minimise the need for expensive laboratory tests.

The unit of observation and analysis is, in this case, the pond, rather than the individual animal. This means that the diagnosis is being made at the pond level (an infected pond or a non-infected pond) rather than at the animal level.

The survey is therefore a survey to demonstrate that no villages are infected (using a random sample of villages and making a village-level diagnosis). The test used to make a village-level diagnosis is, in fact, another survey, this time to demonstrate that no ponds in the village are affected. A test is then performed at the pond level (farmer observation followed, if necessary, by further laboratory testing).

## d) Survey standards

- i) The confidence to be achieved by the survey is 95%. The power is set at 95% (but is likely to be virtually 100% if the test system used achieves nearly 100% *specificity*, as demonstrated in the previous example).
- ii) The target population is all ponds stocked with shrimp in the country during the study period. The *study population* is the same, except that those remote areas to which access is not possible are excluded. As outbreaks can occur at any time of year, and at any stage of the production cycle, it is decided not to further refine the definition of the population to target a particular time or age.
- iii) Three tests are used. The first is farmer observation, to determine if mass mortality is occurring in a particular pond. If a pond is positive to the first test (i.e. mass mortality is detected), a second test is applied. The second test used is polymerase chain reaction (PCR). Cases positive to PCR are further tested using transmission experiments.
- iv) Farmer observation can be treated as a test just like any other. In this case, the observation of mass mortality is being used as a test for the presence of Disease Y. As there are a variety of other diseases that are capable of causing mass mortality, the test is not very specific. On the other hand, it is quite unusual for Disease Y to be present, and not result in mass mortality, so the test is quite sensitive. A standard case definition is established for 'mass mortality' (for instance, greater than 20% of the pond's population of shrimp observed dead in the space of less than 1 week). Based on this definition, farmers are able to 'diagnose' each pond as having mass mortality. Some farmers may be over-sensitive and decide that mass mortality is occurring when only a small proportion of shrimp are found dead (false positives, leading to a decrease in *specificity*) while a small number of others fail to recognise the mortalities, decreasing *sensitivity*.

In order to quantify the *sensitivity* and *specificity* of farmer observation of mass mortalities, as a test for Disease Y, a separate study is carried out. This involves both a retrospective study of the number of mass mortality events in a population that is thought to be free from disease, as well as a study of farmers presented with a series of mortality scenarios, to assess their ability to accurately identify a pond with mass mortality. By combining these results, it is estimated that the *sensitivity* of farmer-reported mass mortalities as a test for Disease Y is 87% while the *specificity* is 68%.

- v) When a farmer detects a pond with mass mortality, specimens are collected from moribund shrimp following a prescribed protocol. Tissue samples from 20 shrimp are collected, and pooled for PCR testing. In the laboratory, the ability of pooled PCR to identify a single infected animal in a pool of 20 has been studied, and the *sensitivity* of the procedure is 98.6%. A similar study of negative specimens has shown that positive results have occasionally occurred, probably due to laboratory contamination, but maybe also because of the presence of non-viable genetic material from another source (shrimp-based feed stuffs are suspected). The *specificity* is therefore estimated at 99%.
  - vi) Published studies in other countries have shown that the *sensitivity* of transmission tests, the third type of test to be used, is 95%, partly due to variability in the load of the agent in inoculated material. The *specificity* is agreed to be 100%.
  - vii) Based on these figures, the combined test system *sensitivity* and *specificity* are calculated using the formulae presented in Example 1, first with the first two tests, and then with the combined effect of the first two tests and the third test. The result is a *sensitivity* of 81.5% and a *specificity* of 100%.
  - viii) The design prevalence must be calculated at two levels. First, the pond-level design prevalence (the proportion of ponds in a village that would be infected if disease were present) is determined. In neighbouring infected countries, experience has shown that ponds in close contact with each other are quickly infected. It is unusual to observe an infected village with fewer than 20% of ponds infected. Conservatively, a design prevalence of 5% is used. The second value for design prevalence applies at the village level, or the proportion of infected villages that could be identified by the survey. As it is conceivable that the infection may persist in a local area without rapid spread to other parts of the country, a value of 1% is used. This is considered to be the lowest design prevalence value for which a survey can be practically designed.
  - ix) The population of villages in the country is 65,302, according to official government records. Those with shrimp ponds number 12,890, based on records maintained by the aquaculture authorities. These are generated through a five-yearly agricultural census, and updated annually based on reports of fisheries officers. There are no records available of the number of ponds in each of these villages.
- e) Sample size

Sample size is calculated for the two levels of sampling, first the number of villages to be sampled and then the number of ponds to be sampled. The number of villages to be sampled depends on the *sensitivity* and the *specificity* of the test used to classify villages as infected or not infected. As the 'test' used in each village is really just another survey, the *sensitivity* is equal to the confidence and the *specificity* is equal to the power of the village-level survey. It is possible to adjust both confidence and power by changing the sample size in the village survey (number of ponds examined), which means that we can determine, within certain limits, what *sensitivity* and *specificity* we achieve.

Annex XVIII (contd)Annex IV (contd)

This allows a flexible approach to sample size calculation. If a smaller first-stage sample size is desired (a small number of villages), a high *sensitivity* and *specificity* are needed, which means that the number of ponds in each village that need to be examined is larger. A smaller number of ponds will result in lower *sensitivity* and *specificity*, requiring a larger number of villages. The approach to determining the optimal (least cost) combination of first- and second-stage sample sizes is described in *Survey Toolbox*.

A further complication is presented by the fact that each village has a different number of ponds. In order to achieve the same (or similar) confidence and power (*sensitivity* and *specificity*) for each village, a different sample size may be required. The authorities choose to produce a table of sample sizes for the number of ponds to sample in each village, based on the total ponds in each village.

An example of one possible approach to determining the sample size follows:

The target *sensitivity* (confidence) achieved by each village-level survey is 95%. The target *specificity* is 100%. Using the *FreeCalc* software, with a design prevalence of 1% (the survey is able to detect disease if 1% or more villages are infected), the first-stage sample size is calculated as 314 villages. Within each village, the test used is the combined test system described above with a *sensitivity* of 81.5% and a *specificity* of 100%. Based on these figures the following table is developed, listing the number of ponds that need to be sampled in order to achieve 95% *sensitivity*.

Population	Sample size
30	29
40	39
60	47
80	52
100	55
120	57
140	59
160	61
180	62
200	63
220	64
240	64
260	65
280	65
300	66
320	66
340	67
360	67
380	67
400	67
420	68
440	68
460	68
480	68
500	68
1000	70

Annex XVIII (contd)Annex IV (contd)

## f) Sampling

First-stage sampling (selection of villages) is done using random numbers and a sampling frame based on the fisheries authorities list of villages with shrimp ponds. The villages are listed on a spreadsheet with each village numbered from 1 to 12,890. A random number table (such as that included in *Survey Toolbox*) or software designed for the generation of random numbers (such as EpiCalc<sup>17</sup>) is used.

The second stage of sampling involves random selection of ponds within each village. This requires a sampling frame, or list of each pond in the village. The fisheries authorities use trained local fisheries officers to coordinate the survey. For each selected village, the officer visits the village and convenes a meeting of all shrimp farmers. At the meeting, they are asked how many ponds they have and a list of farmers' names and the number of ponds is compiled. A simple random sample of the appropriate number of ponds (between 29 and 70, from the table above, depending on the number of ponds in the village) is selected from this list. This is done either using software (such as Survey Toolbox's *RandomAnimal* program), or manually with a random number table or decimal dice for random number selection. Details of this process are described in *Survey Toolbox*. This selection process identifies a particular pond in terms of the name of the owner, and the sequence number amongst the ponds owned (e.g. Mr Smith's 3<sup>rd</sup> pond). Identification of the actual pond is based on the owners own numbering system for the ponds.

## g) Testing

Once ponds have been identified, the actual survey consists of 'testing those ponds'. In practice, this involves the farmers observing the ponds during one complete production cycle. The local fisheries officer makes weekly visits to each farmer to check if any of the selected ponds have suffered mass mortality. If any are observed (i.e. the first test is positive), 20 moribund shrimp are collected for laboratory examination (first PCR, and then, if positive, transmission experiments).

## h) Analysis

Analysis is performed in two stages. First, the results from each village are analysed to ensure that they meet the required level of confidence. If the target sample size is achieved (and only negative results obtained), the confidence should be 95% or greater in each village. At the second stage, the results from each village are analysed to provide a country level of confidence. Again, if the target sample size (number of villages) is achieved, this should exceed 95%.

3. Example 3. – spatial sampling and the use of tests with imperfect specificity

## a) Context

A country has an oyster culture industry, based primarily on rack culture of oysters in 23 estuaries distributed along the coastline. In similar regions in other countries, Disease Z causes mortalities in late summer/early autumn. During an outbreak a high proportion of oysters are affected, however, it is suspected that the agent may be present at relatively low prevalence in the absence of disease outbreaks.

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<sup>17</sup> <http://www.myatt.demon.co.uk/epicalc.htm>

Annex XVIII (contd)Annex IV (contd)

## b) Objective

The national authorities wish to demonstrate national freedom from Disease Z. If the disease should be detected, a secondary objective of the survey is to collect adequate evidence to support zoning at the estuary level.

## c) Approach

The authorities conclude that clinical surveillance for disease outbreaks is inadequate because of the possibility of low level subclinical infections. It is therefore decided to base surveillance on a ~~structured~~ two-stage survey, in which sampled oysters are subjected to laboratory testing. The first stage of the survey is the selection of estuaries. However, due to the objective of providing evidence for zoning (should disease be found in any of the estuaries), it is decided to use a census approach and sample every estuary. In essence this means that there will be 23 separate surveys, one for each estuary. A range of options for sampling oysters are considered, including sampling at harvest or marketing, or using farms (oyster leases) as a level of sampling or stratification. However the peak time of activity of the agent does not correspond to the harvest period, and the use of farms would exclude the significant numbers of wild oysters present in the estuaries. It is therefore decided to attempt to simulate simple random sampling from the entire oyster population in the estuary, using a spatial sampling approach.

## d) Survey standards

- i) The target population is all of the oysters in each of the estuaries. The *study population* is the oysters present during the peak disease-risk period in late summer early autumn. Wild and cultured oysters are both susceptible to disease, and may have associated with them different (but unknown) risks of infection. They are therefore both included in the *study population*. As will be described below, sampling is based on mapping. Therefore the *study population* can more accurately be described as that population falling within those mapped areas identified as oyster habitats.
- ii) A design prevalence value is only required at the oyster level (as a census is being used at the estuary level). While the disease is often recognised with very high prevalence during outbreaks, a low value is used to account for the possibility of persistence of the agent in the absence of clinical signs. A value of 2% is selected.
- iii) The test used is histopathology with immuno-staining techniques. This test is known to produce occasional false-positive results due to nonspecific staining, but is very sensitive. Published studies indicate values of 99.1% for *sensitivity* and 98.2% for *specificity*. No other practical tests are available. This means that it is not possible to definitively differentiate false positives from true positives, and that in a survey of any size, a few false positives are expected (i.e. 1.8%).
- iv) The confidence is set at 95% and the power at 80%. In the previous examples, due to the assumed 100% *specificity* achieved by use of multiple tests, the effective power was 100%. In this case, with imperfect *specificity*, there will be a risk of falsely concluding that a healthy estuary is infected, so the power is not 100%. The choice of a relatively low figure (80%) means that there is a 1 in 5 chance of falsely calling an estuary infected when it is not infected, but it also dramatically decreases the survey costs, through a lower sample size.

Annex XVIII (contd)

Annex IV (contd)

e) Sample size

Based on the assumption that the sampling procedure will mimic simple random sampling, the sample size (number of oysters to sample per estuary) can be calculated with *FreeCalc*. The population size (number of oysters per estuary) is assumed to be very large. The calculated sample size, using the *sensitivity*, *specificity* and design prevalence figures given above, is 450. *FreeCalc* also reports that, based on this sample size and the *specificity* of the test, it is possible to get 10 or fewer false-positive test results, and still conclude that the population is free from disease. This is because, if the population were infected at 2% or greater, the anticipated number of positive reactors from a sample of 450 would be greater than 10. In fact, we would expect 9 true positives ( $450 \times 2\% \times 99.1\%$ ) and 8 false positives ( $450 \times 98\% \times 1.8\%$ ) or a total of 17 positives if the population were infected at a prevalence of 2%.

This illustrates how probability theory and adequate sample size can help differentiate between true- and false-positive results when there is no alternative but to use a test with imperfect *specificity*.

f) Sampling

The aim is to collect a sample of 450 oysters that represent an entire estuary. Simple random sampling depends on creating a sampling frame listing every oyster (not possible) and systematic sampling depends on being able to (at least conceptually) line up all the oysters (again, not possible). The authorities decide to use spatial sampling to approximate simple random sampling. Spatial sampling involves selecting random points (defined by coordinates), and then selecting oysters near the selected points. In order to avoid selecting many points with no oysters nearby, the estuary is first mapped (the fisheries authorities already have digital maps defining oyster leases available). To these maps areas with significant concentrations of wild oysters are also added, based on local expertise. Pairs of random numbers are generated such that the defined point falls within the defined oyster areas. Other schemes are considered (including using a rope marked at regular intervals, laid out on a lease to define a transect, and collecting an oyster adjacent to each mark on the rope) but the random coordinate approach is adopted.

Survey teams then visit each point by boat (using a GPS Global Positioning System unit to pinpoint the location). A range of approaches is available for selecting which oyster to select from a densely populated area, but it should involve some effort at randomness. Survey staff opt for a simple approach: when the GPS receiver indicates that the site has been reached, a pebble is tossed in the air and the oyster closest to the point where it lands is selected. Where oysters are arranged vertically (e.g. wild oysters growing up a post), a systematic approach is used to determine the depth of the oyster to select. First, an oyster at the surface, next, an oyster halfway down, and thirdly, an oyster as deep as can be reached from the boat.

This approach runs the risk of *bias* towards lightly populated areas, so an estimate of the relative density of oysters at each sampling point is used to weight the results (see *Survey Toolbox* for more details).

g) Testing

Specimens are collected, processed, and analysed following a standardised procedure. The results are classified as definitively positive (showing strong staining in a highly characteristic pattern, possibly with associated signs of tissue damage), probably positive (on the balance of probabilities, but less characteristic staining), and negative.

Annex XVIII (contd)Annex IV (contd)

## h) Analysis

The interpretation of the results when using a test with imperfect *specificity* is based on the assumption that, in order to conclude that the population is free from infection, any positive result identified is really a false positive. With a sample size of 450, up to 10 false positives may be expected while still concluding that the population is free from disease. However, if there is reasonable evidence that there is even a single *true* positive, then the population cannot be considered free. This is the reason for the classification of positive results into definitive and probable positives. If there are any definitive positives at all, the population in that estuary must be considered infected. The probable positives are consistent with false positives, and therefore up to 10 may be accepted. Using *FreeCalc* the actual confidence achieved based on the number of (presumed) false positives detected can be calculated. For instance, if 8 'probably positive' results were detected from an estuary, the confidence level for the survey would be 98.76%. On the other hand, if 15 'probably positive' results were detected, the confidence is only 61.9%, indicating that the estuary is likely to be infected.

## i) Discussion

Normally, it may be safely assumed that a surveillance system aimed at demonstrating freedom from disease is 100% specific. This is because any suspected occurrence of disease is investigated until a definitive decision can be made. If the conclusion is that the case is truly a case of disease, then there is no issue of declaring freedom – the disease is known to be present. This example presents a different situation where, due to lack of suitable tests, it is not possible for the surveillance system to be 100% specific. This may represent an unusual situation in practice, but illustrates that methods exist for dealing with this sort of problem. In practice, a conclusion that a country (or estuary) is free from infection, in the face of a small (but statistically acceptable) number of positive results, will usually be backed up by further evidence (such as the absence of clinical disease).

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## APPENDIX X.X.X.

**GUIDELINES FOR AUTHORS OF THE  
DISEASE SPECIFIC SURVEILLANCE  
AQUATIC CODE CHAPTERS**

## Article X.X.X.1.

**Introduction**

PROVIDE RELEVANT DISEASE INFORMATION WHICH SHOULD BE CONSIDERED WHEN DESIGNING A SURVEILLANCE PROGRAMME FOR THIS DISEASE, INCLUDING BUT NOT LIMITED TO THE FOLLOWING:

1. General impact of disease on wild and/or farmed animals
2. Comment about geographical differences in disease expression.
3. Comment on the body of knowledge available for this disease relative to it being recently identified versus an extensively researched pathogen.

**surveillance for early detection****surveillance for self-declaration of freedom****surveillance for estimation of disease occurrence**

## Article X.X.X.2.

**General conditions for surveillance of disease/infection X**

PROVIDE RELEVANT EPIDEMIOLOGICAL INFORMATION WHICH SHOULD BE CONSIDERED WHEN DESIGNING A SURVEILLANCE PROGRAMME FOR THIS DISEASE, INCLUDING BUT NOT LIMITED TO THE FOLLOWING:

1. Populations/host factors
  - a) Describe characteristics of both farmed and wild populations of susceptible hosts
  - b) Describe any difference in structure of population depending on age classes, eg brood stock, seed and grow out that may affect disease distribution in the population
  - c) General information about the disease of relevance to its epidemiology e.g. design prevalence, diagnostic test accuracy, susceptible hosts, susceptible age classes, breadth of hosts, eg. ISA is known to infect only salmonids and within salmonids there are different levels of disease susceptibility versus carrier states
  - d) Potential for zoning and compartmentalization
  - e) Expected proportion immune/susceptible when vaccinated

Annex XVIII (contd)Annex V (contd)

- f) Species (and characteristics) susceptible to infection
  - under natural conditions
  - under experimental conditions.
- g) Evidence for the potential of persistent infection with lifelong carriers
  - Known or suspected wild carriers
  - Vectors
  - Comparative susceptibility/sentinel species (i.e. affecting probability of detection)
  - Prevalence (describe commonly observed prevalence in wild and farmed populations for the detection method used, under different conditions and at different disease stages, if such exist) potential routes of introduction (for farmed and wild populations, legal and illegal)
  - Susceptible host strains, including SPR or SPF
  - Vaccinated versus non vaccinated
  - Susceptible stages of the host
  - Description of movement of susceptible species at different life stages
  - Target organs and infected tissue move
  - Factors affecting susceptibility to infection or disease (eg. stress)
  - Causal web or quantified risk factors/management practices. Risk factors means factors that either increase or decrease the risk of an animal getting infected or for an infected animal developing clinical disease.
  - Differences life stages in terms of susceptibility, risk factors, transmission and prevalence of infection, etc.
  - Sources of stock – are they bought from outside, are they tested
  - Targeted surveillance/risk based surveillance
  - Spatial distribution
    - Within holdings
    - Within farms
    - Among farms
    - Geographical distribution
    - Temporal distribution

Annex XVIII (contd)Annex V (contd)

- Seasonal fluctuations
- Long-term fluctuations
- Periodic changes (population dynamics)
- Climatic conditions
- Mortality and morbidity
- Host population distribution, intermediate host distribution, and vector distribution
- Wild fish
- Reports of infection
- Prevalence estimates
- Epidemiological role
- Following
- Biosecurity measures

### 5.5. Clustering of infection

### 3.3. Diagnosis in subclinical crustacean carriers

Article X.X.X.3

#### **General Design Considerations**

Deal with targeted and risk based surveillance – clear definitions needed.

Clearly define objective and measurable outcomes

Approaches to surveillance (passive, active, targeted)/Historic/disease freedom

- difficulties/advantages in defining historical time periods for disease freedom
- circumstances which would allow you to draw conclusions/interpretations based on passive programmes, e.g. Historical and absence of obvious signs

Prioritise the diseases to be included in the surveillance system (e.g. using a risk based approach)

Philosophical approach to disease uncertainty (cannot prove disease freedom)

Sources of information, evidence, record keeping

Develop a case definition for each outcome

#### **Case definition**

Include mixed infections when dealing with case definition and specificity e.g. Gyro.

Annex XVIII (contd)Annex V (contd)

(Please start this chapter with a simple definition of the disease)

“For the purpose of this chapter, DISEASE NAME is considered to be INFECTION WITH PATHOGEN NAME.”

Case definition for infection, for diseased animal and for disease holding unit

Case definition in endemic situations and in zero prevalence situations

Relationship of infection to disease (stages, carriers, states)

## CHAPTER 5 - Passive surveillance

- Strengths and weaknesses or situation in which passive surveillance would be sufficient
- Identify useful sources of information and record keeping
- Flow of information
- Identify mechanisms to investigate suspect cases
- Availability of information that could be used in a scenario tree

## CHAPTER 6 – Active surveillance (risk based, targeted)

- Define potential sources of data and their reliability
- Define a sampling strategy including frequency, population(s), what kinds of observations, collection of samples and diagnostic tests
- Identify risk factors that may be of use for targeted or risk based surveillance

## CHAPTER 7 – Sampling Considerations

- Describe opportunities for *probability sampling*
- Opportunities for evidence collection and/or sampling at different points in the production cycle (alternative sources of evidence)
- Sample size considerations (confidence, power) in relation to characteristic of host, eg value, size
- Describe known or suspected sampling bias that will affect risk based surveillance
- Sampling for distribution versus for detection
- Opportunities for sampling of co-habitated animals and implications from a surveillance perspective, e.g. differences in susceptibility (ornamentals, e.g. sampling common carp instead of koi carp)
- Identify other sources of reliable information – complex systems (e.g. diagnostic labs, slaughter houses, private practices)

Annex XVIII (contd)Annex V (contd)

## SAMPLING FOR SURVEILLANCE PURPOSES

STRATIFICATION (STRUCTURING OF POPULATION IN THE HOLDING UNIT) – HOW CAN IT BE INFLUENCED BY THE DISEASE?

Surveillance to demonstrate the absence of *disease* or *infection*

- Sampling frequency and duration (to establish freedom and to maintain status)
- Optimum water temperature
- Optimum age range or development stage of fish
- Selection of individual specimens

Add some more information on different methods (castnet, feedtray, harvest) to be used for sampling and discuss potential bias of each method.

Diagnosis in disease situations

Diagnosis in subclinical crustacean carriers

Selection of epidemiological units and sampling methods

## CHAPTER 8 - Statistical considerations

Sample size

Number of fish to be sampled

Minimum expected prevalence or maximal allowable prevalence to declare freedom in that epidemiological unit

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

- The sensitivity and specificity of the *test*, or *test system*;
- The design prevalence (or prevalences where a multi-stage design is used);
- A suitable design prevalence value at the animal level (e.g. prevalence of infected animals in a cage) may be:
  - between 1% and 5% for infections that are present in a small part of the population e.g. are transmitted slowly or are at the early stages of an outbreak, etc.;
  - over 5% for highly transmissible infections.

If reliable information on the expected prevalence in an infected population is not available, a value of 2% should be used for the design prevalence.

Annex XVIII (contd)Annex V (contd)

- At higher levels (e.g. cage, pond, farm, village, etc.) the design prevalence usually reflects the prevalence of infection that is practically and reasonably able to be detected by a *surveillance system*. Detection of infection at the lowest limit (a single infected *unit* in the *population*) is rarely feasible in large *populations*. The expected behaviour of the infection may also play a role. Infections that have the ability to spread rapidly between farms may have a higher farm-level design prevalence than slow-moving infections.

A suitable design prevalence value for the first level of clustering, (e.g. proportion of infected farms in a zone) may be up to 2%.

## CHAPTER 9 – Diagnostic tests

## Diagnostics

PROVIDE RELEVANT EPIDEMIOLOGICAL INFORMATION REGARDING DIAGNOSTIC TEST CHARACTERISTICS WHICH SHOULD BE CONSIDERED WHEN DESIGNING A SURVEILLANCE PROGRAMME FOR THIS DISEASE, INCLUDING BUT NOT LIMITED TO THE FOLLOWING:

- Comment on the presence or absence of pathognomonic signs
- Is there anything specific about agent survival outside the host

Three level diagnosis (simple, moderate and advanced)

Agent detection versus exposure host response

Accuracy/precision/uncertainty/agreement

Factors influencing SENS/SPEC, eg viral load, age, season, water temperature

Predictive values/low prevalence situations

Effect of false positives/false negatives on surv.

Assessment of test and situations to which they are applied, eg low prevalence situations

Assessment of tests without gold standard

Group level testing versus individual, eg pooled samples, group level sens/spec.

Selection of cut-offs for interpretation of test results

Combinations of tests

Apparent prevalence versus true prevalence

Adopting new diagnostic tests can have unpredictable consequences

Vaccination affecting diagnostic test performance

Tests available for use in environments rather than hosts, eg. equipment, water or soil samples and intermediate hosts/carrier

Annex XVIII (contd)Annex V (contd)

Can samples be preserved and how?

Analytical sensitivity and specificity – clinical and epidemiological relevance

Possibility to use pooled testing. Effect of pooling requires assessment as new test

Laboratory variability – agreement

Serology

### **Field diagnostic methods**

(This includes observation of the animal and its environment, and gross clinical examinations)

- Clinical signs
- Behavioural changes: specify which animals are likely to be affected or not affected by the disease or infection within a tank? If different for acute or chronic conditions, provide details.

#### **a) Agent factors**

- Aetiological agent, agent strains
- Survival outside the host under different environmental conditions including salinity, temperature, pH, concentration of organic material, water depth, survival on fomites, etc. (ponds, raceways, rivers, recirculated systems with or without disinfection)
- Duration of survival
- Stability of the agent (describe effective inactivation methods)
- Existence of strains with different molecular composition. Their geographical distribution, pathogenicity and their possible epidemiological importance. Are there tests available to differentiate these strains?
- Life cycle/intermediate hosts
- Incubation time
- Comment on fish / tissues that are not appropriate (i.e. when it is never possible to detect)
  - Priority areas for testing (fish; tissues, etc to be sampled for prevalence comparisons)
  - Optimal diagnostic test combinations to determine prevalence (include ranking of costs)

CHAPTER 10 – Flow of Information and Tools/Methods

CHAPTER 12 – Data Management, Analysis and Interpretation

Data quality (cross check with other sources)

Interpreting uncertainty in claims of disease freedom

Annex XVIII (contd)

Annex V (contd)

Following

Biosecurity measures

CHAPTER 15 – Surveillance in Wild Populations

Species selection (example, lack of susceptible species)

Optimising detection

Sentinel fish (using known susceptible species)

CHAPTER 16 – Surveillance of Ornamentals

Sampling in very small populations

Co-habitation (ornamentals, e.g. sampling common carp instead of koi carp)

## UPDATE ON DEVELOPMENTS IN AQUATIC ANIMAL HEALTH

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### Summary:

The importance of aquatic animal health continues to increase, not least because of the steady expansion of aquaculture production (mainly the farming of fish, molluscs and crustacean species) throughout the world. The latest figures (FAO, 2007) show that the contribution of aquaculture to global supplies of fish, crustaceans, molluscs and other aquatic animals has increased from 3.9 percent of total production by weight in 1970 to 27.1 percent in 2000 and 32.4 percent in 2004. Countries in the Asia and the Pacific region accounted for 91.5 percent of the production quantity and 80.5 percent of the value in 2004. Of the world total, China is reported to account for 69.6 percent of the total quantity and 51.2 percent of total value of aquaculture production. The top ten producing countries for food fish supply from aquaculture in 2004 are indicated in the table below along with the top ten countries in terms of annual growth in aquaculture production for the two-year period 2002-04.

Top ten aquaculture producers of food fish supply: quantity and emerging growth (FAO, 2007)

Producer	2002	2004	APR
	(Tonnes)		(Percentage)
Top ten producers in terms of quantity, 2004			
China	27,767,251	30,614,968	5.0
India	2,187,189	2,472,335	6.3
Vietnam	703,041	1,198,617	30.6
Thailand	954,587	1,172,866	10.8
Indonesia	914,071	1,045,051	6.9
Bangladesh	786,604	914,752	7.8
Japan	826,715	776,421	-3.1
Chile	545,655	674,979	11.2
Norway	550,209	637,993	7.7
United States of America	497,346	606,549	10.4
TOP TEN SUB TOTAL	35,732,648	40,114,531	6.0
REST OF THE WORLD	4,650,830	5,353,825	7.3
<b>TOTAL</b>	<b>40,383,478</b>	<b>45,468,356</b>	<b>6.1</b>
Top ten producers in terms of growth, 2002-04			
Myanmar	190,120	400,360	45.1
Vietnam	703,041	1,198,617	30.6
Turkey	61,165	94,010	24.0
Netherlands	54,442	78,925	20.4
Republic of Korea	296,783	405,748	16.9
Iran (Islamic Rep. of)	76,817	104,330	16.5
Egypt	376,296	471,535	11.9
Chile	545,655	674,979	11.2
Thailand	954,567	1,172,866	10.8
United States of America	497,346	606,549	10.4

Note: Data exclude aquatic plants. APR refers to the average annual percentage growth rate for 2002-04 (FAO (2007). *The State of World Fisheries and Aquaculture 2006*. Food and Agriculture Organisation of the United Nations, Rome.

Annex XIX (contd)

All regions showed increased growth rates in production from 2002 to 2004 but were led by the Near East and North Africa region with 13.5 percent average annual growth. In the region, Turkey has seen the biggest rate of increased production with an annual growth rate of 24 percent, followed by Iran with 16.5 percent, but Egypt is by far the dominant country in terms of total production (providing 92 percent of the regional total) and is now the world's top producer of mullets and the second biggest tilapia producer after China.

Worldwide, aquaculture production continues to grow more rapidly than all other animal food-producing sectors. The aquaculture sector has grown at an average rate of 8.8 percent per year since 1970, compared with only 1.2 percent for capture fisheries and 2.8 percent for terrestrial farmed meat production systems over the same period. However, diseases continue to impact heavily on aquaculture production, and international trade in aquaculture animals is still causing spread of major infectious diseases. Several new diseases have emerged in recent years and some have spread internationally, particularly in shrimp aquaculture. The OIE international health standards for international trade in aquatic animals are continuously reviewed and updated by the Aquatic Animal Health Standards Commission (AAHSC) with the assistance of internationally renowned experts. The current editions of the Aquatic Animal Health Code (OIE 2007) and the Manual of Diagnostic Tests for Aquatic Animals (OIE 2006) incorporate several important modifications agreed during the 74th General Session in May 2006, including amendments to the listed aquatic animal diseases. It is important that Members are aware of these changes and meet their obligations on reporting the occurrence of the listed (and emerging) aquatic animal disease to the OIE. Work has commenced in new areas such as aquatic animal welfare for which a draft set of guidelines has been prepared, and aquatic animal disease surveillance for which a Code chapter has been drafted for Members' comments. Also, the OIE International Committee agreed at the 75<sup>th</sup> General Session in May 2007 that amphibian diseases should be included in the remit of OIE. An ad hoc group of the AAHSC has identified two diseases that meet the OIE criteria for listing, and draft Code chapters for these diseases have been prepared and will be distributed for Members' comments. There have been continuing efforts to encourage greater involvement of veterinary services in the field of aquatic animal disease and to improve cooperation between veterinary and other authorities with competence for aquatic animal health. In this regard, an OIE Global Conference on Aquatic Animal Health 'Defining Roles and Responsibilities' was held in Bergen Norway in October 2006 to provide an opportunity for OIE and its Members to exchange the latest information on developing a science-based approach to the management of aquatic animal health and welfare. This will assist in the evaluation and improvement of the current standards and guidelines for better control of infectious aquatic animal health and countries' capabilities to prepare for, and respond to, aquatic animal disease emergencies, as well as better defining roles and responsibilities. The proceedings of the conference will be published in the near future. In addition, there will be a special multi-author issue of the Scientific and Technical Review Series on 'Changing Trends in Managing Aquatic Animal Disease Emergencies' due for publication in April 2008. Finally, the AAHSC pages on the OIE website ([www.oie.int/aac/eng/en\\_fdc.htm](http://www.oie.int/aac/eng/en_fdc.htm)) are kept continuously updated to provide easy access to the current OIE standards for aquatic animal health as well as the latest reports of the Commission and its ad hoc groups, and aquatic animal disease occurrence reports submitted by Members.

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### AQUATIC ANIMALS COMMISSION WORK PLAN FOR 2008/2009

<b><i>Aquatic Animal Health Code</i></b>
<ul style="list-style-type: none"> <li>• Ongoing review of the list of diseases</li> <li>• Review emerging diseases</li> </ul>
<ul style="list-style-type: none"> <li>• Prepare draft disease chapters for AVM complex</li> </ul>
<ul style="list-style-type: none"> <li>• Finalise revised disease Chapter for Crayfish plague</li> </ul>
<ul style="list-style-type: none"> <li>• Prepare text for disease chapters for gaining and regaining freedom for compartments</li> </ul>
<ul style="list-style-type: none"> <li>• Harmonise horizontal chapters with those in the <i>Terrestrial Code</i></li> </ul>
<ul style="list-style-type: none"> <li>• Finalise Template for surveillance for individual diseases</li> </ul>
<ul style="list-style-type: none"> <li>• Revise Aquatic Animal Health Model Certificates</li> </ul>
<ul style="list-style-type: none"> <li>• Finalise Guidelines for handling and disposal of carcasses and wastes of aquatic animals</li> </ul>
<ul style="list-style-type: none"> <li>• Prepare welfare guidelines for farmed fish (excluding ornamental species)</li> </ul>
<ul style="list-style-type: none"> <li>• Antimicrobial resistance in the field of aquatic animals – contribute to OIE work</li> </ul>
<ul style="list-style-type: none"> <li>• Consider development of text on commodities considered safe for trade</li> </ul>
<ul style="list-style-type: none"> <li>• Consider development of text on trade in vaccinated fish</li> </ul>
<b><i>Manual of Diagnostic Tests for Aquatic Animals</i></b>
<ul style="list-style-type: none"> <li>• Update individual disease chapters using the new template</li> </ul>
<ul style="list-style-type: none"> <li>• Revise chapter on methods for disinfection</li> </ul>
<ul style="list-style-type: none"> <li>• Prepare disease chapters for amphibian diseases if listing is approved</li> </ul>
<ul style="list-style-type: none"> <li>• Prepare disease chapters for IMN and WTD</li> </ul>
<ul style="list-style-type: none"> <li>• Prepare disease chapters for AVM complex</li> </ul>
<b>Meetings</b>
<ul style="list-style-type: none"> <li>• Make presentations on the activities of the Aquatic Animals Commission at the Conferences of the OIE Regional Commissions</li> </ul>
<b>Other issues</b>
<ul style="list-style-type: none"> <li>• Keep the Commission's web pages up to date</li> </ul>
<ul style="list-style-type: none"> <li>• Consider new candidates for OIE Reference Laboratories for listed diseases</li> </ul>
<ul style="list-style-type: none"> <li>• Provide input into the PVS to ensure that there is scope to address the evaluation of aquatic animal health systems</li> </ul>
<ul style="list-style-type: none"> <li>• Review manuscript for OIE Handbook on Aquatic Animal Health Surveillance</li> </ul>
<ul style="list-style-type: none"> <li>• Contribute to FAO/OIE Regional Aquatic Biosecurity Framework Project for Africa</li> </ul>

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