CHAPTER 1.4.

AQUATIC ANIMAL HEALTH SURVEILLANCE

Article 1.4.1.

Introduction and objectives

1. *Surveillance* activities may be performed to achieve any of the following objectives:

   a) demonstrating the absence of *disease*;

   b) identifying events requiring *notification* as listed in Article 1.1.3. of the *Aquatic Code*;

   c) determining the occurrence or distribution of endemic disease, including changes to their *incidence* or *prevalence* (or its contributing factors), in order to:

      i) provide information for domestic *disease* control programmes,

      ii) provide relevant *disease* occurrence information to be used by trading partners for qualitative and quantitative risk assessment.

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

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

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

2. Essential prerequisites to enable a Member to provide information for the evaluation of its animal health status are:

   a) that the particular Member complies with the provisions of Chapter 3.1. of the *Aquatic Code* on the quality and evaluation of the *Competent Authorities*;

   b) that, where possible, *surveillance* data be complemented by other sources of information (e.g. scientific publications, research data, documented field observations and other non-survey data);

   c) that transparency in the planning and execution of *surveillance* activities and the analysis and availability of data and information, be maintained at all times, in accordance with Chapter 1.1. of the *Aquatic Code*.

3. The following recommendations may be applied to all *diseases*, their agents, and *susceptible species* as listed in the *Aquatic Manual*, and are designed to assist with the development of *surveillance* methodologies. Where possible, the development of *surveillance* systems using these recommendations should be based on the relevant information in the individual *disease* chapters in the *Aquatic Manual*. These recommendations are also applicable to non *OIE-listed diseases* that

may be of importance to a country or region, such as new or *emerging diseases*. There is sometimes a perception that *surveillance* can only be conducted using sophisticated methodologies. However, an effective *surveillance* system can also be developed by making use of gross observations and already available resources.

4. It would be impractical to try to develop a *surveillance* system for all the known *aquatic animal diseases* for which a country has *susceptible species*. Therefore prioritising the *diseases* to be included in a *surveillance* system should be conducted considering:

   a) the needs to provide assurance of disease status for trade purposes;

   b) the resources of the country;

   c) the financial impact or threat posed by the different *diseases*;

   d) the importance of an industry-wide *disease* control programme within a country or region.

5. More detailed information in each *disease* chapter (where it exists) of the *Aquatic Manual* may be used to further refine the general approaches described in this chapter. Where detailed *disease* specific information is not available, *surveillance* can also be conducted following the recommendations in this chapter. Access to epidemiological expertise would be invaluable for the design, implementation of the system and interpretation of results derived from a *surveillance* system.

Article 1.4.2.

Principles of surveillance

1. *Surveillance* may be based on many different data sources and can be classified in a number of ways, including:

   a) the means by which data are collected (targeted versus non-targeted);

   b) the *disease* focus (pathogen-specific versus general *surveillance*); and

   c) the way in which units for observation are selected (surveys versus non-random data sources).

2. *Surveillance* activities include:

   a) population-based surveys, such as:

      i) systematic sampling at slaughter;

      ii) random surveys;

   b) non-random *surveillance* activities, such as:

      i) *disease* reporting or *notifications*;

      ii) control programmes/health schemes;

      iii) targeted testing/screening;

      iv) post-mortem inspections;

      v) laboratory investigation records;

      vi) biological specimen banks;

      vii) sentinel units;

      viii) field observations;

      ix) farm production records.

3. In addition, *surveillance* data should be supported by related information, such as:

   a) data on the epidemiology of the *disease*, including environmental, and host and wild reservoir population distributions;

   b) data on farmed and wild animal movements and trading patterns for *aquatic animals* and *aquatic animal products*, including potential for exposure to populations of wild *aquatic animals*, water sources or other contacts;

   c) national animal health regulations, including information on compliance with them and their effectiveness;

   d) history of imports of potentially infected material; and

   e) biosecurity measures in place.

4. The sources of evidence should be fully described. A survey should include a description of the sampling strategy used for the selection of units for testing. For non-random data sources, a full description of the system is required including the source(s) of the data, when the data were collected, and a consideration of any *biases* that may be inherent in the system.

Article 1.4.3.

Critical elements of surveillance

In assessing the quality of a *surveillance* system, the following critical elements need to be addressed in conjunction with an evaluation of the *Competent Authority* (Chapter 3.1.).

1. Populations

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

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

2. Epidemiological unit

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

3. Clustering

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

4. Case and outbreak definitions

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

5. Analytical methodologies

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

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

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

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

6. Testing

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

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

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

7. Quality assurance

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

8. Validation

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

9. Data collection and management

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

a) the distribution of, and communication between, those involved in generating and transferring data from the field to a centralised location;

b) motivation of the people involved in the *surveillance* system;

c) the ability of the data processing system to detect missing, inconsistent or inaccurate data, and to address these problems;

d) maintenance of disaggregated data rather than the compilation of summary data;

e) minimisation of transcription errors during data processing and communication.

Article 1.4.4.

Population-based surveys

In addition to the principles for *surveillance* discussed in Article 1.4.6., the following recommendations should be used when planning, implementing and analysing surveys.

1. Types of surveys

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

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

2. Survey design

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

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

3. Sampling

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

4. Sampling methods

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

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

5. Sample size

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

Article 1.4.5.

Non-random data sources used in surveillance

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

1. Common non-random surveillance data sources

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

a) Disease reporting or notification system

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

b) Control programmes/health schemes

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

c) Targeted sampling

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

d) Post-harvest inspections

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

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

e) Laboratory investigation records

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

f) Biological specimen banks

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

g) Sentinel units

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

ornamental fish) or in animal *subpopulations* where sampling techniques are incapable of detecting the presence of *disease* or *infection* (e.g. where vaccination means that serological tests are inapplicable).

h) Field observations

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

i) Farm production records

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

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

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

3. Analytical methodologies

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

4. Combination of multiple sources of data

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

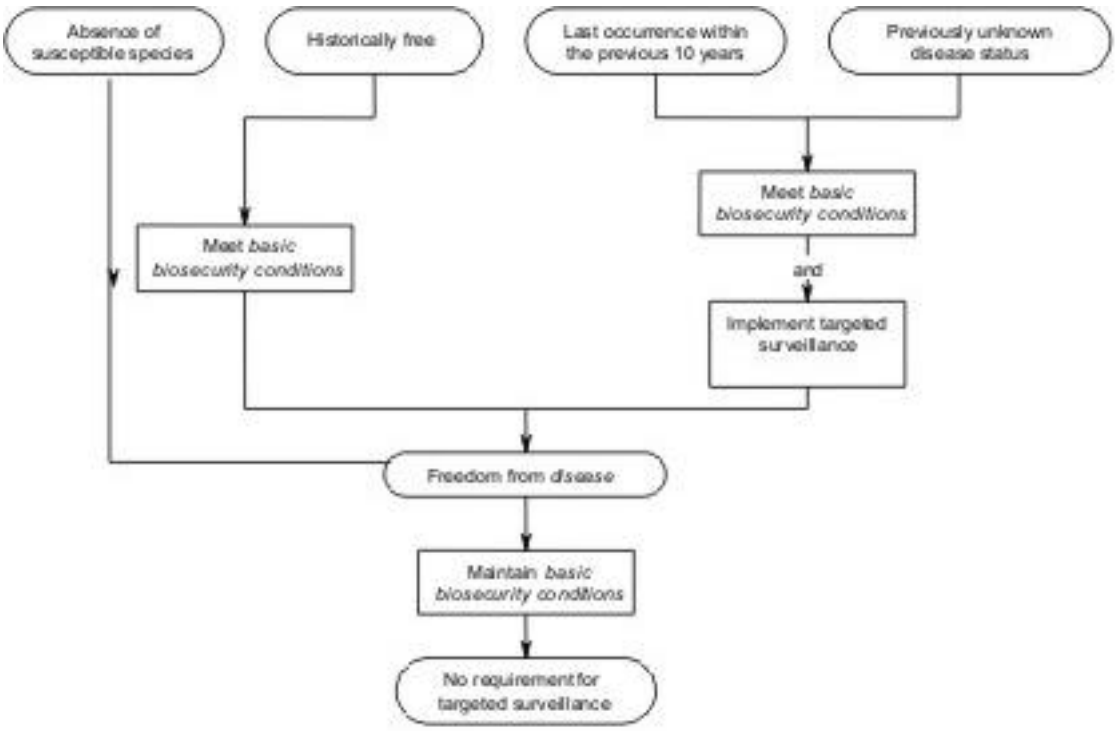
Surveillance information gathered from the same country, *zone* or *compartment* at different times (e.g. repeated annual surveys) may provide cumulative evidence of animal health status. Such evidence gathered over time may be combined to provide an overall level of confidence. However, a single larger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

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

Article 1.4.6.

Pathways to demonstrate freedom from disease

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



1. Absence of susceptible species

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

2. Historically free

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

   a) there has never been a substantiated occurrence of *disease* reported officially or in the scientific literature (peer reviewed), or

   b) *disease* has not occurred for at least 10 years, provided that the *disease agents* are likely to produce identifiable clinical signs in observable susceptible animals,

   and for at least the past 10 years:

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

   d) no vaccination against the *disease* has been carried out unless otherwise allowed for in the *Aquatic Code*;

   e) *disease* is not known to be established in wild *aquatic animals* within the country or *zone* intended to be declared free. (A country or *zone* cannot apply for historical freedom if there

is any evidence of *disease* in wild *aquatic animals*. However, specific *surveillance* in wild *aquatic animals* is not necessary.)

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

f) the country, *zone* or *compartment* of origin was declared free of the *disease* at the time of introduction;

g) *basic biosecurity conditions* were introduced prior to the introduction;

h) no vaccination against the *disease* has been carried out unless otherwise allowed for in the *disease* specific chapter of this *Aquatic Code*.

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

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

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

b) no vaccination against the *disease* has been carried out unless otherwise provided in the *Aquatic Code*;

c) *disease* is not known to be established in wild *aquatic animals* within the country or *zone* intended to be declared free. (A country or *zone* cannot apply for freedom if there is any evidence of *disease* in wild *aquatic animals*. Specific *surveillance* in wild *aquatic animals* of *susceptible species* is necessary to confirm absence.)

Article 1.4.7.

Maintenance of disease free status

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

1. if present, the pathogen is likely to produce identifiable clinical signs in observable *susceptible species*;

2. the *basic biosecurity conditions* are in place and effectively enforced;

3. no vaccination against the *disease* has been carried out unless otherwise provided in the *Aquatic Code*;

4. where applicable, *surveillance* has previously demonstrated that *disease* is not present in populations of wild *aquatic animal* of *susceptible species*.

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

Article 1.4.8.

Design of surveillance programmes to demonstrate freedom from disease

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

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

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

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

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

1. Objectives

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

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

2. Population

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

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

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

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

3. Sources of evidence

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

a) population-based surveys using one or more tests to detect the aetiological agent or evidence of *infection*;

b) other non-random sources of data, such as:

   i) sentinel sites;

   ii) *disease notifications* and laboratory investigation records;

   iii) academic and other scientific studies;

c) a knowledge of the biology of the agent, including environmental, host population distribution, known geographical distribution, vector distribution and climatic information;

d) history of imports of potentially infected material;

e) biosecurity measures in place;

f) any other sources of information that provide contributory evidence regarding *disease* in the country, *zone* or *compartment*.

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

4. Statistical methodology

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

a) the survey design;

b) the *sensitivity* and *specificity* of the test, or test system;

c) the design *prevalence* (or *prevalences* where a multi-stage design is used);

d) the results of the survey.

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

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

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

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

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

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

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

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

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

- over 5% for highly transmissible *infections*.

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

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

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

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

5. Clustering of infection

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

6. Test characteristics

All *surveillance* involves performing one or more tests for evidence of the presence of current or past *infection*, ranging from detailed laboratory examinations to farmer observations. The performance level of a test at the population level is described in terms of its *sensitivity* and *specificity*. Imperfect *sensitivity* and/or *specificity* impact on the interpretation of *surveillance* results and must be taken into account in the analysis of *surveillance* data. For example, in the case of a test with imperfect *specificity*, if the population is free of *disease* or has a very low *prevalence* of *infection*, all or a large proportion of positive tests will be false. Subsequently, samples that test positive can be confirmed or refuted using a highly specific test. Where more than one test is used in a *surveillance* system (sometimes called using tests in series or parallel), the *sensitivity* and *specificity* of the test combination must be calculated.

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

the *Aquatic Manual* may be used but the increased uncertainty associated with these estimates should be incorporated into the analysis of results.

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

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

7. Multiple sources of information

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

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

a) must be scientifically valid, and fully documented, including references to published material; and

b) should, where possible, take into account any lack of statistical independence between different data sources.

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

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

8. Sampling

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

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

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

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

9. Sample size

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

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

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

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

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

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

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

10. Quality assurance

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

Design prevalence	**Sensitivity (%)**	**Specificity (%)**	**Sample size**	**Maximum number of false positive if the population is free**
2	100	100	149	0
2	100	99	524	9

Design prevalence (contd)	Sensitivity (%)	Specificity (%)	Sample size	Maximum number of false positive if the population is free
2	100	95	1,671	98
2	99	100	150	0
2	99	99	528	9
2	99	95	1,707	100
2	95	100	157	0
2	95	99	542	9
2	95	95	1,854	108
2	90	100	165	0
2	90	99	607	10
2	90	95	2,059	119
2	80	100	186	0
2	80	99	750	12
2	80	95	2,599	148
5	100	100	59	0
5	100	99	128	3
5	100	95	330	23
5	99	100	59	0
5	99	99	129	3
5	99	95	331	23
5	95	100	62	0
5	95	99	134	3
5	95	95	351	24
5	90	100	66	0
5	90	99	166	4
5	90	95	398	27
5	80	100	74	0
5	80	99	183	4
5	80	95	486	32
10	100	100	29	0
10	100	99	56	2
10	100	95	105	9
10	99	100	29	0
10	99	99	57	2
10	99	95	106	9
10	95	100	30	0

Design prevalence (contd)	Sensitivity (%)	Specificity (%)	Sample size	Maximum number of false positive if the population is free
10	95	99	59	2
10	95	95	109	9
10	90	100	32	0
10	90	99	62	2
10	90	95	123	10
10	80	100	36	0
10	80	99	69	2
10	80	95	152	12

Article 1.4.9.

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

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

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

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

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

Article 1.4.10.

Surveillance for distribution and occurrence of disease

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

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

- *prevalence* or *incidence* of *disease* in wild or cultured animals;

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

This article describes *surveillance* to estimate parameters of *disease* occurrence.

1. Objectives

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

A single such survey can contribute evidence adding to an on-going collection of health data.

2. Population

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

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

In larger populations where a sampling frame is not available, or when there is a likelihood of clustering of *disease*, multi-stage sampling is required. For example, a multi-stage sampling process may involve sampling of farms or villages followed by sampling of fish from selected ponds within the sampled farms/villages.

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

3. Sources of evidence

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

a) population-based surveys using one or more tests to detect the agent;

b) other non-random sources of data, such as:

   i) sentinel sites;

   ii) *disease notifications* and laboratory investigation records;

   iii) academic and other scientific studies;

c) a knowledge of the biology of the agent, including environmental, host population distribution, known geographical distribution, vector distribution and climatic information;

d) history of imports of potentially infected material;

e) biosecurity measures in place;

f) any other sources of information that provide contributory evidence regarding *disease* or *infection* in the country, *zone* or *compartment*.

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

4. Statistical methodology

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

a) the survey design;

b) the *sensitivity* and *specificity* of the test, or test system;

c) the results of the survey.

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

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

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

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

5. Clustering of infection

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

infection. For endemic diseases, it is important to identify characteristics of the population which contribute to clustering and thus provide efficiency in *disease* investigation and control.

6. Test characteristics

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

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

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

Test results from *surveillance* for endemic disease will provide estimates of apparent *prevalence* (AP). Using diagnostic sensitivity (DSe) and diagnostic specificity (DSp), true *prevalence* (TP) should be calculated with the following formula:

$$TP = (AP + DSp - 1)/(DSe + DSp - 1)$$

In addition, it should be remembered that different laboratories may obtain conflicting results for various tests, host, or procedure-related reasons. Therefore, sensitivity and specificity parameters should be validated for the particular laboratory and process.

7. Multiple sources of information

Where multiple different data sources providing information on *infection* or *disease* are generated, each of these data sources may be analysed and presented separately.

Surveillance information gathered from the same country, *zone* or *compartment* at different times and similar methodology (e.g. repeated annual surveys) may provide cumulative evidence of animal health status and changes. Such evidence gathered over time may be combined (e.g. using Bayesian methodology) to provide more precise estimates and details of *disease* distribution within a population.

Apparent changes in *disease* occurrence of endemic diseases may be real or due to other factors influencing detection proficiency.

8. Sampling

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

When sampling below the level of the *epidemiological unit* (e.g. individual animal), the method used should be probability-based sampling. Collecting a true probability-based sample is often very difficult and care should therefore be taken in the analysis and interpretation of results obtained using any other method, the danger being that inferences could not be made about the sampled population.

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

9. Sample size

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

- the *sensitivity* and *specificity* of the diagnostic test (single or in combination);
- expected *prevalence* or *incidence* in the population (or *prevalences/incidences* where a multi- stage design is used);
- the level of confidence that is desired of the survey results;
- the precision desired (i.e. the width of the confidence or probability intervals).

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

- the size of the population (but it is acceptable to assume that the population is infinitely large);
- uncertainty about *sensitivity* and *specificity*.

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

A number of software packages, e.g. Survey Tool Box (www.aciar.gov.au; www.ausvet.com.au), WinPEPI (www.sagebrushpress.com/pepibook.html) can be used for the calculation of sample sizes.

In the case in which the values of Se and Sp are not known (e.g. no information is available in the specific *disease* chapter in the *Aquatic Manual*), they should not automatically be assumed to be 100%. Assumed values should be produced in consultation with subject-matter experts.

10. Quality assurance

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

Article 1.4.11.

Examples of surveillance programmes

The following examples describe *surveillance* systems and approaches to the analysis of evidence for demonstrating freedom from *disease*. The purpose of these examples is:

- to illustrate the range of approaches that may be acceptable;
- to provide practical guidance and models that may be used for the design of specific *surveillance* systems; and
- to provide references to available resources that are useful in the development and analysis of *surveillance* systems.

While these examples demonstrate ways in which freedom from *disease* may be successfully demonstrated, they are not intended to be prescriptive. Countries are free to use different approaches, as long as they meet the requirements of this chapter.

The examples deal with the use of surveys and are designed to illustrate different survey designs, sampling schemes, the calculation of sample size, and analysis of results. It is important to note that alternative approaches to demonstrating freedom using complex non-survey-based data sources are also currently being developed and may soon be published [2].

1. Example 1. – One-stage structured survey (farm certification)

   a) Context

      A freshwater aquaculture industry raising fish in tanks has established a farm certification scheme. This involves demonstrating farm-level freedom from a particular (hypothetical) disease (Disease X). The *disease* does not spread very quickly, and is most common during the winter months, with adult fish at the end of the production cycle being most severely affected. Farms consist of a number of grow-out tanks, ranging from 2 to 20, and each tank holds between 1,000 and 5,000 fish.

   b) Objective

      The objective is to implement *surveillance* that is capable of providing evidence that an individual farm is free from Disease X. (The issue of national or *zone* freedom, as opposed to farm freedom, is considered in the next example.)

   c) Approach

      The accreditation scheme establishes a set of standard operating procedures and requirements for declaration of freedom, based on the recommendations given in this chapter. These require farms to undertake a survey capable of producing 95% confidence that the *disease* would be detected if it were present. Once farms have been surveyed without detecting *disease*, they are recognised as free, as long as they maintain a set of minimum biosecurity standards. These standards are designed to prevent the introduction of Disease X into the farm (through the implementation of controls specific to the method of spread of that *disease*) and to ensure that the *disease* would be detected rapidly if it were to enter the farm (based on evidence of adequate health record keeping and the prompt investigation of unusual *disease* events). The effective implementation of these biosecurity measures is evaluated with annual on-farm audits conducted by independent auditors.

d) Survey standards

Based on the recommendations given in this chapter, a set of standards are established for the conduct of surveys to demonstrate freedom from *infection* with causative agent of Disease X. These standards include:

i) The level of confidence required of the survey is 95% (i.e. Type I error = 5%).

ii) The power of the survey is arbitrarily set at 95% (i.e. Type II error = 5%, which means that there is a 5% chance of concluding that a non-diseased farm is infected).

iii) The *target population* is all the fish on the farm. Due to the patterns of *disease* in this production system, in which only fish in the final stages of grow-out, and only in winter are affected, the *study population* is defined as grow-out fish during the winter months.

iv) The issue of clustering is considered. As fish are grouped into tanks, this is the logical level at which to consider clustering. However, when a farm is infected, the *disease* often occurs in multiple tanks, so there is little evidence of strong clustering. Also, the small number of tanks on a single farm means that it is difficult to define a design *prevalence* at the tank level (i.e. the proportion of infected tanks that the survey should be able to detect on the farm). For these reasons, it is decided to treat the entire grow-out population of each farm as a single homogenous population.

v) Stratification is also considered. In order to ensure full representation, it is decided to stratify the sample size by tank, proportional to the population of each tank.

vi) The design *prevalence* at the animal level is determined based on the epidemiology of the *disease*. The *disease* does not spread quickly; however, in the defined *target population*, it has been reported to affect at least 10% of fish if the population is infected. In order to take the most conservative approach, an arbitrarily low design *prevalence* of 2% is used. A *prevalence* of 10% may have been used (and would result in a much smaller sample size), but the authorities were not convinced by the thought that the population could still be infected at a level of say 5%, and *disease* still not be detected.

vii) The test used involves destructive sampling of the fish, and is based on an antigen- detection enzyme-linked immunosorbent assay (ELISA). Disease X is present in some parts of the country (hence the need for a farm-level accreditation programme). This has provided the opportunity for the *sensitivity* and the *specificity* of the ELISA to be evaluated in similar populations to those on farms. A recent study (using a combination of histology and culture as a gold standard) estimated the *sensitivity* of the ELISA to be 98% (95% confidence interval 96.7–99.2%), and the *specificity* to be 99.4% (99.2–99.6%). Due to the relatively narrow confidence intervals, it was decided to use the point estimates of the *sensitivity* and *specificity* rather than complicate calculations by taking the uncertainty in those estimates into account.

e) Sample size

The sample size required to meet the objectives of the survey is calculated to take the population size, the test performance, the confidence required and the design *prevalence* into account. As the population of each farm is relatively large, differences in the total population of each farm have little effect on the calculated sample size. The other parameters for sample size calculation are fixed across all farms. Therefore, a standard sample size (based on the use of this particular ELISA, in this population) is calculated. The sample size calculations are performed using the FreeCalc software. Based on the parameters listed above, the sample size required is calculated to be 410 fish per farm. In addition, the programme calculates that, given the imperfect *specificity*, it is still possible for the test to produce up to five

false-positive reactors from an uninfected population using this sample size. The authorities are not comfortable with dealing with false-positive reactors, so it is decided to change the test system to include a confirmatory test for any positive reactors. Culture is selected as the most appropriate test, as it has a *specificity* that is considered to be 100%. However, its *sensitivity* is only 90% due to the difficulty of growing the organism.

As two tests are now being used, the performance of the test system must be calculated, and the sample size recalculated based on the test system performance.

Using this combination of tests (in which a sample is considered positive only if it tests positive to both tests), the *specificity* of the combined two tests can be calculated by the formula:

$Sp_{combined} = Sp_1 Sp_2 - (Sp_1 1 \times Sp_2)$

which produces a combined specificity of $1 + 0.994 - (1 \times 0.994) = 100\%$.

The *sensitivity* may be calculated by the formula:

$Spe_{combined} = Se_1 \times Se$

which produces a combined sensitivity of $0.9 \times 0.98 = 88.2\%$.

These new values are used to calculate the survey sample size yielding a result of 169 fish. It is worth noting that attempts to improve the performance of a test (in this case increase *specificity*) generally result in a decrease in the performance of the other aspect of the test performance (*sensitivity* in this example). However, in this case, the loss of *sensitivity* is more than compensated for by the decreased sample size due to the improved *specificity*.

It is also worth noting that, when using a test system with 100% *specificity*, the effective power of the survey will always be 100%, regardless of the figure used in the design. This is because it is not possible to make a Type II error, and conclude that the farm is infected when it is not.

A check of the impact of population size on the calculated sample size is worthwhile. The calculated sample size is based on an infinitely large population. If the population size is smaller, the impact on sample size is shown in the following table:

Population size	**Sample size**
1,000	157
2,000	163
5,000	166
10,000	169

Based on these calculations, it is clear that, for the population sizes under consideration, there is little effect on the sample size. For the sake of simplicity, a standard sample size of 169 is used, regardless of the number of grow-out fish on the farm.

f) Sampling

The selection of individual fish to include in the sample should be done in such a manner as to give the best chance of the sample being representative of the *study population*. A fuller description of how this may be achieved under different circumstances is provided in Survey Toolbox[3]. An example of a single farm will be used to illustrate some of the issues.

One farm has a total of eight tanks, four of which are used for grow-out. At the time of the survey (during winter), the four grow-out tanks have 1,850, 4,250, 4,270 and 4,880 fish, respectively, giving a total population of 15,250 grow-out fish.

Simple random sampling from this entire population is likely to produce sample sizes from each tank roughly in proportion to the number of fish in each tank. However, proportional stratified sampling will guarantee that each tank is represented in proportion. This simply involves dividing the sample size between tanks in proportion to their population. The first tank has 1,850 fish out of a total of 15,250, representing 12.13%. Therefore 12.13% of the sample (21 fish) should be taken from the first tank. Using a similar approach the sample size for the other three tanks is 47, 47 and 54 fish, respectively.

Once the sample for each tank is determined, the problem remains as to how to select 21 fish from a tank of 1,850 so that they are representative of the population. Several options exist.

i) If the fish can be handled individually, random systematic sampling may be used. For example, samples can be collected at harvest or during routine management activities involving handling the fish (such as grading or vaccination).

ii) If fish are handled, systematic sampling simply involves selecting a fish at regular intervals. For instance, to select 21 from 1,850, the sampling interval should be 1,850/21 = 88. This means that every 88th fish from the tank should be sampled. To ensure randomness, it is good practice to use a random number between 1 and 88 (in this case) to select the first fish (e.g. using a random number table), and then select every 88th fish after that.

iii) If fish cannot be handled individually (by far the most common, and more difficult, circumstance) then the fish to be sampled must be captured from the tanks. Fish should be captured in the most efficient and practical way possible; however, every effort should be made to try to ensure that the sample is representative. In this example, a dip net is the normal method used for capturing fish. Using a dip net, convenience sampling would involve capturing 21 fish by repeatedly dipping at one spot and capturing the easiest fish (perhaps the smaller ones). This approach is strongly discouraged. One method of increasing the representativeness is to sample at different locations in the tank – some at one end, some at either side, some at the other end, some in the middle, some close to the edge. Additionally, if there are differences among the fish, an attempt should be made to capture fish in such a way as to give different groups of fish a chance of being caught (i.e. do not just try to catch the small ones, but include big ones as well).

This method of collecting a sample is far from the ideal of random sampling, but due to the practical difficulties of implementing random sampling of individual fish, this approach is acceptable, as long as the efforts made to increase the representativeness of the sample are both genuine and fully documented.

g) Testing

Specimens are collected, processed and tested according to standardised procedures developed under the certification programme and designed to meet the requirements of the *Aquatic Manual*. The testing protocol dictates that any specimens that test positive to ELISA be submitted for culture, and that any positive culture results indicate a true positive specimen (i.e. that the farm is not free from *disease*). It is important that this protocol be adhered to exactly. If a positive culture is found, then it is not acceptable to retest it, unless further testing is specified in the original testing protocol, and the impact of such testing accounted for in the test system *sensitivity* and *specificity* estimates (and therefore the sample size).

h) Analysis

If the calculated sample size of 169 is used, and no positive reactors are found, then the survey will have a confidence of 95%. This can be confirmed by analysing the results using the FreeCalc software mentioned above (which reports a confidence level of 95.06%).

It may happen in some cases that the survey is not conducted exactly as planned, and the actual sample size is less than the target sample size. However, the size of the farm may also be smaller. In these cases, it is advisable to analyse the farm data on a farm-by-farm basis. For example, if only 165 specimens were collected from a farm with only 2,520 fish, the resulting confidence would still be 95%. If only 160 fish were collected, the confidence is only 94.5%. If a rigid target of 95% confidence is used, then this survey would fail to meet that target and more evidence would be required.

2. Example 2 – Two-stages structured survey (national freedom)

a) Context

A country aims to declare freedom from Disease Y of crustaceans. The industry in this country is based largely on small-holder ponds, grouped closely together in and around villages. The *disease* is reasonably highly contagious, and causes mass mortality mid to late in the production cycle, with affected animals becoming moribund and dying in a matter of days. Affected animals show few characteristic signs, but an infected pond will almost invariably break down with mass mortality unless harvested beforehand. It is more common in late summer, but can occur at any time of year. It also occurs occasionally early in the production cycle. In this country, there are some limitations to the availability of laboratory facilities and the transport infrastructure. However, there is a relatively large government structure, and a comprehensive network of fisheries officers.

b) Objective

The objective is to establish national freedom from Disease Y. The *surveillance* system must meet the requirements of this chapter, but must also be able to be practically implemented in this small-holder production system.

c) Approach

The aquaculture authorities decide to use a survey to gather evidence of freedom, using a two-stage survey design (sampling villages at the first level, and ponds at the second). Laboratory testing of specimens from a large number of farms is not considered feasible, so a combined test system is developed to minimise the need for expensive laboratory tests.

The unit of observation and analysis is, in this case, the pond, rather than the individual animal. This means that the *diagnosis* is being made at the pond level (an infected pond or a non-infected pond) rather than at the animal level.

The survey is therefore a survey to demonstrate that no villages are infected (using a random sample of villages and making a village-level *diagnosis*). The test used to make a village-level *diagnosis* is, in fact, another survey, this time to demonstrate that no ponds in the village are affected. A test is then performed at the pond level (farmer observation followed, if necessary, by further laboratory testing).

d) Survey standards

i) The confidence to be achieved by the survey is 95%. The power is set at 95% (but is likely to be virtually 100% if the test system used achieves nearly 100% *specificity*, as demonstrated in the previous example).

ii) The *target population* is all ponds stocked with shrimp in the country during the study period. The *study population* is the same, except that those remote areas to which access is not possible are excluded. As *outbreaks* can occur at any time of year, and at any stage of the production cycle, it is decided not to further refine the definition of the population to target a particular time or age.

iii) Three tests are used. The first is farmer observation, to determine if mass mortality is occurring in a particular pond. If a pond is positive to the first test (i.e. mass mortality is detected), a second test is applied. The second test used is polymerase chain reaction (PCR). Cases positive to PCR are further tested using transmission experiments.

iv) Farmer observation can be treated as a test just like any other. In this case, the observation of mass mortality is being used as a test for the presence of Disease Y. As there are a variety of other *diseases* that are capable of causing mass mortality, the test is not very specific. On the other hand, it is quite unusual for Disease Y to be present, and not result in mass mortality, so the test is quite sensitive. A standard *case definition* is established for mass mortality' (for instance, greater than 20% of the pond's population of shrimp observed dead in the space of less than one week). Based on this definition, farmers are able to 'diagnose' each pond as having mass mortality. Some farmers may be over-sensitive and decide that mass mortality is occurring when only a small proportion of shrimp are found dead (false positives, leading to a decrease in specificity) while a small number of others fail to recognise the mortalities, decreasing sensitivity.

In order to quantify the sensitivity and specificity of farmer observation of mass mortalities, as a test for Disease Y, a separate study is carried out. This involves both a retrospective study of the number of mass mortality events in a population that is thought to be free from *disease*, as well as a study of farmers presented with a series of mortality scenarios, to assess their ability to accurately identify a pond with mass mortality. By combining these results, it is estimated that the *sensitivity* of farmer-reported mass mortalities as a test for Disease Y is 87% while the *specificity* is 68%.

v) When a farmer detects a pond with mass mortality, specimens are collected from moribund shrimp following a prescribed protocol. Tissue samples from 20 shrimp are collected, and pooled for PCR testing. In the laboratory, the ability of pooled PCR to identify a single infected animal in a pool of 20 has been studied, and the sensitivity of the procedure is 98.6%. A similar study of negative specimens has shown that positive results have occasionally occurred, probably due to laboratory contamination, but maybe also because of the presence of non-viable genetic material from another source (shrimp-based feed stuffs are suspected). The *specificity* is therefore estimated at 99%.

vi) Published studies in other countries have shown that the *sensitivity* of transmission tests, the third type of test to be used, is 95%, partly due to variability in the load of the agent in inoculated material. The *specificity* is agreed to be 100%.

vii) Based on these figures, the combined test system *sensitivity* and *specificity* are calculated using the formulae presented in Example 1, first with the first two tests, and then with the combined effect of the first two tests and the third test. The result is a *sensitivity* of 81.5% and a *specificity* of 100%.

viii) The design *prevalence* must be calculated at two levels. First, the pond-level design *prevalence* (the proportion of ponds in a village that would be infected if *disease* were present) is determined. In neighbouring infected countries, experience has shown that ponds in close contact with each other are quickly infected. It is unusual to observe an infected village with fewer than 20% of ponds infected. Conservatively, a design *prevalence* of 5% is used. The second value for design *prevalence* applies at the village

level, or the proportion of infected villages that could be identified by the survey. As it is conceivable that the *infection* may persist in a local area without rapid spread to other parts of the country, a value of 1% is used. This is considered to be the lowest design *prevalence* value for which a survey can be practically designed.

ix) The population of villages in the country is 65,302, according to official government records. Those with shrimp ponds number 12,890, based on records maintained by the aquaculture authorities. These are generated through a five-yearly agricultural census, and updated annually based on reports of fisheries officers. There are no records available of the number of ponds in each of these villages.

e) Sample size

Sample size is calculated for the two levels of sampling, first the number of villages to be sampled and then the number of ponds to be sampled. The number of villages to be sampled depends on the *sensitivity* and the *specificity* of the test used to classify villages as infected or not infected. As the 'test' used in each village is really just another survey, the *sensitivity* is equal to the confidence and the *specificity* is equal to the power of the village-level survey. It is possible to adjust both confidence and power by changing the sample size in the village survey (number of ponds examined), which means that we can determine, within certain limits, what *sensitivity* and *specificity* we achieve.

This allows a flexible approach to sample size calculation. If a smaller first-stage sample size is desired (a small number of villages), a high *sensitivity* and *specificity* are needed, which means that the number of ponds in each village that need to be examined is larger. A smaller number of ponds will result in lower *sensitivity* and *specificity*, requiring a larger number of villages. The approach to determining the optimal (least cost) combination of first- and second-stage sample sizes is described in Survey Toolbox.

A further complication is presented by the fact that each village has a different number of ponds. In order to achieve the same (or similar) confidence and power (*sensitivity* and *specificity*) for each village, a different sample size may be required. The authorities choose to produce a table of sample sizes for the number of ponds to sample in each village, based on the total ponds in each village.

An example of one possible approach to determining the sample size follows:

The target sensitivity (confidence) achieved by each village-level survey is 95%. The target specificity is 100%. Using the FreeCalc software, with a design *prevalence* of 1% (the survey is able to detect *disease* if 1% or more villages are infected), the first-stage sample size is calculated as 314 villages. Within each village, the test used is the combined test system described above with a *sensitivity* of 81.5% and a *specificity* of 100%. Based on these figures the following table is developed, listing the number of ponds that need to be sampled in order to achieve 95% sensitivity.

f) Sampling

First-stage sampling (selection of villages) is done using random numbers and a sampling frame based on the fisheries authorities list of villages with shrimp ponds. The villages are listed on a spreadsheet with each village numbered from 1 to 12,890. A random number table (such as that included in Survey Toolbox) or software designed for the generation of random numbers (such as EpiCalc [4]) is used.

Population	Sample size
30	29
40	39
60	47
80	52
100	55
120	57
140	59
160	61
180	62
200	63
220	64
240	64
260	65
280	65
300	66
320	66
340	67
360	67
380	67
400	67
420	68
440	68
460	68
480	68
500	68
1,000	70

The second stage of sampling involves random selection of ponds within each village. This requires a sampling frame, or list of each pond in the village. The fisheries authorities use trained local fisheries officers to coordinate the survey. For each selected village, the officer visits the village and convenes a meeting of all shrimp farmers. At the meeting, they are asked how many ponds they have and a list of farmers' names and the number of ponds is

compiled. A simple random sample of the appropriate number of ponds (between 29 and 70, from the table above, depending on the number of ponds in the village) is selected from this list. This is done either using software (such as Survey Toolbox's Random Animal Programme), or manually with a random number table or decimal dice for random number selection. Details of this process are described in Survey Toolbox. This selection process identifies a particular pond in terms of the name of the owner, and the sequence number amongst the ponds owned (e.g. Mr Smith's 3rd pond). Identification of the actual pond is based on the owners own numbering system for the ponds.

g) Testing

Once ponds have been identified, the actual survey consists of 'testing those ponds'. In practice, this involves the farmers observing the ponds during one complete production cycle. The local fisheries officer makes weekly visits to each farmer to check if any of the selected ponds have suffered mass mortality. If any are observed (i.e. the first test is positive), 20 moribund shrimp are collected for laboratory examination (first PCR, and then, if positive, transmission experiments).

h) Analysis

Analysis is performed in two stages. First, the results from each village are analysed to ensure that they meet the required level of confidence. If the target sample size is achieved (and only negative results obtained), the confidence should be 95% or greater in each village. At the second stage, the results from each village are analysed to provide a country level of confidence. Again, if the target sample size (number of villages) is achieved, this should exceed 95%.

3. Example 3. – Spatial sampling and the use of tests with imperfect specificity

a) Context

A country has an oyster culture industry, based primarily on rack culture of oysters in 23 estuaries distributed along the coastline. In similar regions in other countries, Disease Z causes mortalities in late summer/early autumn. During an *outbreak* a high proportion of oysters are affected; however, it is suspected that the agent may be present at relatively low *prevalence* in the absence of *disease outbreaks*.

b) Objective

The national authorities wish to demonstrate national freedom from Disease Z. If the *disease* should be detected, a secondary objective of the survey is to collect adequate evidence to support zoning at the estuary level.

c) Approach

The authorities conclude that clinical *surveillance* for *disease outbreaks* is inadequate because of the possibility of low level subclinical *infections*. It is therefore decided to base *surveillance* on a two-stage survey, in which sampled oysters are subjected to laboratory testing. The first stage of the survey is the selection of estuaries. However, due to the objective of providing evidence for zoning (should disease be found in any of the estuaries), it is decided to use a census approach and sample every estuary. In essence this means that there will be 23 separate surveys, one for each estuary. A range of options for sampling oysters are considered, including sampling at harvest or marketing, or using farms (oyster leases) as a level of sampling or stratification. However, the peak time of activity of the agent does not correspond to the harvest period, and the use of farms would exclude the significant numbers of wild oysters present in the estuaries. It is therefore decided to attempt to simulate simple random sampling from the entire oyster population in the estuary, using a spatial sampling approach.

d) Survey standards

i) The *target population* is all of the oysters in each of the estuaries. The *study population* is the oysters present during the peak disease-risk period in late summer early autumn. Wild and cultured oysters are both susceptible to *disease*, and may have associated with them different (but unknown) *risks* of *infection*. They are therefore both included in the *study population*. As will be described below, sampling is based on mapping. Therefore the *study population* can more accurately be described as that population falling within those mapped areas identified as oyster habitats.

ii) A design *prevalence* value is only required at the oyster level (as a census is being used at the estuary level). While the *disease* is often recognised with a very high *prevalence* during *outbreaks*, a low value is used to account for the possibility of persistence of the agent in the absence of clinical signs. A value of 2% is selected.

iii) The test used is histopathology with immuno-staining techniques. This test is known to produce occasional false-positive results due to nonspecific staining, but is very sensitive. Published studies indicate values of 99.1% for sensitivity and 98.2% for specificity. No other practical tests are available. This means that it is not possible to definitively differentiate false positives from true positives, and that in a survey of any size, a few false positives are expected (i.e. 1.8%).

iv) The confidence is set at 95% and the power at 80%. In the previous examples, due to the assumed 100% *specificity* achieved by use of multiple tests, the effective power was 100%. In this case, with imperfect specificity, there will be a risk of falsely concluding that a healthy estuary is infected, so the power is not 100%. The choice of a relatively low figure (80%) means that there is a 1 in 5 chance of falsely calling an estuary infected when it is not infected, but it also dramatically decreases the survey costs, through a lower sample size.

e) Sample size

Based on the assumption that the sampling procedure will mimic simple random sampling, the sample size (number of oysters to sample per estuary) can be calculated with FreeCalc. The population size (number of oysters per estuary) is assumed to be very large. The calculated sample size, using the sensitivity, specificity and design *prevalence* figures given above, is 450. FreeCalc also reports that, based on this sample size and the *specificity* of the test, it is possible to get 10 or fewer false-positive test results, and still conclude that the population is free from *disease*. This is because, if the population were infected at 2% or greater, the anticipated number of positive reactors from a sample of 450 would be greater than 10. In fact, we would expect 9 true positives (450 × 2% × 99.1%) and 8 false positives (450 × 98% × 1.8%) or a total of 17 positives if the population were infected at a *prevalence* of 2%.

This illustrates how probability theory and adequate sample size can help differentiate between true- and false-positive results when there is no alternative but to use a test with imperfect *specificity*.

f) Sampling

The aim is to collect a sample of 450 oysters that represent an entire estuary. Simple random sampling depends on creating a sampling frame listing every oyster (not possible) and systematic sampling depends on being able to (at least conceptually) line up all the oysters (again, not possible). The authorities decide to use spatial sampling to approximate simple random sampling. Spatial sampling involves selecting random points (defined by coordinates), and then selecting oysters near the selected points. In order to avoid selecting many points with no oysters nearby, the estuary is first mapped (the fisheries authorities already have

digital maps defining oyster leases available). To these maps areas with significant concentrations of wild oysters are also added, based on local expertise. Pairs of random numbers are generated such that the defined point falls within the defined oyster areas. Other schemes are considered (including using a rope marked at regular intervals, laid out on a lease to define a transect, and collecting an oyster adjacent to each mark on the rope) but the random coordinate approach is adopted.

Survey then visit each point by boat (using a GPS Global Positioning System unit to pinpoint the location). A range of approaches is available for selecting which oyster to select from a densely populated area, but it should involve some effort at randomness. Survey staff opt for a simple approach: when the GPS receiver indicates that the site has been reached, a pebble is tossed in the air and the oyster closest to the point where it lands is selected. Where oysters are arranged vertically (e.g. wild oysters growing up a post), a systematic approach is used to determine the depth of the oyster to select. First, an oyster at the surface, next, an oyster halfway down, and thirdly, an oyster as deep as can be reached from the boat.

This approach runs the risk of *bias* towards lightly populated areas, so an estimate of the relative density of oysters at each sampling point is used to weight the results (see Survey Toolbox for more details).

g) Testing

Specimens are collected, processed, and analysed following a standardised procedure. The results are classified as definitively positive (showing strong staining in a highly characteristic pattern, possibly with associated signs of tissue damage), probably positive (on the balance of probabilities, but less characteristic staining), and negative.

h) Analysis

The interpretation of the results when using a test with imperfect *specificity* is based on the assumption that, in order to conclude that the population is free from *infection*, any positive result identified is really a false positive. With a sample size of 450, up to 10 false positives may be expected while still concluding that the population is free from *disease*. However, if there is reasonable evidence that there is even a single true positive, then the population cannot be considered free. This is the reason for the classification of positive results into definitive and probable positives. If there are any definitive positives at all, the population in that estuary must be considered infected. The probable positives are consistent with false positives, and therefore up to 10 may be accepted. Using FreeCalc the actual confidence achieved based on the number of (presumed) false positives detected can be calculated. For instance, if 8 'probably positive' results were detected from an estuary, the confidence level for the survey would be 98.76%. On the other hand, if 15 'probably positive' results were detected, the confidence is only 61.9%, indicating that the estuary is likely to be infected.

i) Discussion

Normally, it may be safely assumed that a *surveillance* system aimed at demonstrating freedom from *disease* is 100% specific. This is because any suspected occurrence of *disease* is investigated until a definitive decision can be made. If the conclusion is that the *case* is truly a *case* of *disease*, then there is no issue of declaring freedom – the *disease* is known to be present. This example presents a different situation where, due to lack of suitable tests, it is not possible for the *surveillance* system to be 100% specific. This may represent an unusual situation in practice, but illustrates that methods exist for dealing with this sort of problem. In practice, a conclusion that a country (or estuary) is free from *infection*, in the face of a small (but statistically acceptable) number of positive results, will usually be backed up by further evidence (such as the absence of clinical disease).

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

2 International EpiLab, Denmark, Research Theme 1: Freedom from disease. http://www.vetinst.dk/high_uk.asp?page_id=196

3 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

4 http://www.myatt.demon.co.uk/epicalc.htm