

The role of OIE aquatic standards and OIE Reference Laboratories in aquatic animal disease prevention and control

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Summary

The World Organisation for Animal Health (OIE) develops normative documents relating to rules that Member Countries and Territories can use to protect themselves from diseases without setting up unjustified sanitary barriers. For aquatic animal disease, the *Aquatic Animal Health Code* and the *Manual of Diagnostic Tests for Aquatic Animals* are prepared by the Aquatic Animal Health Standards Commission, with the assistance of internationally renowned experts, the other Specialist Commissions of the OIE, and in consultation with OIE Members. The role of these standards in aquatic animal disease prevention and control is described in detail. There are currently 27 OIE Reference Laboratories and one Collaborating Centre for aquatic animal diseases, providing a network of expertise in aquatic animal health. These laboratories play a key role in aquatic animal disease prevention and control through providing diagnostic services and expert advice that is particularly useful in emergency situations.

Keywords

Aquatic animals – Aquatic animal health – Diagnosis – Disease control – Disease risk – Standards – Trade – World Organisation for Animal Health.

Introduction

The World Organisation for Animal Health (OIE) is an intergovernmental organisation, created in 1924, which currently has 172 Member Countries and Territories. Its main objectives are:

- to ensure transparency in the global animal disease situation
- to collect, analyse and disseminate veterinary scientific information
- to provide expertise and encourage international solidarity in the control of animal diseases
- within its mandate under the World Trade Organization (WTO) Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement), to safeguard world trade by publishing health standards for international trade in animals and animal products
- to improve the legal framework and resources of national Veterinary Services
- to provide a better guarantee for the safety of food of animal origin and to promote animal welfare through a science-based approach.

The OIE's remit has covered aquatic animals for almost fifty years. The Fish Diseases Commission was established as a Specialist Commission in 1960 because of the increasing awareness of the importance of international trade in fish. In 1988, the scope of the Commission was extended to include diseases of molluscs and crustaceans, and in 2003 the Commission was renamed the Aquatic Animal Health Standards Commission (in brief, Aquatic Animals Commission).

The OIE develops normative documents relating to rules that Members can use to protect themselves from diseases. By applying these rules, Members can be assured that their measures do not represent unjustified sanitary barriers, because the OIE normative documents enjoy recognition as 'international standards' under the agreements of the WTO.

There are currently 27 OIE Reference Laboratories for aquatic animal diseases. Under the guidance of an expert whose competence is recognised internationally, they function as centres of expertise and standardisation of diagnostic techniques. Additionally, they may provide scientific and technical training for personnel from Member Countries and Territories and coordinate scientific and technical studies in collaboration with other laboratories or organisations.

This paper describes the development of the latest versions of the OIE *Aquatic Animal Health Code (Aquatic Code)* and the OIE *Manual of Diagnostic Tests for Aquatic Animals (Aquatic Manual)*, and how both standards assist in preventing and managing aquatic animal disease emergencies. Since the publication of the first editions of these standards in 1995, several editions of each document have been produced, but the changes made over the last three years in particular have significantly altered the scope and format of those standards and increased their usefulness. The role of OIE Reference Laboratories in the diagnosis of aquatic animal diseases will be explained.

The OIE standards for aquatic animals

The standards for aquatic animals (the *Aquatic Code* and the *Aquatic Manual*) are prepared by the Aquatic Animals Commission with the assistance of internationally renowned experts, individually, or through *ad hoc* groups. The views of Member Countries and Territories are systematically sought through the circulation of draft and revised texts to OIE Member Delegates. Delegates are then expected to seek the comments of the relevant national experts before informing the OIE of their country's response. The standards are finally adopted by the OIE International Committee at the annual General Assembly of all Delegates from all Member Countries and Territories.

The International Committee is the highest authority of the OIE. It comprises the Delegates of all Member Countries and Territories. The General Session of the International Committee lasts five days and is held every year during the last week of May in Paris, France. Voting by Delegates within the International Committee respects the democratic principle of 'one country, one vote'. However,

most decisions are made on the basis of consensus, avoiding the need for the casting of votes by its Delegates.

The value of the OIE standards is twofold:

- the measures published in the standards are the result of consensus among the veterinary authorities of OIE Members
- the standards constitute a reference within the SPS Agreement for international standards for animal health and zoonoses.

The OIE *Aquatic Animal Health Code*

The aim of the *Aquatic Code* is to assure the sanitary safety of international trade in aquatic animals (fish, molluscs and crustaceans) and their products. This is achieved through the detailing of health measures to be used by veterinary or other competent authorities of importing and exporting countries. These measures are designed to minimise the transfer of agents that are pathogenic for aquatic animals while ensuring that unjustified sanitary barriers are avoided. The *Aquatic Code* makes reference only to the aquatic animal health situation in the exporting country, assuming that either the disease is not present in the importing country or is the subject of a control or eradication programme. Therefore, when determining its import measures, an importing country should do so in a way that is consistent with the principle of national treatment and the other provisions of the SPS Agreement.

The *Aquatic Code* is updated regularly, and a new edition is published each year, both in hard copy and on-line. At the time of writing, the latest edition is from 2007 (13).

Part 1 of the *Aquatic Code* contains general provisions for aquatic animal health, while Part 2 details recommendations applicable to specific diseases. Appendices on blood sampling and vaccination and on inactivation of pathogens are contained in Part 3, and Part 4 provides model international aquatic animal health certificates. The next section describes in more detail how the provisions of Parts 1 and 2 can assist in aquatic animal disease prevention and control.

Part 1 of the *Aquatic Code*: General provisions

Section 1.1 General definitions

This section provides definitions of the terms or expressions used. It is important to realise that these definitions are contextual, i.e. 'for the purpose of the

Aquatic Code, and not stand-alone definitions of general applicability. The need to define terms that on the surface appear straightforward (e.g. 'disease', 'infection') arose with the formal recognition in 1998 of the OIE standards as reference documents within the SPS Agreement. It is also noteworthy that not all of the definitions in the *Aquatic Code* are identical to those used in the OIE *Terrestrial Animal Health Code (Terrestrial Code)*. Whilst harmonisation of both *Codes* is an ongoing priority for the OIE, biological differences between terrestrial and aquatic animals sometimes warrant different definitions. An example is the definition of 'zone' which in the *Aquatic Code* – but not in the *Terrestrial Code* – refers to water catchments.

Section 1.2 Notification systems

Chapter 1.2.1 in Section 1.2 describes notification systems, commencing with the statement that 'Countries shall make available to other countries, through the OIE, whatever information is necessary to minimise the spread of aquatic animal diseases and their aetiological agents and to assist in achieving better world-wide control of these diseases'. This important ground rule is followed by detailed reporting requirements for OIE Member Countries and Territories. These rules specify the disease events that must be reported to the OIE within 24 hours ('immediate' notification). The overall purpose of these provisions is transparency in the animal health situation worldwide. While the necessity for such transparency is particularly obvious in clinical outbreaks of disease, this section of the *Aquatic Code* points out that the presence of an infectious agent, even in the absence of clinical disease, should also be reported.

Chapter 1.2.2 in Section 1.2 presents the criteria for including an aquatic animal disease on the OIE list of notifiable diseases. These criteria are consistent with those used in the *Terrestrial Code*. Diseases proposed for listing must meet all of the relevant parameters under the topics 'consequences', 'spread' and 'diagnosis'. It is important to note that a disease may be listed if it has been shown to, or scientific evidence indicates that it is likely to, negatively affect wild aquatic animal populations that are an asset worth protecting for economic or ecological reasons. This means that primary production losses in aquaculture are not the only consequence that would support a listing.

For some new and emerging diseases, it may not be possible to conduct such a full assessment against the listing criteria, because the scientific information is not yet available. For such diseases, this chapter provides a shorter, less stringent set of listing criteria to facilitate their listing and enable information on the occurrence of these diseases to be collected. It is understood that after an appropriate time period, the Aquatic Animals Commission will re-assess the listing of this disease, with a view to

either conducting a full assessment, or – if the problem has declined – proposing that the disease be delisted.

Chapter 1.2.3 of Section 1.2 shows the diseases listed by the OIE. There are currently (2007) nine diseases of fish, seven diseases of molluscs and nine diseases of crustaceans listed in this chapter.

Section 1.3 Obligations and ethics in international trade

The first chapter in Section 1.3 begins by stating that 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. The chapter then continues with responsibilities of the importing country, for example, that the import requirements included in the international aquatic animal health certificate should assure that commodities introduced into the importing country comply with the national appropriate level of protection (ALOP), and that importing countries should restrict their requirements to those justified by this ALOP. If these are stricter than the OIE standards, guidelines and recommendations, then they should be based on an import risk analysis (see description in Section 1.4 below). Also detailed are the responsibilities of the exporting country, notably, that it must be prepared to supply certain information to importing countries on request. Responsibilities in the case of an incident occurring after importation are also laid down.

The second chapter in Section 1.3 specifies certification procedures.

Section 1.4 Risk analysis

The importation of animals and animal products, whether of aquatic or terrestrial origin, involves a degree of disease risk to the importing country, therefore, in Section 1.4, the *Aquatic Code* describes the principles of conducting risk analyses. The principal aim of import risk analysis is to provide importing countries with an objective and defensible method of assessing the disease risks associated with the importation of animals, animal products, animal genetic material, feedstuffs, biological products and pathological material. The principles and methods are the same whether the commodities are derived from aquatic or from terrestrial animal sources. The analysis should be transparent. This is necessary so that the exporting country is provided with clear reasons for the imposition of import conditions or any refusal to import.

Chapter 1.4.1 outlines the role of the OIE with respect to the SPS Agreement, provides definitions and describes the OIE procedure for settlement of disputes. Chapter 1.4.2 provides guidelines and principles for conducting

transparent, objective and defensible risk analyses for international trade. The components of risk analysis described in Chapter 1.4.2 are hazard identification, risk assessment, risk management and risk communication, as shown in Figure 1.

Chapter 1.4.3 provides guidance on the evaluation of competent authorities, which is an important element of assessing the likelihood of hazards being present in the aquatic animal population of the exporting country.

Given the difficulty of establishing and maintaining freedom from a particular disease for an entire country, especially for diseases whose entry is difficult to control, there may be benefits to some countries in establishing and maintaining a subpopulation with a distinct aquatic animal health status. Subpopulations may be separated by natural or artificial geographical barriers or, in certain situations, by the application of appropriate management practices.

In May 2007, the OIE International Committee adopted a revised version of Chapter 1.4.4 on zoning and compartmentalisation. Zoning and compartmentalisation are procedures implemented by a country to define subpopulations of distinct aquatic animal health status for the purpose of disease control or international trade. Compartmentalisation applies to a subpopulation when management practices related to biosecurity are the defining factors, while zoning applies when a subpopulation is defined on a geographical basis. In practice, spatial considerations and good management play important roles in the application of both concepts.

Zoning and compartmentalisation may not be applicable to all diseases, but the OIE will develop separate requirements for each disease for which the application of zoning or compartmentalisation is considered appropriate. More detailed information on how compartmentalisation

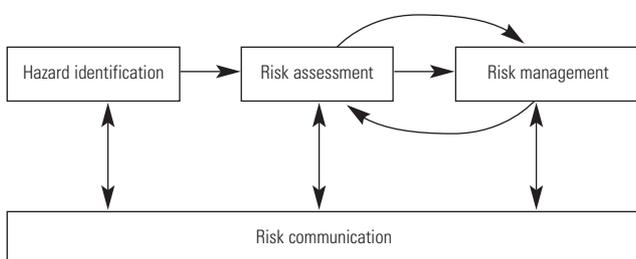


Fig. 1
The four components of risk analysis

Note the reiterative process of re-assessing residual risk after risk management measures have been identified. Also note the importance of risk communication at each step in the process

Source: Section 1.4 – Risk analysis (Article 1.4.1.1). *Aquatic Animal Health Code* (13)

may assist in disease control is provided elsewhere in this publication (14).

More information on import risk analysis in general is contained in the OIE *Handbook on Import Risk Analysis for Animals and Animal Products* which comes in two volumes, the first providing an introduction and details of qualitative risk analysis (7), and the second providing detailed information on quantitative risk analysis (8).

Section 1.5 Import/export procedures

This section describes aquatic animal health measures applicable before departure, during transit, and on arrival. Chapter 1.5.1 makes recommendations for transport and provides detailed technical requirements regarding disinfection of transporters, treatment of transportation water, and discharge of infected water. Chapter 1.5.2 spells out aquatic animal health measures applicable before and at departure, for example, each country should only authorise the export of live aquatic animals and aquatic animal products if they are correctly identified and have been inspected according to the procedures outlined in the *Aquatic Code* and *Aquatic Manual*. Chapter 1.5.3 describes aquatic animal health measures applicable during transit, Chapter 1.5.4 provides details of the responsibilities of frontier posts in the importing country, and Chapter 1.5.5 describes aquatic animal health measures applicable on arrival. The latter includes, for example, guidance that an importing country should only accept into its territory, live aquatic animals that have been examined by a member of the personnel of the competent authority of the exporting country or a certifying official approved by the importing country, and that are accompanied by an international aquatic animal health certificate. Finally, Chapter 1.5.6 recommends measures concerning international transport of aquatic animal disease agents and pathological material. It goes into details on packaging and documentation for transport, with the aim of reducing the risk of accidental release of aquatic animal pathogens during international transport of packaged materials.

Section 1.6 Contingency plans

A number of diseases are regarded as posing a potential threat to aquaculture as well as to wild stocks of aquatic animals in some countries. The introduction of such diseases into countries declared free from these diseases or into countries with an established control system and eradication programme for such diseases, may result in significant losses. To diminish such losses, the veterinary administration or other competent authority responsible for aquatic animal health may need to act quickly and should develop contingency plans before such events occur. This short section, which contains only one chapter, provides guidelines for contingency planning.

More information on contingency planning can be found in another paper in this publication (4).

Section 1.7 Fallowing

Discontinuation of an agricultural pursuit (for example, by crop rotation or by fallowing) is commonly recognised to be of value in resting or restoring the local environment. In the aquatic environment, fallowing can also assist in breaking re-infection cycles by reducing the amount of a disease agent on a site, simply through removing its host. Consequently, fallowing is often carried out as a regular disease management measure in aquaculture, especially prior to the introduction of new populations of aquatic animals into a previously used site. This section, which also contains just one chapter, provides guidelines for fallowing in aquaculture.

Part 2 of the *Aquatic Code*: Recommendations applicable to specific diseases

Part 2 contains recommendations applicable to specific diseases of fish, molluscs and crustaceans. Until 2005, those recommendations focused almost entirely on demonstrating freedom from infection with the diseases listed. The requirements that needed to be fulfilled before a country, zone or individual aquaculture establishment could be declared free were largely identical for each listed disease. They required, for example, two years of targeted surveillance and laboratory testing of a prescribed number of animals, irrespective of the nature of the disease and the epidemiological circumstances.

In May 2003, the International Committee adopted a new *Aquatic Manual* chapter on surveillance requirements for declaration of freedom from infection. This chapter suggested that disease-specific information be used to underpin sampling and surveillance schemes and consequently the provisions for declaration of freedom from listed diseases. It also provided for alternative pathways to declaration of freedom, taking into account historical freedom as well as the absence of susceptible species. With the adoption of that chapter, a major overhaul of Part 2 of the *Aquatic Code* was warranted.

Moreover, prior to 2005, the disease chapters in the *Aquatic Code* did not offer much advice regarding protective measures in international trade in susceptible animals or their products, apart from a general recommendation that the importing country should require a health certificate from the exporting country. The chapters did not suggest any disease-specific measures to manage the risk associated with the importation of certain commodities. Neither did they reflect the realities of trade, such as the fact that more than 90% (45 million tonnes [live weight equivalent]) of world fish trade is in processed

fish (2), some of which can probably be traded quite safely with few – if any – sanitary measures.

In 2004, the Aquatic Animals Commission, in close consultation with the OIE Terrestrial Animal Health Standards Commission, developed a template on which to base individual disease chapters for the *Aquatic Code*. This approach was adopted by the OIE International Committee in 2004 (6). Apart from addressing the issues around commodities and declaration of freedom as described above, the new template also introduced the concept of compartments in addition to zones for establishing and maintaining subpopulations of aquatic animals with a distinct health status (see above).

The International Committee adopted three chapters in the new format in 2005 (one for a disease of fish, one for a disease of molluscs and one for a disease of crustaceans) (9), and in 2006, most other fish and mollusc disease chapters were adopted in the new format (10). By mid 2007, new versions of all disease chapters (except those on de-listed diseases) for fish, mollusc and crustaceans had been adopted (12) and were included in the tenth edition (2007) of the *Aquatic Code* (13). Modifications of a technical nature are made on an ongoing basis to continuously improve the accuracy and applicability of those chapters. (Chapters on diseases that no longer met the listing criteria were maintained if they were still considered of some importance for international trade – these chapters will also be updated over time.)

All disease chapters are now presented in a consistent format, and the recommendations in each of the disease chapters are designed to minimise the risk of the disease under consideration being introduced and established in the importing country, taking into account the nature of the traded commodity (e.g. live animals or dead product), its intended purpose (e.g. for aquaculture or for human consumption) and the aquatic animal health status of the exporting country (declared free or not declared free). This means that, correctly applied, the recommendations ensure that the intended importation can take place with an optimal level of animal health security, incorporating the latest scientific findings and available techniques.

Article 1 of the disease chapters in the *Aquatic Code* defines the disease under consideration 'for the purposes of the *Aquatic Code*'. Articles 2 to 12 of each chapter warrant some closer inspection regarding how they can assist in aquatic animal disease prevention and control.

Article 2 Scope

Article 2 of each disease chapter describes those host species that are susceptible to the disease in question and are traded internationally. A more comprehensive list of susceptible species (that is, including those that are not currently traded internationally) is contained in the

corresponding chapter of the *Aquatic Manual* for reference. Once one of those species becomes relevant in international trade, their addition to Article 2 of the *Aquatic Code* chapter may be proposed for adoption to the OIE International Committee.

Article 3 Commodities

Articles 7 to 12 Recommendations for trade

Essentially, there are two broad categories of traded commodities: the ones that are considered 'safe' for trade, and the ones for which a risk analysis is recommended. For the latter, specific measures are suggested wherever possible.

When authorising import or transit of 'safe' commodities, competent authorities should not require any conditions relating to the disease in question, regardless of the status of the exporting country, zone or compartment for that disease or its intended end-use. Typically, these commodities are 'sterile' products derived from the susceptible species, that is, products that have been treated in such a way that the infective agent has been inactivated. However, for some diseases (for example some mollusc diseases caused by parasites), gametes, eggs and larvae of the susceptible species may also be safe to trade, regardless of their origin or the intended end-use. For some diseases, it is also possible to list as 'safe' any commodities derived from host species 'known not to be susceptible' (as opposed to 'not known to be susceptible'). For example, regarding the molluscan infection with *Marteilia refringens*, the importation of all commodities from the Pacific oyster *Crassostrea gigas* – including the live animal – from anywhere (including countries that are not declared free of this disease) is considered safe.

Another group of commodities that can be assumed to be safe for trade – regardless of where they originate – are those that are destined for human consumption and have been prepared and packaged for direct retail trade. This usually means chemically preserved products, but for some diseases, chilled or frozen eviscerated fish, chilled or frozen fillets or cutlets and dried eviscerated fish are also considered safe.

For the second, bigger group of commodities, i.e. those for which some form of analysis is recommended, specific guidance is provided in Articles 7 to 12. These recommendations for disease-specific risk management measures depend on the status of the exporting country, zone or compartment for that disease and take into account the intended end-use for the traded commodity, for example, release into aquaculture, or direct human consumption, the latter being generally regarded as of lower risk.

For example, for the importation of live aquatic animals, for any purpose, from countries, zones or compartments

declared free of koi herpesvirus disease (KHVD), Article 7 recommends that the importing country require a health certificate from the exporting country that confirms that the place in which the fish were produced has been declared free of KHVD. This is the only measure recommended.

If the live fish are intended for aquaculture purposes and originate from a place not declared free of KHVD, Article 8 applies and recommends that the importing country assess the risk. This article further suggests suitable risk mitigation measures, for example, the direct delivery to, and lifelong holding of the consignment in, biosecure facilities for continuous isolation from the local environment. Also, it is recommended that all effluent and waste material from the processing procedure be treated in a manner that ensures inactivation of KHV.

If these live animals (from a place not declared free of KHVD) were intended for processing for human consumption (Article 9), it is recommended that they be delivered directly to, and held in, quarantine facilities before being slaughtered and processed into one of the 'safe' products listed earlier in Article 3 (or other products authorised by the competent authority). All effluent and waste material from the processing procedure will still need to be treated in a manner that ensures inactivation of KHV. Article 10 has similar provisions that apply when the live animals are intended for use in animal feed, or for agricultural, industrial or pharmaceutical use.

Acknowledging that KHVD may be transmitted even by dead product derived from infected species, the recommendation for the importation of product from countries, zones or compartments not declared free of KHVD (Article 12) is the direct delivery into, and holding of the consignment in, biosecure/quarantine facilities before being processed to become one of the 'safe' products listed in Article 3 (or other products authorised by the competent authority). All effluent and waste material will need to be treated in a manner that ensures inactivation of KHV. If the dead product is from a place declared free of KHVD (Article 11), it is recommended that the importing country require a health certificate from the exporting country that attests that the place in which the fish were produced has been declared free of KHVD. This is the only measure recommended.

Table I summarises the various recommendations for trade in commodities derived from susceptible species.

As well as the categories of species already covered (the species that are known to be susceptible and those that are known not to be susceptible) there is a third category – species that are not known to be susceptible, i.e. they may be, but it is not known for certain. For the importation of a live commodity of this type of species from a place not declared free of KHVD, a risk analysis is recommended

Table I
Recommendations on trade in commodities derived from species susceptible to a given disease

Nature of the commodity	Place of origin declared free of the disease in question		Purpose of importation	General recommendation	Specific measures suggested
	Yes	No			
Live aquatic animals (all live stages, including eggs and gametes)					
Certain life stages that are 'safe'	•	•	Any	No restrictions	n/a
Any life stage that is not considered 'safe'	•		Any	Request aquatic animal health certificate	Yes
Any life stage that is not considered 'safe'		•	Aquaculture	Risk assessment	Yes
Any life stage that is not considered 'safe'		•	Human consumption	Risk assessment	Yes
Any life stage that is not considered 'safe'		•	Animal feeds, etc.	Risk assessment	Yes
Aquatic animal products (non-viable aquatic animals and products from aquatic animals)					
Treated so that the disease agent is inactivated	•	•	Any	No restrictions	n/a
Certain products that have been prepared and packaged for direct retail trade	•	•	Human consumption	No restrictions	n/a
Other products	•		Any	Request aquatic animal health certificate	Yes
Other products		•	Any	Risk assessment	No

n/a: not applicable

only if the species could reasonably be expected to be a potential KHV vector.

Article 4 Disease-free country

This Article describes the requirements for self-declaration of country freedom from the disease under consideration. In general, four different pathways are described:

- absence of susceptible species
- historical freedom
- targeted surveillance with negative results (to be underpinned with scientifically based, disease-specific surveys)
- regaining freedom after an outbreak in a previously free country.

This constitutes a graded, risk-based approach. For example, where there are no susceptible species, there is no need for targeted surveillance (indeed, the value of targeted surveillance in non-susceptible species is difficult to imagine!). The four different pathways are described in more detail below.

Absence of susceptible species

For the purpose of self-declaration of freedom following this pathway, susceptible species are all those species listed in Article 2. However, the *Aquatic Code* recognises that the absence of these species alone may not provide sufficient assurance to allow declaration of freedom from the disease in question. Rather, a set of defined basic biosecurity conditions must have been met continuously for a

specified time period before such a declaration is made. These basic biosecurity conditions are defined in the *Aquatic Code*. They require, *inter alia*, that an early detection system (also defined) is in place that must include 'veterinarians or aquatic animal health specialists trained in recognising and reporting suspicious disease occurrence'. The pertinent definitions are shown in Appendix I.

The number of years for which these basic biosecurity conditions must have been met depends on the disease. Whilst two years is set as a default, the period may be longer or shorter, depending on the biology and life cycle of the agent and the susceptible species; the requirement for, and presence of, intermediate hosts; direct transmission and incubation periods; and the seasonality of the disease. For example, the mollusc disease caused by infection with *Marteilia refringens* is seasonal, therefore, the period for which basic biosecurity conditions must have been met is three years, as this is the optimal period for enabling cases to be detected.

For diseases with a large number and wide range of susceptible host species (for example, the fish diseases viral haemorrhagic septicaemia [VHS] and epizootic ulcerative syndrome, and the mollusc disease caused by infection with *Perkinsus olseni*), the pathway via 'absence of susceptible species' is not recommended.

Historical freedom

Two time periods are critical, first, the 'historical' period during which the disease has not been observed and second, that part of this period during which the basic

biosecurity conditions must have been met continuously (acknowledging that this is unlikely to be the entire historical period). For most fish diseases, the default values are currently 25 years (no observed disease) and ten years (basic biosecurity conditions), respectively. For mollusc and crustacean diseases, these time periods are generally shorter (default values are ten years and two years, respectively), recognising the much shorter life cycles of the host species.

Non-observance of disease can only be used as a pathway for self-declaration of freedom if the conditions in the country have been 'conducive to clinical expression', that is, there must be confidence that if the disease agent had been present, it would have caused clinical signs of disease at some stage. These conducive conditions are further described in the corresponding disease chapters in the *Aquatic Manual*.

Targeted surveillance

The third pathway (targeted surveillance with negative results) can be used for countries that would not meet the requirements of the first two pathways, e.g. countries in which the presence of subclinical infection is likely (i.e. countries in which susceptible species are present but conditions are not conducive to clinical expression of the disease). In such a situation, not only must the basic biosecurity conditions have been met for a number of years, but targeted surveillance for the disease agent in question must have been conducted for that period without detection of the agent.

As with the other pathways, there is a two-year default time period for biosecurity conditions and targeted surveillance; however, for some diseases, more specific time periods can be stipulated to take into account the nature of the disease. For the mollusc disease caused by infection with *Marteilia refringens* the time period for biosecurity measures is three years, but negative results from targeted surveillance must have been obtained for at least the last two of those three years; starting the targeted surveillance in the second year of the biosecurity measures ensures that new cases of infection with *M. refringens* are more likely to be detected.

The OIE *Aquatic Manual* provides guidance on how to design and conduct targeted surveillance. Besides the general guidelines in Chapter 1.1.4, there is also disease-specific information contained in each of the disease chapters (see below). These disease-specific chapters provide information that is crucial for the design and conduct of a sampling and testing programme, e.g. seasonality of the infection, host species and tissue predilections, and the diagnostic specificity and sensitivity of screening tests.

Over time, it will be possible to provide disease-specific time periods in more and more disease chapters of the *Aquatic Code*, consistent with the epidemiological information becoming available in the corresponding disease chapters in the *Aquatic Manual*.

Regaining freedom

The provisions of the fourth pathway apply to those countries that were declared free but then detected the disease in question. In this case, historical freedom and absence of susceptible species cannot apply, so to regain disease-free status targeted surveillance is mandatory for a specified time period (default two: years) after the detection. In addition, before an area can recover its disease-free status, there are several other requirements to fulfil:

- declaration of the affected area as an infected zone
- establishment of a buffer zone
- implementation of measures such as removal and destruction of infected populations and use of appropriate disinfection methods
- review and modification of the previously existing biosecurity conditions (after disease has been detected the newly reviewed biosecurity measures must be implemented for a substantial period of time before disease-free status can be regained (default period: two years).

However, it is possible to maintain part of the non-affected area as a free zone provided it meets the criteria of Article 5 of the pertinent disease chapter in the *Aquatic Code* (see below).

Article 5 Disease-free zone or compartment

This article provides the requirements for self-declaration of freedom for zones and compartments. Essentially, the same four pathways as for country freedom are described. The provisions for regaining freedom after the disease has been detected in a previously free compartment are still under development.

Article 6 Maintenance of status

This article provides the requirements for maintaining disease-free status in a country, zone or compartment. Generally, targeted surveillance may be discontinued provided that basic biosecurity conditions are continuously maintained. However, this does not apply where a free zone or compartment is located in an infected country or in all cases where conditions are not conducive to clinical expression of the disease; in such cases, targeted surveillance needs to be continued at a level determined on the basis of likelihood of infection.

The OIE *Manual of Diagnostic Tests for Aquatic Animals*

The aim of the *Aquatic Manual* is to provide a uniform approach to the diagnosis of the diseases listed in the *Aquatic Code* and of other diseases that may be of importance to international trade, so that the requirements for health certification in connection with trade in aquatic animals and aquatic animal products can be met. This is achieved through the detailing of pathogen identification methods that are suitable for the diagnosis of isolated cases of disease as part of national aquatic animal health surveillance or control programmes, or as part of a programme to underpin claims of freedom from a specific disease.

Although many publications exist on the diagnosis and control of aquatic animal diseases, the *Aquatic Manual* is a key document describing the methods that can be applied in aquatic animal health laboratories all over the world, thus increasing efficiency and promoting improvements in aquatic animal health worldwide. The requirements published in this *Aquatic Manual* are recognised as international standards by the WTO.

The *Aquatic Manual* is updated regularly, and a new edition is published every 2 to 3 years in hard copy and on-line (11). More frequent updates (in highlighted format) are made to the on-line version.

Part 1 of the *Aquatic Manual* contains general provisions, while Part 2 details recommendations applicable to specific diseases. Part 3 contains the list of OIE Reference Laboratories and Collaborating Centres for diseases of fish, molluscs and crustaceans.

Part 1 of the *Aquatic Manual*

Chapter 1.1.1 is about quality management in veterinary testing laboratories. Chapter 1.1.2 explains the principles of validation of diagnostic assays for infectious diseases, and Chapter 1.1.3 provides guidance specifically for the validation and quality control of polymerase chain reaction methods. These three chapters are largely identical to those provided in the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*.

Chapter 1.1.4 of the *Aquatic Manual* describes the requirements for surveillance for declaration of freedom from infection. As mentioned earlier, the OIE International Committee adopted this chapter in May 2003 as a fundamentally new approach to disease surveillance. Perhaps not surprisingly, this chapter was soon found to require refinement and increased user-friendliness. While the fifth edition of the *Aquatic Manual* (2006) still contains

the original (2003) version of that chapter, in 2005 the OIE decided to convene an *Ad hoc* Group on Aquatic Animal Health Surveillance. The terms of reference of this group include the revision of this chapter, and a first draft was circulated to OIE Members with the Aquatic Animals Commission's March 2007 meeting report. To increase consistency with the terrestrial standards, revised guidelines on aquatic animal health will soon be contained in the *Aquatic Code* rather than the *Aquatic Manual*.

Chapter 1.1.5 of the *Aquatic Manual* details methods for disinfection of aquaculture establishments.

Part 2 of the *Aquatic Manual*

Part 2 contains recommendations applicable to specific diseases of fish, molluscs and crustaceans. Just like for the *Aquatic Code*, the template for disease-specific chapters for the *Aquatic Manual* was completely overhauled in preparation for the fifth edition (2006), so these chapters are now presented in a consistent format that links back to the corresponding chapters in the *Aquatic Code*. However, not surprisingly, some teething problems occurred; for example, not all the disease chapters contain the same level of detail. The OIE now engages a consultant editor to, *inter alia*, revise the disease chapter template as well as the actual disease chapters for improved clarity and consistency of content. This is being done in preparation for the next (sixth) hard-copy edition of the *Aquatic Manual* (due in 2009).

Section 1 of each chapter provides a case definition of the disease in question. The following Sections 2 to 6 of each chapter warrant some closer inspection regarding how they can assist in aquatic animal disease prevention and control.

Section 2 Design of surveillance programmes

This section provides information for the design of surveillance programmes. Subsections cover the following:

- agent factors (aetiological agent, agent strains, survival outside the host, stability)
- host factors (susceptible species and their stages, target organs and infected tissue, vectors)
- the disease pattern (occurrence and transmission mechanisms, prevalence, geographical distribution, mortality and morbidity, economic and/or production impact)
- control and prevention (vaccination, chemotherapy, immunostimulation, resistance breeding, restocking with resistant species, blocking agents, and general husbandry practices).

Information on susceptible species and disease pattern is crucial when a country wishes to declare itself free of a

certain disease, regardless of which pathway is chosen. Information on the agent and prevention and control clearly assists in managing the disease.

Section 3 Diagnostic methods

As the 'core part' of each disease chapter, this section provides information on diagnostic methods for the disease in question. Its subsections are field diagnostic methods (usually a short description of clinical signs), clinical methods (including gross and microscopic pathology, electron microscopy and cytopathology) and agent detection and identification methods – usually by far the largest single subsection in each entire chapter.

The clinical signs expressed by fish, molluscs and crustaceans infected with the diseases listed in the *Aquatic Code* are rarely pathognomonic. Moreover, animals may be subclinically infected with the causative agents of these diseases, that is, they may not show any clinical signs. The only reliable approach for diagnosis of aquatic animal diseases lies, therefore, in the specific identification of the pathogens, using laboratory methods. These methods, which are suitable for the diagnosis of isolated cases of disease as part of national aquatic animal health surveillance or control programmes, form the main contents of the *Aquatic Manual*.

These agent detection and identification methods are further divided into direct detection methods (microscopic methods, agent isolation and identification through cell culture or artificial media, antibody-based antigen detection methods, molecular techniques) and indirect methods (serology). However, there is usually little information provided under indirect methods, due to the insufficient development of serological methodology and the resulting lack of acceptance of the detection of antibodies to pathogens in fish as a routine method for assessing the health status of fish populations. For diseases of fish, the validation of some serological techniques could arise in the near future, rendering the use of serology more widely acceptable for diagnostic purposes, but molluscs and crustaceans do not produce antibodies as a response to infection.

In earlier editions of the *Aquatic Manual*, the methods described for screening or diagnosis of fish diseases were based either on isolation of the pathogen followed by its specific identification, or on the demonstration of pathogen-specific antigens using an immunological detection method. However, in recent years, molecular techniques such as the polymerase chain reaction (PCR), DNA probes and *in-situ* hybridisation have been increasingly developed for these purposes.

The experiences of the last decade indicate that the PCR techniques will eventually supersede many of the classical

direct methods of infectious agent detection. It is clear that in many laboratories, PCR is replacing virus isolation or bacteria cultivation for the detection of agents that are difficult or impossible to culture. There are several reasons for this trend, including the fact that virus isolation requires:

- the presence of replicating viruses
- expensive cell culture and maintenance facilities
- as long as several weeks to complete the diagnosis
- special expertise, which is missing or diminishing today in many laboratories.

PCR assays have now become relatively inexpensive, safe and user-friendly tools in diagnostic laboratories. Where a PCR method has been standardised sufficiently to become widely and reliably available, it has been added to the more traditional methods in the *Aquatic Manual*.

Section 4 Rating of tests against purpose of use

This section provides a rating of some of the tests described in Section 3 against a specific purpose of use (surveillance, presumptive diagnosis, confirmatory diagnosis). For many fish diseases, histology – while recommended as a standard method for presumptive diagnosis – would not be recommended for surveillance. In general, the ratings are as follows:

- 'recommended method for reasons of availability, utility, and diagnostic specificity and sensitivity'
- 'standard method with good diagnostic sensitivity and specificity'
- 'the method has application in some situations, but cost, accuracy, or other factors severely limit its application'
- 'presently not recommended for this purpose'.

Although few of the methods listed in the first two categories would have undergone formal standardisation and validation (see Chapters 1.1.2 and 1.2.3 in Part 1 of the *Aquatic Manual*), their routine nature and the fact that they have been used widely without dubious results make them acceptable. It is acknowledged, however, that these ratings are somewhat subjective, as ratings for suitability involve issues of reliability, sensitivity, specificity and utility.

For the most part, molecular methods for fish diseases are recommended for either direct detection of the pathogen in clinically diseased fish or for the confirmatory identification of a disease agent isolated using the traditional method. However, with a few exceptions, molecular techniques are currently not acceptable as screening methods to demonstrate the absence of a specific

disease agent in a fish population for the purpose of health certification in connection with international trade of live fish or their products. There is a need for more validation of molecular methods for this purpose before they can be recommended in the *Aquatic Manual*.

Because of the general unavailability of the traditional pathogen isolation methods for mollusc and crustacean diseases, molecular techniques, particularly PCR, have increasingly supplemented the more traditional histological and tissue smear methods described in the *Aquatic Manual*, not only for diagnosis of clinical cases but also for screening programmes to demonstrate the absence of the specific disease agent for health certification purposes.

Section 5 Corroborative diagnostic criteria

This section provides corroborative diagnostic criteria for the definition of a suspect case and a confirmed case, respectively. For many of the diseases, these definitions are a combination of clinical parameters and laboratory test results, the latter referring back to Section 4 and the rating of tests against purpose of use. An example is shown in Appendix II for infectious salmon anaemia.

Section 6 Diagnostic/detection methods to declare freedom

This section recommends diagnostic or detection methods to declare freedom from the disease under consideration. As for the corroborative diagnostic criteria, these criteria also should refer back to Section 4 and the rating of tests against purpose of use.

OIE Reference Laboratories and Collaborating Centres

There are currently 27 OIE Reference Laboratories for aquatic animal diseases. Under the guidance of an expert whose competence is recognised internationally, they function as centres of expertise and standardisation of diagnostic techniques. Additionally, they may provide scientific and technical training for personnel from OIE Member Countries and Territories and coordinate scientific and technical studies in collaboration with other laboratories or organisations.

The network of OIE Reference Laboratories is also well equipped to address generic problems arising with the diagnosis of animal diseases. One example is the ongoing, contentious issue of whether it is feasible to differentiate between genotypes of certain agents of listed diseases for the purposes of notifying the OIE and applying control measures. Examples from the aquatic field include the fish

disease VHS and the crustacean disease infectious haematopoietic and hypodermal necrosis.

The Aquatic Animals Commission organised a special workshop on pathogen strain differentiation and listing and notification of diseases by strain/genotype during the First International Conference of OIE Reference Laboratories and Collaborating Centres, which was held in Florianopolis, Brazil, from 3 to 5 December 2006. As this issue is a problem for terrestrial as well as aquatic animal diseases the workshop was attended by representatives from both aquatic and terrestrial Reference Laboratories, with both sides providing constructive contributions to the debate.

The workshop concluded that the issue of differentiating genotypes of an agent of a listed disease is of increasing relevance, especially for listing and reporting of certain diseases of aquatic animals, and that this will be an ongoing issue for the foreseeable future and will require further scientific debate. The workshop also passed a number of recommendations to ensure that progress is made with regard to these issues.

The OIE Collaborating Centre for Information on Aquatic Diseases is located at the Centre for Environment, Fisheries and Aquaculture Science in Weymouth in the United Kingdom. The Centre created and maintains an international database on the occurrence of the OIE listed aquatic animal diseases, which is freely available via the internet (www.collabcen.net). This database can also be accessed through the web pages of the Aquatic Animals Commission (www.oie.int/aac/eng/en_fdc.htm). The Director of the Collaborating Centre is also currently the chief editor of these pages, which provide easy access to technical information on aquatic animal health issues, including recent disease reports from OIE Members, and a 'latest news' service.

Challenges ahead

Over recent years, new issues have arisen for the OIE to address in the field of aquatic animals, e.g. aquatic animal welfare and the development of antimicrobial resistance in aquaculture. The safety of aquatic feeds for aquatic animals is another novel topic that requires detailed consideration before recommendations can be developed for the *Aquatic Code*.

Another important new initiative of the OIE is the inclusion of diseases of amphibians in its remit. Between late 2006 and early 2007, Member Countries and Territories responded to a questionnaire on amphibian

diseases, and over 70% of the respondents (total number of responses = 65) supported the inclusion of amphibian diseases in the remit of the OIE. Based on this supportive majority, the International Committee, at its 75th General Session in 2007, agreed to expand the OIE remit to include amphibian diseases. The first steps in the implementation of this expansion will be to make certain diseases of amphibians notifiable to the OIE, to provide diagnostic guidance for those diseases, and to prepare recommendations for international trade in amphibian species that are susceptible to these diseases.

Unlike the terrestrial animal farming sector, which relies on a comparatively small number of usually well-domesticated animals worldwide, aquaculture continues to trial 'new' species for their suitability to breed and grow under controlled conditions. Typically, little is known about the health status of such new candidate species, their nutritional and other husbandry requirements, or their life cycle. Frequently, broodstock are taken from the wild, increasing the risk of introducing pathogens into hatcheries. New diseases therefore continue to emerge, and developing diagnostic techniques is a 'catching-up' game.

Multi-agent disease syndromes are common in the aquatic environment and are increasingly described, particularly in crustacean hosts. Most crustacean viruses cannot be grown in cell culture, infectivity trials are therefore hampered severely, and it is difficult to ascertain which role – if any – a particular infectious agent plays in the syndrome. This is far from being an academic or research problem; rather, it severely compromises the development of case definitions for disease reporting purposes and the development of control strategies.

Currently, nearly half of the fish consumed as food worldwide originate in aquaculture rather than in the wild, compared to less than 10% in 1980, and while consumer

demand for fish continues to climb worldwide, levels of capture from the wild have remained roughly stable since the mid 1980s (3). Therefore, there will be increasing pressure on aquaculture to meet the demand, and with increased production will come increased disease risks. These will arise not only through the intensification of culture but also through the switch to 'new' species (see above) and the translocation of live animals that have successfully been cultured in one part of the world to new destinations. The accompanying risk of disease transfer is well documented, but it appears that there is still not enough attention paid to preventing spread of such diseases.

The *Aquatic Code* and *Aquatic Manual* have a major role to play not only in the prevention and control of aquatic animal diseases, but also more generally in the improvement of aquatic animal health worldwide. The Aquatic Animals Commission, with the assistance of experts, will continue to do its best to ensure that the provisions of those standards are science-based and globally applicable. With the support of the OIE Central Bureau, the Commission will also continue raising awareness worldwide about OIE aquatic standards and the Organisation's role in aquatic animal health more generally. Recent examples are presentations at the OIE Global Conference on Aquatic Animal Health, held in Bergen (Norway) in October 2006 (1) and the First International Conference of OIE Reference Laboratories and Collaborating Centres, held in Florianopolis (Brazil) in December 2006 (5).



Le rôle des normes et des Laboratoires de référence de l'OIE dans la prévention et le contrôle des maladies des animaux aquatiques

E.-M. Bernoth

Résumé

L'Organisation mondiale de la santé animale (OIE) élabore des textes normatifs décrivant les règles applicables par les Pays et Territoires Membres pour que ceux-ci puissent se prémunir contre les maladies animales sans dresser des barrières sanitaires injustifiées aux échanges commerciaux. S'agissant des maladies des animaux aquatiques, c'est la Commission des normes sanitaires pour les animaux aquatiques qui est chargée de préparer le *Code sanitaire pour*

les animaux aquatiques et le *Manuel des tests de diagnostic pour les animaux aquatiques*, assistée d'experts internationalement reconnus et des autres Commissions spécialisées de l'OIE, en consultation avec les Membres de l'Organisation. Le rôle de ces normes dans la prévention et le contrôle des maladies des animaux aquatiques est décrit en détail. Il existe actuellement 27 Laboratoires de référence de l'OIE et un Centre collaborateur pour les maladies des animaux aquatiques, qui forment un réseau d'expertise dans le domaine de la santé des animaux aquatiques. En fournissant des prestations de diagnostic et de conseil qui s'avèrent particulièrement utiles lors des situations d'urgence, ces laboratoires jouent un rôle crucial dans la prévention et le contrôle des maladies des animaux aquatiques.

Mots-clés

Animal aquatique – Commerce international – Diagnostic – Norme – Organisation mondiale de la santé animale – Prophylaxie – Risque sanitaire – Santé des animaux aquatiques.



El rol de las normas y los Laboratorios de Referencia de la OIE en la prevención y control de las enfermedades de los animales acuáticos

E.-M. Bernoth

Resumen

La Organización Mundial de Sanidad Animal (OIE) elabora textos normativos referentes a las reglas que los Países y Territorios Miembros pueden aplicar para protegerse de las enfermedades sin por ello erigir barreras sanitarias injustificadas. La Comisión de Normas Sanitarias para los Animales Acuáticos, con la ayuda de expertos de renombre internacional y de otras comisiones especializadas de la OIE, y en consulta con los Miembros de la Organización, es la encargada de preparar tanto el *Código sanitario para los animales acuáticos* como el *Manual de pruebas de diagnóstico para los animales acuáticos*. La autora describe con todo detalle la función que cumplen estas normas en cuanto al control y la prevención de enfermedades de los animales acuáticos. Hay actualmente 27 Laboratorios de Referencia y un Centro Colaborador de la OIE que trabajan sobre el tema y configuran, en su conjunto, una gran red de especialistas en la materia. Con los servicios de diagnóstico y asesoramiento especializado que prestan, especialmente útiles en situaciones de emergencia, dichos establecimientos son una pieza cardinal en el control y la prevención de las enfermedades de los animales acuáticos.

Palabras clave

Animales acuáticos – Comercio – Control de enfermedades – Diagnóstico – Normas – Organización Mundial de Sanidad Animal – Riesgo de enfermedad – Sanidad de los animales acuáticos.



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Appendix I

OIE *Aquatic Animal Health Code* definitions relating to the declaration of freedom from certain diseases

Terms displayed in italics are further defined in the *Aquatic Animal Health Code* (13)

Basic biosecurity conditions means a set of conditions applying to a particular *disease*, and a particular *zone* or country, required to ensure adequate disease security, such as:

- a) the *disease*, including suspicion of the *disease*, is compulsorily notifiable to the *Competent Authority*; and
- b) an *early detection system* is in place within the *zone* or country; and
- c) import requirements to prevent the introduction of *disease* into the country or *zone*, as outlined in the *Aquatic Code*, are in place.

Early detection system means an efficient system for ensuring the rapid recognition of signs that are suspicious of a *listed disease*, or an *emerging disease* situation, or unexplained mortality, in *aquatic animals* in an *aquaculture establishment* or in the wild, and the rapid communication of the event to the *Competent Authority*, with the aim of activating diagnostic investigation with minimal delay. Such a system will include the following characteristics:

- a) broad awareness, e.g. among the personnel employed at *aquaculture establishments* or involved in *processing*, of the characteristic signs of the *listed diseases* and *emerging diseases*;
- b) veterinarians or *aquatic animal* health specialists trained in recognising and reporting suspicious disease occurrence;
- c) ability of the *Competent Authority* to undertake rapid and effective disease investigation;
- d) access by the *Competent Authority* to laboratories with the facilities for diagnosing and differentiating listed and emerging diseases.

Emerging disease means a newly recognised serious *disease*, the cause of which may or may not yet be established, that has the potential to be spread within and between populations, for example by way of trade in *aquatic animals* and/or *aquatic animal products*.



Appendix II

Corroborative diagnostic criteria for infectious salmon anaemia (ISA), as contained in the ISA chapter of the *OIE Manual of Diagnostic Tests for Aquatic Animals*

5. Corroborative diagnostic criteria

Reasonable grounds to suspect fish of being infected with ISAV are outlined in Section 5.a below. The Competent Authority shall ensure that, following the suspicion of fish on a farm being infected with ISAV, an official investigation to confirm or rule out the presence of the disease will be carried out as quickly as possible, applying inspection and clinical examination, as well as collection and selection of samples and using the methods for laboratory examination as described in Section 3.

a) Definition of suspect case

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

- i) Clinical and/or pathological changes consistent with ISA (Section 3.a and b), with or without clinical signs of disease
- ii) Isolation and identification of ISAV in cell culture from a single sample from any fish on the farm as described in Section 3.c.ii
- iii) Evidence for the presence of ISAV from two independent laboratory tests such as RT-PCR (Section 3.c.ii) and IFAT on tissue imprints (Section 3.c.ii)
- iv) Transfer of live fish from a farm where ISA may be suspected to be present to farms without suspicions of ISA
- v) Any other epidemiological links to ISA-suspected or confirmed farms
- vi) Detection of antibodies to ISAV.

b) Definition of confirmed case

The following criteria in i) or ii) or iii) should be met for confirmation of ISA:

- i) Mortality, clinical signs and pathological changes consistent with ISA (Section 3.a and b), and detection of ISAV by one or more of the following methods:
 - a) isolation and identification of ISAV in cell culture from at least one sample from any fish on the farm as described in Section 3.c.ii
 - b) detection of ISAV by RT-PCR by the methods described in Section 3.c.ii
 - c) detection of ISAV in tissue preparations by means of specific antibodies against ISAV (e.g. IFAT on tissue imprints or fixed sections as described in Section 3.c.i)
- ii) Isolation and identification of ISAV in cell culture from at least two independent samples from any fish on the farm tested on separate occasions as described in Section 3.c.ii
- iii) Isolation and identification of ISAV in cell culture from at least one sample from any fish on the farm with corroborating evidence of ISAV in tissue preparations using either RT-PCR (Section 3.c.ii) or IFAT (Section 3.c.ii).

Source: (11).
