PART 2

RECOMMENDATIONS APPLICABLE TO SPECIFIC DISEASES

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GENERAL INTRODUCTION

A. OVERALL APPROACH TO AQUATIC ANIMAL HEALTH MANAGEMENT

A comprehensive approach for animal health control in aquatic animal culture requires:

- Assessment of the health status of aquatic animals using methods based on the guidelines in the *Aquatic Animal Health Code* (the *Aquatic Code*) and the related objectives of surveillance systems in the OIE *Guide for Aquatic Animal Health Surveillance* (2009).

- The constraint of restocking open waters and farming facilities only with aquatic animals having a health status higher than or equal to that of aquatic animals already living in the considered areas.

- Eradication of disease in aquaculture facilities when possible, by slaughtering infected stocks, disinfecting facilities and restocking with aquatic animals from approved specific disease-free sources.

- The implementation of biosecurity measures at the production site level; this is important in health management and is necessary for the prevention of entry and spread of aquatic animal diseases.

- Notification by every Member Country upon request from its trading partners of its particular requirements, besides those provided by the *Aquatic Code*, for importation of aquatic animals and aquatic animal products.

If the above procedures are followed, it becomes possible to give adequate assurance of the health status of aquaculture products for specified diseases, according to their country, zone or compartment of origin.

The issue of a health certificate by the appropriate official, based on a health status report and examinations of aquatic animals, provides adequate assurance that the aquaculture products in a defined consignment originate from a whole country, a zone or a compartment free of one or more of the specified diseases listed in the *Aquatic Code* and possibly of other specified diseases (see model of international certificate in the *Aquatic Code*, Part 5).

Surveillance is also aimed at determining the occurrence or distribution of endemic disease or infection, including changes to their incidence or prevalence (or its contributing factors), in order to provide information for domestic disease control programmes, as well as provide relevant disease occurrence information to be used by trading partners for qualitative and/or 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.

The assessment of the health status of aquatic animal stocks is based on inspection of production sites and further laboratory examination of samples originating from specimens taken among the stock of a defined aquatic animal population. This endeavour requires the sample to be collected and processed according to accepted methods. Sample sizes based on statistical methodology for surveillance or for issuing health certificates should be calculated using the guidelines in the *Aquatic Code* and the OIE *Guide for Aquatic Animal Health Surveillance* (2009).

Among several techniques applicable to aquatic animal pathogens, the *Aquatic Manual* has established two types of examination procedures that will be acceptable for such work: 1) Screening methods, and 2) Diagnostic methods. The accepted methods are listed under each disease chapter. The sample size required varies with the intended purpose of the disease diagnostic or pathogen detection method. The sensitivity and specificity of the accepted methods listed in each disease-specific chapter should be taken into account, especially when a test is used to demonstrate disease freedom.

The guidelines in the specific disease chapters may be applied to all the aquatic animal diseases, their agents and susceptible species as listed in the *Aquatic Manual*, and are designed to assist with the development of surveillance

methodologies. These guidelines are also applicable to other aquatic animal diseases that are not included in the *Aquatic Manual* but which may be of importance to a country or region, such as new or emerging diseases. Where possible, the development of surveillance systems using these guidelines should be based on the relevant information in the individual disease chapters.

Sometimes more than one susceptible species are present in a country/zone/compartment. Unless otherwise specified in the individual disease chapter in this *Aquatic Manual*, all susceptible species should be considered for sampling.

The assessment of the health status of aquatic animal stocks is based on surveillance, which includes sampling and testing of epidemiological units. This endeavour requires the design and implementation of surveillance systems as described in the OIE *Guide for Aquatic Animal Health Surveillance* (2009), sampling and assessing the health status of epidemiological units, as described in this chapter, and the testing of samples according to methods described in the individual disease chapters.

B. TARGET PATHOGENS AND DISEASES

Target pathogens and aquatic animal diseases are included in the *Aquatic Code* according to the following basic considerations: the disease has been shown to cause significant production losses at a national or multinational (zonal or regional) level or the disease has been shown to or scientific evidence indicates that it is likely to negatively affect wild aquatic animal populations or the agent is of public health concern, and infectious aetiology of the disease is proven or an infectious agent is strongly associated with the disease, but the aetiology is not yet known, and the likelihood exists of international spread, including via live animals, their products or fomites, and several countries or countries with zones may be declared free of the disease based on the general surveillance principles outlined in *Aquatic Code* Chapter 1.4 *Aquatic animal health surveillance,* and a repeatable and robust means of detection/diagnosis exists. For the OIE list of diseases in vigour, please consult the current edition of the *Aquatic Code.* This *Aquatic Manual* includes chapters on specific diseases, including all diseases listed in the *Aquatic Code*.

Surveillance can also be developed for non-listed diseases, especially for emerging diseases.

It would be impractical to try to develop a surveillance system for all the known aquatic animal diseases in a country. Therefore, prioritising the diseases to be included in a surveillance system should be conducted by considering:

- the need to provide assurance of disease status for trade purposes,
- the resources of the country, taking into account that, even if a country has few resources, there should not be different testing standards. Meeting the standards to support trade should be a trade requirement subject to variance only when agreed by the importing country,
- the risk posed by the different diseases to external trade (destination country) and internal movements (different region/zone in the origin country).

The concept of risk encompasses the probability of the disease occurring, the capacity to spread and the severity of its consequences. These aspects are dealt with in further detail in *Aquatic Code* Chapter 2.1 *Import risk analysis*.

C. SAMPLING

1. Sampling objectives

There are at least three purposes for which aquatic animal stocks may be sampled. These are: 1) surveillance; 2) issuing health certificates, and 3) disease outbreak investigation. The number and type of samples to be taken for analysis and the sampling procedure varies greatly according to which of these purposes applies.

2. Number of epidemiological units to be sampled

Sample sizes based on statistical methodology for surveillance or for issuing health certificates should be calculated using the guidelines in chapter 1.4 of the *Aquatic Code* (and the OIE *Guide for Aquatic Animal Health Surveillance* (2009).

When conducting disease outbreak investigations, the number and status of units to be sampled should be determined in accordance with the appropriate chapter of the *Aquatic Manual* dealing with the case definition.

Aquatic animal collection should encompass a statistically adequate number of specimens, although it is obvious that failure to detect certain pathogens from the sample does not guarantee the absence of these agents in the specimen examined or in the stock. This is particularly true of free-ranging or feral stocks from which it is difficult to collect a representative and random sample. However, the risk of a pathogen escaping the surveillance system is reduced in production facilities whose stocks have been inspected and checked for pathogens over a period of several years (at least 2), insofar as they are not exposed to possible recontamination by feral populations.

For all diseases, it is highly recommended that the scheduling of sampling be planned (i.e. by farm schedule, season, etc.) so that the particular life-stage(s) is(are) sampled at a time when the pathogen of concern is most likely to be detected. This is especially important when the available diagnostic methods are dependent on simple microscopy or histological methods and do not include molecular methods.

Any issues related to clustering of infection, test characteristics, and multiple sources of information, as well as quality assurance, are described in the OIE *Guide for Aquatic Animal Health Surveillance* (2009).

2.1. Sampling to demonstrate disease freedom

To demonstrate disease freedom, aquatic animals should be selected in a way that increases the chances of finding the pathogen if it is there, therefore increasing the sensitivity of the adopted diagnostic method. For example, sampling crustaceans or fish that are lethargic or approach the edge of ponds, tanks and raceways increases the chances of finding infected individuals. Moribund animals with clinical signs consistent with the disease in question should therefore be preferentially selected and the remainder of the sample should comprise randomly selected animals from the population of interest. Similarly, when information on the risk factors for the disease/infection in question is known, sampling should be done in the highest risk units to increase the chances of detecting the pathogen.

The goal of this targeted sampling strategy is to increase the pre-test probability of samples containing infected individuals. The effect is to 'bias' towards disease detection. It is important to remember that when sampling is biased, positive test results for a pathogen cannot be used to obtain an accurate estimate of the disease prevalence in the population of interest. For example, a sample of 100 moribund animals resulting in a single positive individual does not necessarily indicate a prevalence of 1% for the population in general.

Test sensitivity and specificity are important for sample size calculation, for analysis of results and for drawing conclusions. Test sensitivity is particularly important when demonstrating disease freedom.

2.2. Sampling to assess disease occurrence and distribution

To assess disease occurrence (e.g. estimate prevalence or incidence), the epidemiological units selected should comprise a representative sample of the population of interest concerning the parameter being estimated. Such a representative sample can only be produced using probability sampling. Sampling units for convenience or attempting to purposely create a representative sample by selecting units will not yield representative samples.

While selection of a representative sample of farms is more feasible using random sampling methods or geographical sampling, opportunities for selection of a truly random sample are rare in aquaculture settings. For example, simple random sampling can be used if the aquatic animal can be identified individually and enumerated (this is sometimes possible for some broodstock animals).

Innovative methods to attain randomness should be sought whenever possible. Such methods may be possible when access to all individuals/units in the population is available. For example, during processing each animal may, in some cases, be handled individually and a systematic random sampling scheme can be designed.

Although non-random samples are commonly used in aquaculture, they are technically impossible to use in statistical analyses. In the instances in which a true random sample cannot be collected, convenience or haphazard sampling can be conducted, although it cannot produce a truly representative sample, and, therefore, the parameter estimates will be biased, often in an unknown direction. Methods for non-random sampling that may produce relatively more representative results, for instance, include cast-net sampling in the case of crustaceans. The use of feed trays or pellets should be avoided as the animals selected would be relatively more healthy, therefore often producing an underestimate of the occurrence and prevalence of the disease or pathogen in question. Given the bias recommended by the sampling procedures outlined in this text, it is important to note that the results cannot be used to determine outbreak risk or outbreak rates as the data are highly biased toward disease prevalence. Risk assessment requires a true randomised sampling scheme.

When the objective is to estimate the prevalence of infected farms in a country, the process will be done in two steps. In the first step, a representative sample of farms will be selected using methods such as those outlined

in Survey Toolbox[1]. In the second step, a survey will be conducted on each selected farm to assess whether the farm is free from the disease or not using the methods that have been outlined in the OIE *Guide for Aquatic Animal Health Surveillance* (2009). The proportion of infected farms in this survey can be used to estimate the prevalence of infected farms in the country.

2.3. Sampling to investigate suspected disease outbreaks

The investigation of a clinical outbreak requires a logical and systematic approach, based on clinical experience, to select individuals and the correct diagnostic tests. Sufficient numbers of moribund (or high risk) individuals should be selected for diagnostic testing based on clinical signs suggesting the occurrence of likely disease processes. It should be remembered that many diseases require multiple factors to create sufficient conditions to cause clinical disease and identification of a pathogen in moribund individuals does not, in itself, confirm that the pathogen was the inciting cause of the outbreak. In addition, it does not mean that non-moribund individuals in the same population are free of this pathogen.

In clinical disease episodes, carefully selected specimens with representative lesions should be obtained from live or moribund animals. Every effort should be made to sample those specimens for diagnoses that are representative of the disease(s) that is (are) affecting the stock of interest, and that are moribund or clinically diseased. Collection of dead specimens should be avoided. When cultured or wild populations are presenting clinical signs of an active disease that are consistent with, or suggestive of, any one of the OIE listed diseases, care should be taken to ensure that the samples collected are preserved appropriately for the anticipated diagnostic tests (see Section 4 *Diagnostic Methods* in the specific disease chapters).

Regardless of the surveillance objectives, the sampling methods used at all levels must be fully documented and justified.

Test sensitivity is important for disease outbreak investigation, even if the number of false positives is increased, because identifying cases is of utmost importance. As stated before, test sensitivity and specificity are also important for sample size calculation, for analysis of results and for drawing conclusions.

3. Sampling protocol

3.1. Selection of epidemiological units and sampling methods

Surveillance data may originate from a number of different sources.

Mortality records are usually available for cultured aquatic animal facilities. However, in the case of mollusc sites, they are either rarely available or they are available with a lower frequency of observation (e.g. a cumulative mortality observed over a 2-week period). The existence of records can be used to identify periods of unexpectedly high mortality or unexplained events, which would direct sampling to higher probability components of the population or time periods if the objective was to detect a specific disease or infection.

Many aquatic animal diseases share risk factors related to disease introduction or transmission, such as exposure between age classes in a region. Known age pattern distributions within production areas assist in designing surveys.

The value of individual units in aquaculture production facilities often warrants routine health monitoring visits. Interaction with the farm's usual veterinarian or aquatic animal health specialist represents a valuable source of evidence for identification of outbreaks and also for verification of the absence of unexplained clinical events occurring in a population.

Aquatic animals sampled should be alive when collected, unless otherwise specified in the individual disease chapters.

During disease outbreak investigations, if any moribund animals are present in the population to be sampled, they should be selected first and the remainder of the sample should comprise randomly selected live aquatic animals from all rearing units that represent the lot being examined. In the case of wild populations of sessile

1 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 .

animals (e.g. molluscs), a higher suspect component of the population may comprise individuals located in close proximity to mortalities.

3.2. Post-sampling procedures

Organ, tissue and fluid aquatic animal samples should be taken and processed as soon as possible after specimen collection. Sample freezing should be avoided if histological examination is required. However, freezing samples is an option for several specific testing purposes, such as virus bioassays, PCR testing, toxicology testing and even some bacteriological purposes. Consequently, the most appropriate samples should be preserved in the most appropriate manner for the intended diagnostic test(s).

Samples should be sent to the laboratory and packed separately in sealed aseptic refrigerated containers or on ice but, where freezing is not appropriate (see above), direct contact with the ice or freezer packs should be avoided. However, it is highly preferable and recommended to collect samples from the aquatic animals immediately after they have been selected at the aquaculture production site and to store and process the samples as described in the individual chapters or in the introductory chapters for each species. An identification label that includes information on the place, time, date, species, number of samples collected, dead/moribund state on collection, and the name and contact information of the individual collecting the sample(s) should be attached to the sample(s). An indelible (waterproof and alcohol resistant) marker or a number 2 pencil should be used to label the samples using durable water resistant or plastic paper.

KEY REFERENCE FOR FURTHER READING

World Organisation for Animal Health (OIE) (2021). Chapter 1.4. Aquatic animal health surveillance. *In:* Aquatic Animal Health Code, 23rd edition. OIE, Paris, France.

World Organisation for Animal Health (OIE) (2009). OIE *Guide for Aquatic Animal Health Surveillance.* OIE, Paris, France.

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