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# A 12-point checklist for surveillance of diseases of aquatic organisms: a novel approach to assist multidisciplinary teams in developing countries

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## Abstract

A 12-point checklist in the design and practical application of active surveillance of diseases in aquatic organisms (farmed and wild) has been developed to serve as a methodological approach and guidance for a multidisciplinary team particularly in countries where surveillance expertise is limited. The checklist is based on a review of available main aquatic surveillance references and scientific literature and was further developed based on the outcomes of several aquaculture biosecurity project-related workshops hosted by the Food and Agriculture Organization of the United Nations. The checklist includes the following: (1) scenario setting; (2) defining surveillance objective; (3) defining the populations; (4) disease clustering; (5) case definition; (6) diagnostic testing; (7) study design and sampling; (8) data collection and management; (9) data analysis; (10) validation and quality assurance; (11) human and financial resources and logistics requirements; and (12) surveillance in the bigger picture. For a multidisciplinary approach to disease control, knowledge of fish biology, aquaculture systems and many aspects of aquaculture health management are required. Surveillance needs significant financial investment and must be supported by adequate diagnostic capability, information system management, legal framework and communication networks, with transparent reporting mechanisms to allow rapid disease response for serious diseases of aquatic organisms. It is a stepwise and pragmatic approach that offers a good starting point for addressing disease issues especially in developing countries. It can be used as a model to build targeted surveillance competency and a basic reference when implementing a surveillance programme or improving existing programmes.

**Key words:** 12-point surveillance checklist, active disease surveillance, aquaculture, aquatic diseases control, aquatic organism, epidemiology.

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## Introduction

Of the 17 United Nations sustainable development goals (SDGs), addressing biosecurity and health management in aquaculture is more or less relevant to SDG numbers 1, 2, 3, 5, 8, 12, 13, 14 and 17 that pertains to no poverty, zero hunger, good health and well-being, gender equality, decent work and economic growth, responsible production and consumption, climate action, life below water and partnerships for the goals, respectively (https://sdgs.un.org/goals).

Recognized as the fastest growing food-producing sector in the world, aquaculture had an average annual growth rate of 7.5% since 1970, compared with only 0.9% for capture fisheries and 2.6% for terrestrial farmed meat production systems over the same period (FAO 2020). In 2018, the total world aquaculture production (including aquatic plants) was reported to be 114.5 million tonnes by volume and USD 263.6 billion by value. Of the world total, China produced 57.8% of the total volume and 58.6% of the total value of aquaculture production. Other top producers during 2018 were Indonesia, India, Viet Nam, Bangladesh, the Philippines, South Korea, Egypt, Norway and Chile (FAO 2020). In 2050, edible food from the sea, including aquaculture, could increase by 21 to 44 million tonnes, which is a 36 to 74% increase compared with current yields, representing about 12 to 25% of the estimated increase in all meat needed to feed an expected population of 9.8 billion people (Costello *et al*. 2020).

The disease situation in aquaculture is changing rapidly and very difficult to predict due to the current period of accelerated change in the international trading environment – affected by globalization, increasing aquaculture production, microbial adaptation and climate change (Subasinghe *et al*. 2004; Bondad-Reantaso *et al*. 2018; Woo *et al*. 2020). Disease has been a primary constraint to the culture of many aquatic species, impeding both economic and social development in many countries (FAO/NACA 2000; Bondad-Reantaso *et al*. 2005).

Disease is a major limiting factor for successful aquaculture production, with lasting effects on socio-economic development in many countries (FAO/NACA 2000; Bondad-Reantaso *et al*. 2005). Country-level impacts of a significant disease can be estimated indirectly through the level of income, production losses, employment, international trade, investments and consumer confidence (Bondad-Reantaso *et al*. 2005). In the 1990s, production losses in 15 countries in Asia, due to epizootic ulcerative syndrome (EUS), penaeid shrimp diseases and a variety of other diseases, were estimated at USD 1.36 million (ADB/NACA 1991). Total estimated losses in production due to shrimp diseases on a global scale (i.e. 11 countries) reached USD 3 019 million from 1987 to 1994 (Israngkura & Sae-Hae, 2002). At the national level, examples of loss estimates due to infectious salmon anaemia (ISA) include that of the following: (1) GBP 20 million loss to the Scottish farming industry due to ISA outbreaks during the 1998/1999; (2) USD 11 million annual loss to Norwegian salmon industry; and (3) USD 14 million annual loss to Canadian salmon industry (Hastings *et al*. 1999).

More recent estimates of economic losses from decreased production and exports caused by acute hepatopancreatic necrosis disease (AHPND) were USD 12 billion and greater than USD 26 million in Thailand (2010–2017 period) and Viet Nam (2015), respectively (Shinn *et al*. 2018). In China, the world's biggest aquaculture producer, losses due to diseases officially reported are substantial and show an increasing trend (BOF *et al*. 2019). For example, in 2018, disease-related losses were approximately USD 6.5 billion, an increase in USD 1.2 billion more than the losses registered in 2017 and USD 2.4 billion more than 2016; these losses involved 66 different cultured species.

Thus, in this age of uncertainty of food security due to negative impacts of diseases, the use and application of 'surveillance' and 'reporting' has become very timely. The sustainability of the sector will be compromised if challenges posed by exotic, endemic and emerging diseases of aquatic organisms are not tackled in a responsible and efficient manner.

Surveillance is a systematic process of gathering information about the occurrence of important diseases and pathogens in order to produce meaningful reports on the disease status of a farm, zone, country or region. Surveillance will thus support import risk analysis, justify import health certification requirements, and enable export health certification by providing evidence to substantiate claims of the absence of a particular disease (FAO/NACA 2000). Surveillance and reporting are important elements of the Asia Regional Technical Guidelines on Health Management for the Responsible Movement of Live Aquatic Animals (FAO/NACA 2000) and fundamental components of any aquatic animal health protection programme or national strategy on aquatic animal health management (FAO 2007).

According to the World Organisation for Animal Health (OIE) Aquatic Animal Health Code (OIE 2019a), surveillance objectives may be one of the following: (1) demonstrating the absence of a disease; (2) identifying events requiring notification; (3) or determining the occurrence or distribution of endemic diseases, including changes to their incidence or prevalence, in order to: (i) provide information for domestic disease control programmes and/or (ii) provide relevant disease occurrence information to be used by trading partners for qualitative and quantitative risk assessments.

Several important transparent and transnational reporting systems exist for aquatic animal diseases, such as the World Animal Health Information System (WAHIS) of the

OIE (https://www.oie.int/wahis_2/public/wahid.php/Wahidhome/Home), the Animal Disease Notification System (ADNS) of the European Union (https://ec.europa.eu/food/animals/animal-diseases/not-system_en) and the Emergency Prevention System for Animal Health (EMPRES-AH) of the Food and Agriculture Organization of the United Nations (FAO) (http://www.fao.org/ag/againfo/programmes/en/empres/home.asp. The WAHIS is globally accepted as a disease information tool to facilitate the World Trade Organization's Sanitary and Phytosanitary Agreement (SPS Agreement).

The first-ever regional aquatic disease reporting system, the Quarterly Aquatic Animal Disease (QAAD) Reports (Asia and Pacific Region) was developed by the Network of Aquaculture Centres in Asia-Pacific (NACA), the FAO and the OIE through an FAO Technical Cooperation Programme (TCP) Project TCP/RAS 6714 (A) and 9065 (A) – 'Assistance for the Responsible Movement of Live Aquatic Animals', implemented by NACA in 1998, with the participation of 21 countries throughout the region (FAO/NACA 2000, 2001). FAO and NACA collaborated closely with OIE with the specific objective of establishing a reliable disease reporting system in the region (Leaño & Mohan 2011). The QAAD reporting system has been a useful mechanism for recognizing emerging and important aquatic animal diseases, providing up-to-date disease information, serving as a guide to participating countries in revising their national list of reportable diseases and providing valuable information to support risk analysis.

In order to respond effectively to disease outbreaks, a national disease surveillance system and means for a collation and analysis of epidemiological data (such as a national database system) is necessary (FAO/NACA 2000, 2001). Three levels of diagnostics, that is Level I, Level II and Level III diagnosis to support a national surveillance system (see Section 3.6), were also developed under the same project and used in QAAD reporting. Extension services are important and need to be integrated to support existing systems and structures. There might be some benefits to be gained through a coordinated and collaborative engagement between authorities dealing with veterinary services and fisheries and aquaculture authorities. The purpose of a surveillance determines its design and structure. Common features include diseases and pathogens of concern; resources and capacities to conduct surveillance; diagnostic capability; an information system for the collection, recording and collation of data; and last but not the least, a system to report findings and analysis of surveillance data.

It is well recognised that aquatic disease surveillance and reporting falls under the responsibility of the veterinary authorities; while in some countries, it is a task delegated, shared or historically undertaken by other administrative bodies. In most developing countries, aquatic disease surveillance responsibility is separate from veterinary services. This has been recognized by the OIE, as evident by their establishment of a country Focal Point for aquatic animal diseases. Reporting to the OIE, nonetheless, is through the country's Chief Veterinary Officer.

The process of designing and implementing an aquatic surveillance programme can be a challenging task, especially for personnel with limited knowledge in disease epidemiology and principles of surveillance. Several FAO projects addressing aquatic animal health, based on the request of Competent Authority (CA) of developing countries, included capacity building on disease detection, reporting according to international standards and training of national stakeholders in aquatic disease surveillance. One of the earliest projects was in the Western Balkan region, a project to strengthen Bosnia and Herzegovina's capacity on aquaculture health management, which recognized the lack of a methodological and systematic approach in the implementation of a surveillance programme, even though the country intended to follow, for example, European Union standards and procedures. The veterinary inspectors' checklist was developed and included information on, parameters to be analysed/tested, activities to be performed, samples to be collected and frequency of inspection. The legal reference to which the inspection procedures was based ensured that appropriate sanitary practices (for fish, water, feed, facilities, etc.) and monitoring of fish health and safety and quality of fishery products were in place (Government of Bosnia & Herzegovina, State Veterinary Office 2009).

Similar issues and needs were identified in other developing countries in Asia and Africa. A methodological approach that can lead to a good understanding of epidemiology, surveillance concepts and principles, as well as how they can be practically applied in the field though simplified tools, and the involvement of a multidisciplinary team for effective implementation, were well recognized. The interactions of aquatic systems and environments present unique challenges requiring multidisciplinary and holistic approaches for addressing aquatic disease problems (Georgiadis *et al.* 2001; Peeler & Taylor 2011).

This paper attempts to address such issues and needs through a 12-point checklist that aims to serve as guidance for a multidisciplinary team with the responsibility of supporting the management and control of diseases of aquatic organisms. These teams may consist of aquaculture officers, biologists, terrestrial veterinarians, laboratory personnel, inspection officers, extension and research officers and other aquaculture stakeholders (e.g. primary producers) who are tasked to perform surveillance of diseases in farmed and wild populations. In many cases, except for veterinarians, they may have no formal training in epidemiology, including aquatic epidemiology and surveillance, but

all will have sufficient technical and practical skills in various disciplines in their respective fields. The experience of the authors in implementing projects aimed to establish aquatic disease surveillance and reporting systems in developing countries highlighted the fact that, in many cases, even terrestrial veterinarians have limited experience in designing a surveillance programme for populations of aquatic organism. The 12 points were formed to make it easier for a multidisciplinary team to follow the necessary steps, consisting of a mixture of scientific, logistical and practical requirements for conducting surveillance. This paper provides an essential summary of the steps required, all in one place.

## Development of the 12-point checklist

The first step in the process of developing the checklist was a thorough review of available main references on surveillance for aquatic animal diseases to understand the scope and key elements that need to be captured when designing a surveillance programme. The main references examined included FAO/NACA (2000, 2001), Cameron (2002), Subasinghe *et al*. (2004), Corsin *et al*. (2009) and the OIE Aquatic Animal Health Code (2019a).

The second step was a review of available scientific literature specific to aquatic animal health surveillance to determine findings or recommendations from specific studies related to the practical application of surveillance principles in aquaculture (see, e.g., Baldock *et al*. 2008; Peeler & Taylor 2011; Oidtmann *et al*. 2013).

As we were not able to identify any study that described all elements of a surveillance programme implemented at local, national or global levels, we reviewed studies on aquatic animal diseases to which surveillance tools were applied. These included the following: estimation of component surveillance sensitivity using scenario tree modelling to demonstrate the freedom from viral haemorrhagic septicaemia (VHS) in farmed Atlantic salmon (*Salmo salar*) in Norway (Lyngstad *et al*. 2016); Australia's national surveillance programme to demonstrate national freedom from white spot disease (WSD) (Hood *et al*. 2019); use of an active surveillance programme to study risk factors of acute hepatopancreatic necrosis disease (AHPND) in shrimp in Bac Lieu province, Viet Nam (Nguyen *et al*. 2019) and the Mekong Delta, Viet Nam (Boonyawiwat *et al*. 2018). Other national-level studies, for example in Chile, were also available (see Section 4).

As a third step in the process, the draft 12-point checklist was presented in several regional workshops related to projects being implemented by FAO in order to gain further perspectives and insights on their application to diseases in aquaculture systems, the utility for a multidisciplinary team and implementation requirements. Recommendations and suggestions were subsequently incorporated in the draft checklist. These projects included four extra-budgetary funded projects that focused on enhancing capacities and strengthening biosecurity governance and health management in aquaculture.

## Results

The draft 12-point checklist for designing an active disease surveillance programme for aquaculture is presented in Figure 1 and Table 1. This checklist includes the steps, their descriptions and the criteria and elements required to complete each step. These steps are presented in a chronological manner, but this checklist is meant to be flexible and certain steps may be implemented at any point and/or in parallel with other steps. More importantly, the 12-point checklist contains all the essential elements needed in designing an active surveillance programme and its implementation by a multidisciplinary team in any aquaculture setting.

A brief narrative of each step in the 12-point checklist is presented below.

### Scenario setting

| | | |
|---|---|---|
| 1 | Scenario setting | National status of the disease in question; including:<br>• Health status of a specific pathogen in the country<br>• Existence of surveillance activities<br>• Health status of a specific pathogen in neighbouring countries and/or trading partners<br>• Health status of a specific pathogen in shared water sheds<br>• Data sources |

Scenario setting is an essential first step when starting to design a surveillance programme. It involves an understanding of the health status of Disease X in a country, zone or farm/compartment. Three likely scenarios are listed below:

- Scenario 1: Infected status: Disease X is present in cultured and/or wild species; as supported by one or more cases reported through existing surveillance or grey and/or peer-reviewed scientific literature. Disease X is officially reported by stakeholders to the Competent Authority (CA) of a country and by the CA to the OIE (in case of OIE-listed diseases) and/or any other existing regional reporting mechanisms (e.g. NACA/FAO/OIE QAAD).

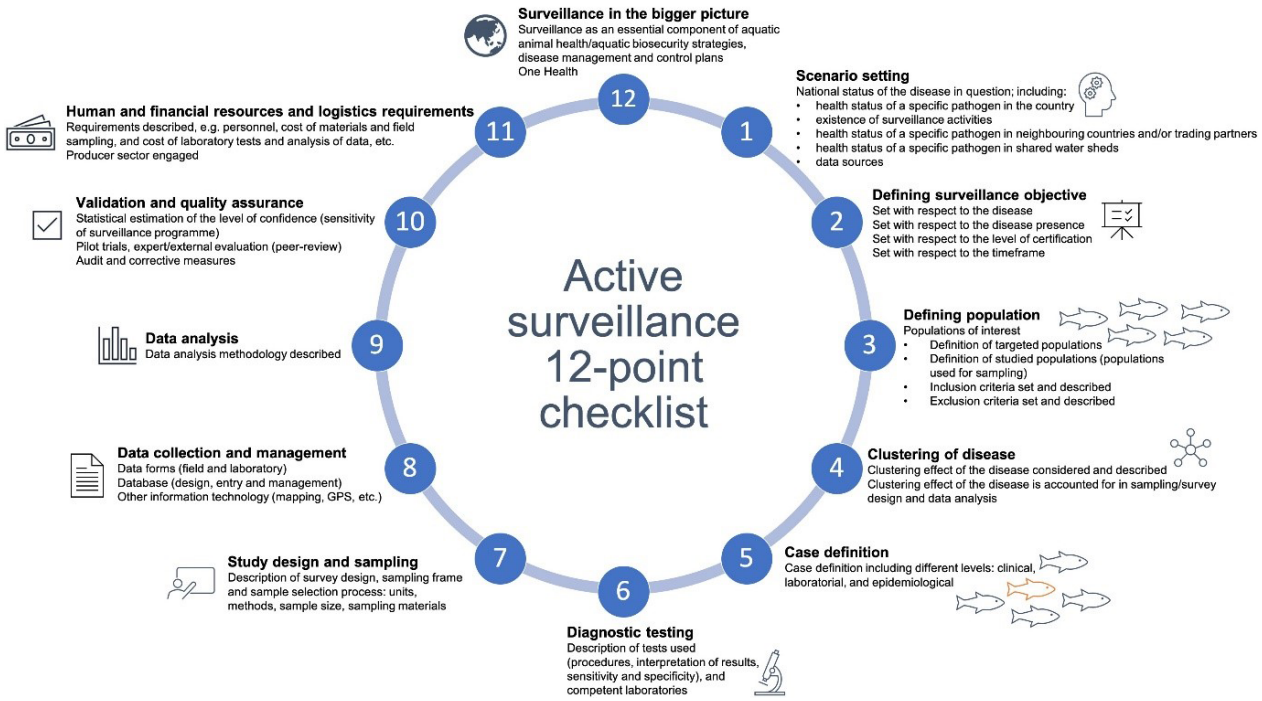


**Figure 1** The 12-point checklist showing the steps, their descriptions and the criteria and elements required to complete each step.

- Scenario 2: Considered free status: no reported cases of Disease X in previous surveillance activities or peer-reviewed scientific literature. Self-declaration of freedom for Disease X is based on transparent and robust evidence, supported by confidence from trading partners; or
- Scenario 3: Unknown status: there are three possibilities under Scenario 3, namely:
  (a) No reported cases and no previous surveillance activities for Disease X;
  (b) Reports of cases of Disease X exist in the grey and/or peer-reviewed scientific literature and/or available information from the private sector but Disease X has not officially been reported to the CA of a country and by the CA to the OIE (in case of OIE-listed diseases) and/or any other existing regional reporting mechanisms (e.g. NACA);
  (c) No reports of Disease X but neighbouring countries with shared water bodies are not considered free.

For the purpose of disease surveillance, the difference between a zone and a compartment is that the former is defined as a portion of a contiguous water system with a distinct health status with respect to certain diseases (Corsin *et al.* 2009; OIE 2019a), while a compartment is based on management and biosecurity practices (Zepeda *et al.* 2008; Corsin *et al.* 2009; OIE 2019a). The latter includes, in addition to surveillance, other criteria established by the CA of a country and the objectives are to facilitate trade in aquatic animals and their products and as a tool for disease management (Zepeda *et al.* 2008; Corsin *et al.* 2009; OIE 2019a).

The selection of a specific scenario will assist in drawing Step 2, that is defining the surveillance objective.

### Defining surveillance objective

| | | |
|---|---|---|
| 2 | Defining surveillance objective | Set with respect to the disease<br>Set with respect to the disease presence<br>Set with respect to the level of certification<br>Set with respect to the timeframe |

Defining a clear surveillance objective is an essential step, as the components and activities of the surveillance programme will be determined based on the surveillance objective. Step 2 requires a good understanding of Disease X (i.e. exotic, endemic, emerging), the status of the presence of Disease X at the desired level of certification (i.e. farm/compartment, zone, country) and the surveillance timeframe, the period during which a surveillance activity will be implemented. In the context of the surveillance objective, certification means documentation of the health

**Table 1** 12-point checklist for designing active disease surveillance in aquaculture

| Step | Step description | Criteria |
|---|---|---|
| 1 | Scenario setting | National status of the disease in question; including:<br>○ Health status of a specific pathogen in the country<br>○ Existence of surveillance activities<br>○ Health status of a specific pathogen in neighbouring countries and/or trading partners<br>○ Health status of a specific pathogen in shared water sheds<br>○ Data sources |
| 2 | Defining surveillance objective | Set with respect to the disease<br>Set with respect to the disease presence<br>Set with respect to the level of certification<br>Set with respect to the timeframe |
| 3 | Defining populations | Populations of interest<br>○ Definition of targeted populations<br>○ Definition of studied populations (populations used for sampling)<br>○ Inclusion criteria set and described<br>○ Exclusion criteria set and described |
| 4 | Disease clustering | Clustering effect of the disease considered and described<br>Clustering effect of the disease is accounted for in sampling/survey design and data analysis |
| 5 | Case definition | Case definition including different levels: clinical, laboratorial, and epidemiological |
| 6 | Diagnostic testing | Description of tests used (procedures, interpretation of results, sensitivity and specificity) and competent laboratories |
| 7 | Study design and sampling | Description of survey design and sample selection process: units, methods, sample size, sampling materials |
| 8 | Data collection and management | Data forms (field and laboratory)<br>Database (design, entry and management)<br>Other information technology (mapping, GPS, etc.) |
| 9 | Data analysis | Data analysis methodology described |
| 10 | Validation and quality assurance | Statistical estimation of the level of confidence (sensitivity of surveillance programme)<br>Pilot trials, expert/external evaluation (peer-review)<br>Audit and corrective measures |
| 11 | Human and financial resources and logistics requirements | Requirements described, e.g. personnel, cost of materials and field sampling, and cost of laboratory tests and analysis of data<br>Producer sector engaged |
| 12 | Surveillance in the bigger picture | Surveillance as an essential component of aquatic animal health/aquatic biosecurity strategies, disease management and control plans<br>One health |

status of the target aquatic population at different levels of aggregation such as at the compartment, zone, pond, farm, village, district or national level. Table 2 shows *examples* of the surveillance objectives based on the health status scenario for Disease X.

### Defining populations

| | | |
|---|---|---|
| 3 | Defining populations | Populations of interest<br>• Definition of targeted populations<br>• Definition of studied populations (populations used for sampling)<br>• Inclusion criteria set and described<br>• Exclusion criteria set and described |

Targeted populations are the populations to which conclusions of the surveillance results will be applied (e.g. absence, presence, or prevalence of Disease X). Usually, they are the same as the populations at risk of being affected by the condition under study, but they can also be high-risk subgroups within a larger population (Salman 2003) such as hatcheries. Studied populations are the aquatic populations from which surveillance data are gathered and may be a subset of, or the same as, targeted populations.

The existence of population data is the most important variable in designing a surveillance plan and interpreting its results. The information required includes a (i) list of susceptible farmed and wild species; (ii) list of water bodies (lakes, rivers or others) and information on aquatic animal populations inhabiting those water bodies; and (iii) list of farms (farm registry can be used if it exists). For farmed

**Table 2** Examples of the objective of Disease X surveillance according to different health status scenarios

| Disease X surveillance scenario | Objective of Disease X surveillance |
|---|---|
| Infected status (one or more Disease X cases reported, e.g. in the previous two years) | • To establish the frequency and distribution of Disease X at the national level in wild and farmed populations for a period of one year<br>• To identify possible risk factors for Disease X spread for the purpose of developing a more targeted disease control programme<br>• To establish a transparent reporting system for Disease X according to national and international notification requirements (e.g. OIE) |
| Unknown status (no reported cases and no previous surveillance activities; however, the country is considered at risk) | • To investigate the presence or absence of Disease X in farmed and/or wild populations<br>• To secure early detection of Disease X |
| Considered free status (no reported cases in previous surveillance activities) | • To confirm freedom of Disease X in the country, zone or farm/compartment<br>• To secure early detection of Disease X |

animals, an overview of the location of farms stocking the susceptible species will be required. Depending on the surveillance aim, population data may need to cover the entire country, or it may be restricted to a specified geographical area of interest, such as a district or river system.

If applicable, surveillance activity for Disease X may include both farmed and wild susceptible populations. This is of particular relevance in relation to the aquatic environment, where a disease may spread from farmed to wild populations and vice versa. Defining populations of interest and targeted populations (as well as relevant spatial and environmental data) are needed in order to understand and recognize risk factors.

Inclusion and exclusion criteria are important in defining the target population. It is essential that they are described and findings evaluated to determine how such findings can affect the external validity of the study results.

Captured or cultured species, management, biosecurity and geographic characteristics are examples of inclusion criteria. They are key features of the target population that are needed to achieve the objectives of the study. Examples of exclusion criteria may include: inability of eligible farmers to: (i) undertake follow-up work, (2) meet scheduled appointments and (3) provide accurate data; farmers that have management practices that could present biases affecting results, or increase their risk for adverse events (Patino & Ferreira 2018).

Information from Step 3 will be the basis for Step 4 and Step 6.

### Disease clustering

| | | |
|---|---|---|
| 4 | Disease clustering | Clustering effect of the disease is considered and described<br>Clustering effect of the disease is accounted for in sampling/survey design and data analysis |

Clustering means an aggregation of cases of a disease, closely grouped in time and place. This can occur at various levels, such as a cluster of infected farms in a zone or cluster of infected ponds on a farm. Clustering must be accounted for in the sampling design (Step 7) and data analysis (Step 9) of a surveillance programme.

For many diseases, there are contributing risk factors related to a host (e.g. susceptibility), environment (e.g. season, climate, contacts) and agent (e.g. virulence, survivability, host specificity) that can lead to clustering of cases; such epidemiological information is documented for most diseases to varying degrees. For example, clustering may occur due to shared exposure to the disease agent (e.g. particular season or location) or host-specific factors that increase susceptibility to the disease (e.g. life stage or increased stress). In aquaculture, the two most important pieces of information needed are the permissive temperature for Disease X and the timing of active aquaculture operations (for life-stages most likely to develop cases). These two elements should be considered when designing the sampling schedule (Step 6). It is not cost-effective to take samples during a period or season when a disease is unlikely to be present.

If information on the aquaculture populations, environment, farming practices and animal movements/contacts is known, it is possible to predict when and where a higher than expected number of disease cases are likely to occur (disease clusters), and direct detection and control activities can be made accordingly (i.e. risk-based surveillance). Even though not all disease clusters represent real outbreaks, keeping track of aggregation of cases, gross signs, mortality, etc., is a very efficient tool for early detection and warning (Rodriguez-Prieto *et al.* 2015).

### Case definition

| | | |
|---|---|---|
| 5 | Case definition | Case definition including different levels: clinical, laboratorial, and epidemiological |

Baldock *et al*. (2005) simplified the understanding of a case definition as an agreed set of rules that permits investigators to uniformly decide that a particular individual has or does not have a particular disease. Thus, a case definition is neither right nor wrong in terms of diagnosing a disease as defined. It is the 'as defined' part that is important here, and it is appropriate to develop a set of rules that will define both suspected and confirmed cases. Active surveillance for the specific disease includes all three requirements (clinical, laboratorial and epidemiological). On the other hand, syndromic surveillance will rely only on a set of defined clinical symptoms; laboratory surveillance will collect only data from laboratory analysis.

A case definition is a set of standard criteria for deciding whether an individual study unit of interest has a particular disease or other outcomes of interest. The study unit may be an individual animal or a group of animals such as a pond of shrimp, cage of fish, entire farm or village. The case definition for Disease X can be based on gross clinical signs, diagnostic test results, mortality rate or a combination of these. It may depend on the surveillance objective and available resources and should be clear and understandable for all involved in the surveillance programme including the primary producers and fishers (in cases where capture fisheries communities are affected).

Table 3 presents examples of the case definition for EUS (infection with *Aphanomyces invadans*), as described by Baldock *et al*. (2005), affecting many species of finfish in both farmed and wild populations.

Thus, the purpose of a case definition is to assure that the surveillance will focus on the disease of concern, in this example, EUS, and not any other disease showing similar clinical signs. A strong consideration in making the case definition is to ensure sufficient surveillance programme sensitivity (the ability of the system to recognize the disease in early stages of onset/introduction) (Hadorn & Stärk, 2008; Oidtmann *et al*. 2013).

**Table 3** The case definition for the suspect and confirmed case for EUS (Baldock *et al* 2005)

| Study unit | Case definition | |
|---|---|---|
| | Suspect case | Confirmed case |
| Animal | • A fish showing red spots, ulcers or lesions similar to the ones associated with EUS, followed by a positive finding of fungal hyphae in tissue squashes. | • A suspected case of fish showing granulomatous mycosis by histopathology (Level II diagnosis) (see Section 3.6).<br>• A suspected case of fish where fungal isolates were isolated (Level II diagnosis) (see Section 3.6).<br>• A suspected case of fish, which tested positive for PCR (Level III diagnosis) (see Section 3.6). |
| Farm | • A location/farm where one or more suspect EUS fish have been found. | • A location/farm where one or more confirmed EUS fish have been found. |

## Diagnostic testing

| | | |
|---|---|---|
| 6 | Diagnostic testing | Description of tests used (procedures, interpretation of results, sensitivity and specificity), and competent laboratories |

Application of diagnostic analyses relies on a broad array of techniques ranging from gross observation to cell culture, histological examination, serological and molecular testing, and genomic sequencing. The choice of which approach to be used for any diagnostic application depends on the objective of surveillance, available laboratory with competent equipment, human and financial resources, and the quality management system for diagnostic tests. Concerning the choice of techniques, it is important to account on *analytical sensitivity* (limit of detection for a disease agent) and *analytical specificity* (ability to distinguish the targeted disease agent from another) of each laboratory test (i.e. 'fit for use, fit for purpose') and *diagnostic sensitivity* (probability of test to correctly identify diseased individuals) and *diagnostic specificity* (probability of test to correctly identify non-diseased individuals).

Diagnostic accuracy relies on a solid case-history. FAO has long promoted the use of Levels I, II, and III for disease diagnosis (FAO/NACA 2000, 2001). None of the levels function in isolation; each one builds on the other, contributing valuable data and information for optimum diagnostic accuracy. Level I provides the foundation and is the basis for accurate interpretation of results obtained from Levels II and III laboratory findings. The different levels are described below.

Level I includes farm/production site observations, record-keeping and gross clinical signs – such information forms the basis for accurate results from Levels II and III diagnostic analyses.

Level II includes the equipment and experience to undertake analyses that can detect and/or identify a range of pathogens. Level II laboratories can do parasitology, histopathology, bacteriology and mycology

examinations, and are, generally speaking, experienced with endemic and opportunistic disease agents in their area, region or country.

Level III diagnostics encompass techniques that target a specialized pathogen or group of pathogens or require highly specialized equipment. Virology, immunology and molecular techniques are included in Level III, although field kits are now available for farm or pond-side use as well as in microbiology or histology laboratories for some pathogens (Walker & Subasinghe 2000).

One of the most important aspects of the effectiveness of the three diagnostic levels is ensuring that Level I observers have access to and know how to contact Levels II and III supports (and at what cost).

Level III laboratories are highly specialized and usually develop in areas where serious disease challenges are recurrent and have warranted research essential to the development of disease-specific expertise and diagnostic technologies. These three diagnostic levels have been used in the NACA/FAO/OIE QAAD Reporting system since 1998.

The quality management system established in laboratories involved in a surveillance programme is also extremely important. This is to ensure all laboratories can accurately apply the diagnostic techniques and reach the same quality level of the results. Routine and periodic examinations of proficiency testing for laboratories participating in the surveillance programme will provide evaluative approaches for the quality of diagnostic results (OIE 2019b) (see Step 10). Cross-sectional studies are also valuable in outbreak investigations whenever there is infectious disease emergence in a farm, region and/or country.

## Study design and sampling

| | | |
|---|---|---|
| 7 | Study design and sampling | Description of survey design, sampling frame and sample selection process: units, methods, sample size, sampling materials |

The most common epidemiological study design used for surveillance is a cross-sectional study, and this is appropriate in an aquaculture setting. In this type of study, cross-sectional observation of a studied population is made assuming representativeness of sampling and statistically justified sample size (i.e. 95% level of confidence) in order to estimate a population parameter or characteristic (i.e. prevalence) (Thrusfield 2003).

Sampling is the act of collecting samples to be analysed via diagnostic testing (Step 6) and is one of the main components of active surveillance. The theory behind sampling is based on the acknowledgment that in most cases when the population is large, it is not cost-effective or feasible to sample the whole population. Sampling when done properly will give a representative samples, which is important for accurately assessing the health status (Step 2) of the population of interest (Step 3). A basic on-farm sampling guideline has recently been made available (Tavornapanich *et al.* 2020).

The sampling frame, defined before initiating sampling, consists of a list of all sampling units (i.e. animals, tanks, net-pens, ponds and farms/premises) included in targeted populations. Choosing a sampling method is often dependent on the surveillance objective. Although random sampling is preferred, it is often impractical to implement in aquatic populations. Common sampling methods used in surveillance can be generally divided into non-random sampling and random sampling. Non-random sampling involves selectively sampling representative individuals/groups, which includes (i) non-probability sampling; (ii) targeted sampling; and (iii) multi-stage sampling. There are different types of random sampling, including (i) simple random sampling; (ii) systematic random sampling; and (iii) stratified random sampling.

For sampling wild populations of aquatic organisms, a non-random spatial sampling can be used. Spatial sampling is similar to random sampling, but instead of selecting individuals from the sampling frame, random locations can be selected from an area. For example, sampling locations can be determined by measuring the length of a river and randomly selecting numbers along that length. This may be adapted to conform to administrative divisions.

Multi-stage sampling can be used in farmed populations of aquatic organisms. Deciding which sampling units (e.g. district, village, zone, compartment, farm, pond, cage) to include in each stage of sampling is dependent on the case definition and planned control measures. Sampling should begin with determining the sampling frame, and if possible, the random selection of an appropriate number of primary sampling units (e.g. farms) from the list. At the farm level, the second stage of sampling involves the selection of ponds/net-pens/tanks or individual animals. This decision relates to the type of aquaculture production, aquatic species farmed, disease of concern, but foremost, the surveillance objective.

If the surveillance objective is set to establishing disease frequency, random sampling is appropriate. If the objective is to demonstrate disease freedom or early detection, secondary sampling units (e.g. ponds, cages, individual animals) that have a higher likelihood of Disease X occurrence (e.g. increased mortality, moribund or showing clinical signs) should be selected first, with the remainder of units selected until the required sample size is achieved.

Statistically valid sample size of random sampling depends on the population size (i.e. the number of farms, ponds and animals), availability of resources, proficiency of diagnostic tests used and the surveillance objective from

which design prevalence is derived. Design prevalence in the absence of disease-specific requirements should be set in accordance to the OIE Aquatic Code (OIE 2019a), but in general, it represents the minimum expected prevalence of infection in the study population or the prevalence of infection that is practically and reasonably able to be detected by a surveillance system (Oidtmann *et al*. 2013). If population size, test performance and design prevalence are known (or estimated) and desired estimation error and surveillance sensitivity are decided upon (or adopted from international standards), formulas or online calculators for sample size calculation are openly and easily available (Step 8).

When using these tools, it becomes evident how an increasing randomized sample reduces sampling error and increases the likelihood that the sample accurately reflects the population of interest. However, the final sample number will depend on the degree of confidence of the results (defined by the statistical significance level and power), the characteristics of non-perfect diagnostics tests (i.e. sensitivity and specificity), and available funding and resources (e.g. trained personnel and laboratory capacity).

The requirement of a large sample size for surveillance dramatically increases the cost of diagnostic tests and human and laboratory resources. Pooling of samples is usually implemented during diagnostic testing. However, there are very few publications that have evaluated the effect of pooling, even for terrestrial animals. The OIE standards allow samples taken for molecular or antibody-based tests to be combined as pooled samples of no more than five specimens per pooled sample, but there is no supporting evidence for this (OIE 2019c). The effect of pooling specimens actually varies with the pathogen load in tissue, the prevalence of diseased animals in the population, the size of specimens and the detection method (Muñoz-Zanzi *et al*. 2006). Recent evaluation on the detection of *Enterocytozoon hepatopenaei* (EHP) in pooled DNA samples based on TaqMan qPCR showed that samples with a 50:1 pooling rate had similar diagnostic sensitivity to samples with a 5:1 pooling rate (Song *et al*. 2019).

**Data collection and management**

| | | |
|---|---|---|
| 8 | Data collection and management | Data forms (field and laboratory)<br>Database (design, entry and management)<br>Other information technology (mapping, GPS, etc.) |

Data collection and management are crucial elements of disease surveillance. Surveillance programmes often incorporate data collection from various sources and use various methodologies, but with respect to surveillance for Disease X, data are collected actively (sampling and field investigations) and passively (disease suspicion reports and investigations). For each Disease X surveillance component, data forms should be prepared in advance in the form of checklists and questionnaires to facilitate and encourage uniform data collection. Questionnaires can be developed separately for wild and farmed aquatic organisms depending on the population sampled. Data collected in the field using prescribed data forms, including laboratory results, need to be entered into a digital database (i.e. spreadsheet). It is of key importance to secure traceability of data (population data with laboratory results). In order to secure proper data collection, questionnaire/data forms should be translated into the local language. Examples of information to be collected in the field can be found in Step 11. Depending on who is undertaking the surveillance and for what purpose, accessibility of the data needs to be defined and agreed upon. As surveillance is an ongoing, continuous activity, a well thought out data capture, repository and management system must be designed. Based on the experience of the National Aquatic Animal Disease Monitoring and Surveillance Programme (NAADMSP) in China, a unified numbering system for sampling sites is highly recommended for repeated sampling at fixed points and continuous comparison of surveillance results (Dong & Huang 2016b).

**Data analysis**

| | | |
|---|---|---|
| 9 | Data analysis | Data analysis methodology described |

Data analysis methodology should be considered in advance (i.e. before sampling and data collection) since it dictates the type of data to be collected and when, where and how data need to be obtained.

Exploratory data analysis is the very first step in the process of analysing a dataset. It is important to know whether the collected or reported values differ because of factors you are interested in (e.g. treatments) or because they are part of 'background' natural variation. It is also important to evaluate what the numbers actually mean and to represent them in a way that readily communicates their meaning to others. Descriptive statistics is required to summarize the information by different representations, which includes, for example, tables, plots or diagrams and statistical measures. This presents a little problem when the data set comprises relatively few observations made on a small group of animals. However, as the quantity of information grows, it becomes increasingly difficult to obtain an overall 'picture' of what is happening.

Quantifying disease outbreaks is important to assist the management or understanding of an aquatic disease. For

example, knowing the prevalence of a disease allows one to determine how large a disease problem is, to compare the prevalence of disease between groups or to monitor the success of a disease control programme. Measuring disease can be done in many ways. For example, by counting disease events or calculating the proportion of a population that is affected. It is also possible to compare the incidence of disease between groups with ratios, and this can be useful to examine the effect of risk factors (Sergeant & Perkins 2015). When the survey is finished, the prevalence of disease can be calculated and expressed as the percentage of infection. The methodology of surveillance design is a tool that gives confidence in the interpretation of surveillance data (set as 95% level of confidence). In practical terms, this means that results gained from selected samples can be confidently interpreted for the whole population.

For qualitative or categorical data, frequency tables are the most commonly used summary statistics. For quantitative data, summary statistics should be calculated for each numeric variable in the data set and subgroups, based on outcome variables and any other variables of interest. Measures of central tendency such as mean or median are commonly reported with measures of spread or variability, including variance, standard deviation and standard error. Ideally, summary statistics should be graphically represented including plots, which is an ideal way of displaying information about data (i.e. its shape of distribution, trend or direction of change over time or relationships).

A particularly useful technique in epidemiology to evaluate the relationship between two variables is the use of 2 × 2 (two-by-two or contingency) tables (Table 4). These tables are used to evaluate the association between a possible risk factor ('Exposure') and an outcome ('Disease'). Counts summarizing the occurrence of the four possible combinations of events in the study population are entered into the appropriate cells. The table can be rotated or flipped so that either rows or columns represent 'Exposure', and the column headings (+) and (−) can be in either order to match common textbooks of epidemiology (Sergeant & Perkins 2015). A single table or multiple strata can be entered. Statistics produced include the Fisher and mid-p exact tests, chi squares, odds ratio, maximum likelihood odds ratio estimate, risk/prevalence ratio (relative risk), risk difference and aetiologic fractions with confidence limits produced by several methods, with stratified analysis (Thrusfield & Christley 2018).

**Table 4** A contingency table of disease status against the practice of sharing equipment in a farm (epidemiological unit)

| Variable | Disease status | | Total |
|---|---|---|---|
| | Diseased | Not diseased | |
| No sharing equipment | a | b | a + b |
| Sharing equipment | c | d | c + d |
| Total | a + c | b + d | n = a + b + c + d |

Finally, multivariate regression modelling techniques are used further to identify associations between risk factors and the outcome of interest, including, to mention some, the occurrence of a disease, the time from the beginning of a production cycle and the development of infection or disease, and the proportion of infected individuals or counting events. Each of those disease outcomes is modelled using distinct probability families (e.g. linear, logistic or Poisson) and approaches (e.g. survival or linear). Although identification of risk factors are not the main purpose for active surveillance, there are several examples of using surveillance data, in which results would promote, for example, risk-based surveillance activities in shrimp or salmon farming industries (Escobar-Dodero *et al.* 2010; Dong & Huang 2016b; Muniesa *et al.* 2017; Qiu *et al.* 2018; Xie *et al.* 2018).

A practical note is the use of online epidemiological calculators such as epitools (https://epitools.ausvet.com.au/), openepi (https://www.openepi.com/Menu/OE_Menu.htm), statulator (http://statulator.com/), winpepi (http://www.brixtonhealth.com/pepi4windows.html) and winepi (http://www.winepi.net/uk/index.htm), amongst others.

## Validation and quality assurance

| | | |
|---|---|---|
| 10 | Quality assurance and validation | Statistical estimation of the level of confidence (sensitivity of surveillance programme)<br>Pilot trials, expert/external evaluation (peer-review)<br>Audit and corrective measures |

Quality assurance and validation are important points as they provide confidence; thus, the level of certainty of the outcomes is established. This step is done throughout the whole process from the design until the actual implementation.

The principles of quality assurance need to be incorporated 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. Administrative and procedural activities need to be conducted to avoid problems and allow corrective measures to be introduced if mistakes occur. These will guarantee good quality implementation of the surveillance plan.

Concerning quality assurance of a diagnostic system, it is expected that the government or its CA will authorise

laboratories involved, and diagnostic methods used for surveillance. Depending on laboratory resources available nationwide, it can consist of a single laboratory or network of laboratories (governmental, university, private). Proficiency ring testing, accreditation of diagnostic laboratories and analytical methods used for surveillance are an essential part of an overall quality assurance system (ISO 17025), and it is very often required for certification of aquatic animal health in international trade (OIE 2019b).

Examples of quality assurance requirements for EUS and TILV active surveillance include the following:

- National surveillance team (NST) established;
- Training and education of NST on pathogen (EUS and TiLV) biology, pathology, diagnostics and surveillance conducted;
- Data collection and a questionnaire described and explained clearly, and common understanding achieved;
- Diagnostic laboratory accredited in line with ISO 17025, if possible;
- Trained field and laboratory personnel;
- Routine proficiency ring testing run for all participating diagnostic laboratories;
- Clear standard operating procedures developed and used during implementation (e.g. aseptic technique procedures for minimizing contamination from potential areas of sample collection developed and made clear to the sampling teams);
- Sampling teams closely supervised; and
- Pilot survey conducted as a sampling exercise.

Validation is the process that determines the fitness of a surveillance programme, which has been properly developed, optimized and standardized for a specific and defined objective. Overestimation or underestimation of parameters of interest is the most common problems identified during disease surveillance. Validation is needed to confirm scientific values, confidence in the system and compliance with international standards. This step is also done throughout the whole process from the design until the actual implementation. The surveillance design and implementation plan might be validated by both data or test validation, proficiency ring testing, pilot studies, external evaluation and peer-reviewed evaluation by experts and other relevant project proponents.

**Human and financial resources and logistics requirements**

| | | |
|---|---|---|
| 11 | Human and financial resources and logistics requirements | Requirements described, e.g. personnel, cost of materials and field sampling, and cost of laboratory tests and analysis of data, etc.<br>Producer sector engaged |

Surveillance is an economic activity, and in this step, it is essential to plan in advance the resources (human and financial) needed based on the surveillance design developed in previous steps. In this step, a checklist of field logistics/operational requirements needs to be completed (e.g. surveillance team, diagnostic team, field support team, communication, farms to be visited, work plan and budget). In addition, awareness raising concerning the surveillance activity is needed for targeted farmers including training of abovementioned teams. If the surveillance is project-based, it has to be reviewed and approved by project proponents. If this is a regular activity of an aquatic animal health programme, it has to be approved by concerned authorities. In both cases, the financial allocation should be provided.

**Surveillance in the bigger picture**

| | | |
|---|---|---|
| 12 | Surveillance in the bigger picture | Surveillance as an essential component of aquatic animal health/aquatic biosecurity strategies, disease management and control plans<br>'One Health' |

This last step puts active surveillance for Disease X in line with overall national strategies for enhancement of aquaculture biosecurity and health of aquatic organisms, aquaculture and international trade, as well as the 'One Health' platform.

As previously mentioned, surveillance is a key element of a national strategy on aquaculture biosecurity and aquatic animal health management and a fundamental element of any aquatic animal health protection programme (FAO 2007). Surveillance and monitoring programmes are essential for the early detection and rapid emergency response to significant disease outbreaks and form the basis for early warning of exotic incursions or newly emerging diseases. Response strategies and contingency plans should be prepared in advance for emergency or routine response to diagnosed disease cases and emerging situations during surveillance.

These programmes are also increasingly demanded by trading partners to support statements of national disease status and are the basis for disease zonation/compartmentalization. Surveillance also provides the building blocks of information necessary to give an accurate picture of the distribution and occurrence of diseases relevant to disease control and international movement of aquatic organisms and their products. At a smaller scale, surveillance is a key

element to provision of disease status guarantees at the compartment or farm level, thus making trade possible from a country or zone that may not be subject to surveillance.

Surveillance to demonstrate freedom from a specific disease requires a well-designed active surveillance programme that meets the standards outlined in the OIE Aquatic Animal Health Code (OIE 2019b). Members of the OIE are requested to provide information regarding its aquatic animal health status, allowing transparency so that planning, surveillance activities, analysis and availability of data and information are maintained at all times in compliance with prescribed standards.

It is also time to consider surveillance from a 'One Health' perspective and, where possible, guide surveillance efforts to consider parameters of human and environmental health paving the way for future 'One Health' national surveillance programmes (Stentiford *et al.* 2020). In this regard, a number of surveillance programmes have been implemented to focus on the possibility of human parasites, human enteric viruses, SARS-CoV-2 and antimicrobial resistance in aquaculture or aquatic animal products (Ming *et al.* 2014; Jiang *et al.* 2018; NACA 2020).

## Discussion

An over-arching theme evident in the peer-reviewed publications examined was the challenges in applying surveillance methodologies to populations of aquatic organisms, particularly the complexity of production systems, accessibility of aquatic organisms for inspection or sampling, large population size and determination of the epidemiological unit due to the connectivity with the environment (Baldock *et al.* 2008; Peeler & Taylor 2011; Oidtmann *et al.* 2013). The importance of risk-based surveillance was emphasized by many authors as a means to improve efficiency by prioritizing data collection and resources (Peeler & Taylor 2011; Oidtmann *et al.* 2013; Gustafson *et al.* 2015; Hood *et al.* 2019). Adequate data are required to implement this approach, such as the risk of infection based on the presence of risk factors, sensitivity and specificity of diagnostic tests (including test validation), the prevalence of infection, and farm locations and animal movements.

Although epidemiological studies in aquatic systems have, and continue to, lag behind those applied to terrestrial systems in terms of scope and complexity, rapid development in the application of epidemiology to aquatic systems has occurred over a relatively short period (Subasinghe 2005; Peeler & Taylor 2011; Oidtman *et al.* 2013).

At the national/country level, some examples of aquaculture surveillance exist and briefly described below are the drivers, scope, objectives and breadth of such programmes, and the indication how the 12-point checklist is in line with these.

In Chile, farmed salmon production is the second largest in the world. Until the sanitary crisis caused by the epidemic of infectious salmon anaemia virus (ISAV) (Mardones *et al.* 2009), few epidemiological studies had been published, mainly because of the lack of epidemiological data and disease reporting from salmon farmers. After the ISAV epidemic, strict mandatory surveillance programmes were in place primarily focused on ISAV, but also for bacterial and viral diseases, parasites and use of antimicrobials (Gallardo Lagno *et al.* 2019). After approximately ten years, researchers have been using such data to characterize epidemiologically most diseases and their risk factors including sea lice (Yatabe *et al.* 2011; Hamilton-West *et al.* 2012; Arriagada *et al.* 2019), infectious pancreatic necrosis virus (Escobar-Dodero *et al.* 2010), *Piscirickettsia salmonis* (Hillman *et al.* 2020) and the use of antimicrobials (Price *et al.* 2016), amongst others. The development and establishment of comprehensive surveillance systems supported by participative salmon farmers and interacting with researchers from the academia has resulted in the advancement of updated science-based policies in Chile (Gallardo Lagno *et al.* 2019).

China, the world's biggest aquaculture producer, accounting for about 58% of the global total production, has suffered economic losses due to diseases. Thus, since early 2005, China has been developing and implementing its national active surveillance on major aquatic animal diseases annually through the NAADMSP, which includes passive and active (targeted) components. The NAADMSP is being managed by the Chinese Ministry of Agriculture and Rural Affairs (MARA) with technical advisory from an Expert Committee comprised of specialists from various fields combining inter-departmental and interdisciplinary cooperation amongst industries, universities, research institutes, extension centres and customs, jointly tackling major problems in aquatic animal health. The passive monitoring programme mostly focuses on known diseases in aquaculture and provides information on disease outbreak and losses statistics. Similar to the 12-point checklist in this paper, targeted surveillance via NAADMSP mostly works on surveillance sites located in different provinces, planned by the National Fisheries Technology Extension Center (NFTEC), according to the importance of aquaculture species in the provinces. With technical support from National Reference Laboratories for designated diseases and operational implementation by more than 100 participating laboratories with recognized competency, the programme is reformulated each year with such considerations as scenario setting, defining surveillance objectives, diagnostic standards (histopathology, cell culture and PCR detection), technical training, proficiency testing, data collection and

analysis methods, and reporting lines. As an example, in 2018, the active surveillance of NAADMSP targeted seven major national listed aquatic animal diseases and four emerging diseases with frequent occurrences in recent years, with 4571 monitoring sites (detected 7854 samples and 1.18 million aquatic animals) covering nearly all the provinces and 66 farmed species. This targeted surveillance work provided robust technical support for aquatic disease forecast and development of measures for disease prevention and control, hence contributing significantly to China's sustainable aquaculture development (BOF *et al*. 2019).

In order to ensure the quality (Step 10) of the NAADMSP, MARA has carried out an annual training course on diagnostic technology of aquatic animal diseases and the Annual Proficiency Testing Programme (PT). In 2018, the programme involved testing 11 aquatic animal pathogens. A total of 153 laboratories nationwide had participated in the PT, while 137 of them were assessed as acceptable with an average satisfaction at 88.0% (BOF *et al*. 2019).

Through the targeted surveillance programme in China, response actions have been surveyed, and control recommendations addressed for each targeted disease (BOF *et al*. 2019). However, during the implementation of the surveillance programme in earlier years, field personnel for site surveys did not always understand how to describe or select control measures. There may be difficulty in implementation of follow-up surveys to determine the effect of response measures (Dong & Huang 2016a, 2016b). The survey questionnaire and design of the surveillance programme, therefore, need to be carefully considered when putting surveillance in the bigger picture (Step 12).

In India, the main driver for the National Surveillance Programme for Aquatic Animal Diseases (NSPAAD) in the country is the significant threat of aquatic animal diseases to the growth of aquaculture. The Government of India's Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture, initiated a National Surveillance Programme in the year 2013. The programme implemented through 31 collaborating centres is coordinated by the Indian Council of Agricultural Research (ICAR)-National Bureau of Fish Genetic Resources. The programme has been successful in strengthening the country's disease surveillance system. The programme identified seven new diseases in India (Rajendran *et al*. 2016; Sahoo *et al*. 2016; Swaminathan *et al*. 2016; Behera *et al*. 2017; Sahul Hameed *et al*. 2017; Sood *et al*. 2017; Girisha *et al*. 2019) and helped to dispel rumours on the presence of AHPND in the country and to date, India is reported to be free of the disease. Active surveillance has provided transparent scientific evidence and allowed for the declaration/statement that four major pathogens of finfish and two of shrimps have not been detected in India. The programme successfully developed a network of aquatic animal health laboratories across the country; diagnostic capability for detection of OIE/NACA-listed and emerging aquatic animal pathogens; mechanisms for first-time confirmation of exotic and emerging diseases and sending alerts/advisories to stakeholders; and provides scientific advice to farmers. In addition, NSPAAD has assisted the CA in a better understanding of the disease situation in aquatic animal populations in the country and has been providing evidence to the CA for fulfilling its obligations under the World Trade Organization (WTO) Agreement on Sanitary and Phytosanitary Measures (the 'SPS agreement') and international disease reporting requirements.

In Norway, all licensed aquaculture operations are required by law to have regular health inspections by authorized veterinarians or aqua medicine biologists. For finfish aquaculture operations, a minimum of six or 12 yearly inspections are typically required, depending on the operation type, and the number of fish stocked. During such inspections, Level I diagnostics are utilized to determine whether there are any health concerns for which additional diagnostic investigations are required. Any suspicion of a notifiable disease must be conveyed to the CA immediately. In addition, there are mandatory investigations of all unexplained mortality events. Such inspections form the cornerstone of the passive disease surveillance system in Norwegian aquaculture. A study assessing the cost-effectiveness of surveillance options for the exotic viral disease viral haemorrhagic septicaemia (VHS) showed that the current surveillance system based on these routine inspections have a high capability for detecting VHS in marine farmed salmonids (Lyngstad *et al*. 2016). Conversely, surveillance for endemic viruses ISAV and Salmonid alphavirus are heavily dependent on Level III diagnostics (e.g. real-time RT-PCR analyses) on monthly samples submitted to appointed laboratories.

At present, the United Kingdom (UK) follows the systems laid out in EU legislation (2006/88/EC) regarding the surveillance and control of aquatic animal diseases, which is well-aligned with the 12-point checklist proposed in this paper. The UK's national status is that they are currently free of most OIE-listed diseases and the aim of their surveillance programme is, therefore, to detect incursions of these pathogens to retain freedom and associated trading status. In terms of active surveillance, all aquaculture sites are visited annually by inspectors from the relevant CA and samples are taken on suspicion of disease. Additionally, random consignments of high-risk ornamental fish imports are tested for pathogens of concern. There is also a passive surveillance programme where investigations are conducted if suspicion of disease is reported. Depending on the circumstances, sample sizes of up to 150 individual

animals may be taken from a selection of units across a site, which provides 95% confidence of detecting a pathogen if present in >2% of the population assuming 95% test sensitivity and 100% specificity. Moribund animals and those showing clinical signs of disease are sampled preferentially to further improve the likelihood of detection. To ensure quality standards are high, testing is conducted according to the methods specified by the OIE in a centralized ISO 9001 accredited laboratory. All data are stored on centralized databases, and analysis is carried out by an epidemiological advice service. Importantly, site visits and surveillance are not just conducted for the purpose of detecting diseases, but also as part of a bigger picture surveillance programme to ensure good biosecurity in a 'OneHealth' context.

Surveillance programmes should be in a constant state of evaluation and adjustment to ensure that they continue to function according to their objectives and serve the needs of their stakeholders, and to confirm that the information gathered is reliable. In addition, evaluations can identify whether the methodologies used for surveillance are still applicable in the face of changing conditions. Sharing outcomes of evaluations is essential and should lead to decision-making and application of corrective measures. There are important approaches from the terrestrial sector that can be used in the context of aquaculture (Dufour & Hendrikx 2009; Hoinville 2013).

In developing countries, government services and officers and aquaculture stakeholders responsible for aquaculture health management often have limited formal education in epidemiological approaches to disease control. In such situations, the development of a country's capacity to design and implement surveillance is challenging, particularly when the resources needed for training and implementation are limited.

Managing health and implementing biosecurity under-the-water in the case of diseases of aquatic organisms, with the high number of farmed species, different environments, farming systems, scale and extent of operation and management and the general dynamic nature of the aquatic environment – require special attention and appropriate approaches. The various workshops organized by FAO and partners paved the way for sharing of experiences that provided clarity on the limitations (especially at country level) that need to be addressed when designing and implementing surveillance. Such workshops also provided a platform to discuss opportunities that can be explored (e.g. existence of specialists, producer clubs/associations and other networks) and recognize the great advantage of working as multidisciplinary teams.

The 12-point checklist provides a starting point for specialists working as members of a multidisciplinary team. It highlights the most common steps and elements that were captured from the major references mentioned in the methodology section. The checklist is presented in a stepwise manner to provide a clear view of the overall picture when implementing a surveillance programme for diseases of aquatic organisms; however, many of the steps have overlapping elements and/or requirements. The checklist will serve as one of the toolkits of the Progressive Management Pathway for Improving Aquaculture Biosecurity (PMP/AB) (FAO 2020).

In many real-life situations that are complex in nature and require differing steps/accommodation (e.g. many aquatic animal species, diseases, production systems, type and quality of data/information available), there needs to be flexibility. Proponents should ensure that the methodology selected is transparent and science-based, with support from scientific literature, experts and available data. Any uncertainties or assumptions made (e.g. sensitivity/specificity for disease freedom testing) and their potential effect on results should be documented. The 12-point checklist serves as guidance; however, it is recommended that specialists in aquatic epidemiology be consulted. More details are available in other surveillance resources previously mentioned.

Countries will be faced with significant challenges with respect to implementing surveillance and disease zoning programmes since these require costly investments. It is therefore essential to weigh the economic benefits of such programmes against the country's aquaculture potential.

Some of the challenges include establishing programmes that are practical, cost-effective and capable of implementation within the constraints of existing disease detection techniques, resource availability, technical capacity and last but not least, sustainability. The need for multidisciplinarity and the application of epidemiology in analysing disease situations are becoming more necessary in aquaculture.

Consequently, at the practical level, successful application of surveillance principles in specific environmental, cultural and political conditions requires not only science-based but also sound, simple practical and adaptable solutions. In addition, another important challenge is engaging especially the producer sector to ensure buy-in or determine incentives that can be made available to encourage participation and collaboration. Farmers are a vital part of any disease surveillance programme for aquaculture, especially in the case of syndromic surveillance, and should actively be involved in the planning and implementation of such programmes (Brugere *et al*. 2017).

Resource availability may limit the extent of surveillance programmes. In this scenario, passive surveillance combined with active surveillance (e.g. many information sources: farm records, private or government laboratory reports, academic studies) can improve its efficiency by

using targeted, risk-based surveillance programme for prioritized diseases of concern and resources.

## Conclusions

The application of disease surveillance and reporting to diseases of aquatic organisms and aquaculture health management is complicated by many factors such as the wide ranges of socio-economic and technological development in many countries, diversity of species cultured, range and complexity of environments, nature of containment, intensity of production, variety of culture systems and types of management. Although there are still problems with respect to accuracy, consistency and timely submission of reports, the system is evolving, and reporting governments are starting to realize the benefits of such a system.

Knowledge of the aquatic species biology, aquaculture systems and practices, interaction with wild aquatic species and many aspects of aquaculture health management are needed for a multidisciplinary approach to aquatic disease control. Monitoring and surveillance activities for diseases of aquatic organisms conducted by governmental offices often remain unpublished or published in domestic languages, thus limiting the transfer of scientific and field information between developed and developing aquatic health surveillance programmes. Publication of design, implementation and results is strongly encouraged. An international/regional network to enhance the communication, training and collaboration in surveillance of diseases of aquatic organisms will greatly facilitate the knowledge sharing and technology refining.

Surveillance, whether passive (reactive and general in nature) or active (proactive and targeted), must be supported by adequate reporting mechanisms so that suspected cases of serious diseases are quickly brought to the attention of the CA. Surveillance and monitoring efforts must be supported by adequate diagnostic capability (including appropriately trained expertise, competent laboratory and rapid-response field diagnostics, and standardized field and laboratory methods), information system management (i.e. a system to record, collate, and analyse data and to report findings), legal support structures, transport and communication networks and linked to national, regional (e.g. NACA/FAO/OIE QAAD) and international (e.g. OIE) disease reporting systems (e.g. pathogen list or list of diseases of concern, disease notification and reporting procedures).

Surveillance is a resource-demanding endeavour and allocation of such resources, and government commitment are essential. Projects may be able to provide the start-up costs in terms of training; however, governments need to find resources for surveillance implementation. Besides knowledge and understanding, surveillance programmes require significant financial support, not usually easily available and mobilized in developing countries. The combined principle of co-financing, support by international development agencies and donors with national contribution and strong government commitment is necessary. If political will and commitment from responsible authorities and cooperation at all levels (especially primary production) can be achieved, surveillance has great potential to effectively minimize the spread of diseases of aquatic organisms and enhance trade.

The 12-point surveillance checklist presented in this paper offers a good starting point for addressing the issues and needs identified during our work in developing countries. The stepwise and pragmatic approach can be used as a model to build targeted surveillance competency (capacity/capability) and a basic reference when starting surveillance or to improve existing surveillance programmes. This approach, according to our knowledge, is not presented or discussed in this form in any peer-reviewed literature, and can be used as an educational tool for multidisciplinary groups involved in aquatic animal health efforts in developing countries and will assist in the development and application of surveillance to manage and control diseases in aquaculture. This 12-point checklist could also benefit other multidisciplinary teams dealing with AMR surveillance in aquaculture and also for integrated 'One Health' surveillance approaches that are presently being conceptualized in many countries covering animal, human and environmental health.

Two important steps planned in the future include the following: (1) development of a detailed manual of procedures for the 12-point checklist and training as one of the toolkits to support the PMP/AB, and (2) surveillance evaluation.

For the former, several countries are now testing the 12-point checklist targeting EHP, EUS and TiLV; outcomes of these initial applications will provide further insights for refinement and hopefully can contribute to the dearth of publications available on comprehensive surveillance for aquatic animal and aquatic plant diseases.

For the latter, the OIE Tool for the Evaluation of Performance of Veterinary Services (OIE PVS Tool), the audit conducted by the Food and Veterinary Office of the EU, and the Surveillance Evaluation Tool (SET), launched by FAO in 2017 (http://www.fao.org/ag/againfo/programmes/en/empres/tools_SET.html), have been widely used in capacity assessments of national surveillance for terrestrial animals. Aquaculture and the aquatic environment provides unique disease challenges as compared to terrestrial systems. However, general principles of epidemiology still apply in the context of aquaculture, and surveillance design and evaluation methodologies can be used with some minor adaptation. In fact, SET was successfully used in 2019 to evaluate and compare freshwater and marine

fisheries systems in Spain with minimal adaptive changes (publication in preparation). The OIE has developed a PVS Tool specific for aquatic animal health (OIE PVS Tool: Aquatic) based on their original PVS Tool (OIE, 2020), and this can be a useful tool for surveillance evaluation.

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