Common Errors In Surveillance And Monitoring Programs On Fish Populations

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Abstract

The presence of a certain pathogen in a fish does not necessary mean development of a disease status in the fish or fish population. In fact, many other factors are involved in the development of the disease, as type of pathogen and type of host, prevalence of the pathogen in the population and load in the tissues, health status of the fish and stress situations, synergism/antagonism with other pathogens and microorganisms, density of the fish in the population, etc. The knowledge of the presence of a specific pathogen may allow us to apply control measures to avoid – or at least reduce the risk of – severe epizootics in both farmed and wild populations. Therefore, surveillance and monitoring (S&M) programs of fish populations are the best tools to reduce the impact that the spreading of epizootics might have on farmed and wild aquatic animals and ecosystems, and to prevent and respond to emerging and re-emerging diseases. However, those S&M programs must be correctly designed and implemented in order to yield reliable data to be further used in the development and application of control measures. As part (WP3) of the DIPNET EU project (Disease Interactions and Pathogen exchange NETwork), the authors of the present report have evaluated the suitability of S&M programs for both farmed and wild fish populations reported internationally. Based on this study, we will point out the common errors observed in most of the S&M projects evaluated, which frequently render the data unusable or confusing. General guidelines for an appropriate aquatic animal S&M programme will be also presented on a hypothetical case.

Theoretical approach to a survey

The first question of concern when designing a survey is its objective, which influences the calculation of the required sample size and sampling methodology. Active and targeted surveillance frequently involves testing for the presence or absence of one or more pathogens in a population, but it is possible also to perform epidemiological surveys to estimate prevalence of infection. In addition, the survey may be focussed on two completely different situations in terms of load and prevalence of the pathogen: i) a population of asymptomatic carrier fish, or ii) a population of suspected infected fish which may correspond to wild or farmed fish populations respectively.

The next but equally important question to answer would be how to select the sample: representative methods for surveys focussed to calculation of prevalence, and targeted methods to detect disease. Simple (one stage) sampling is desirable, but often multistage sampling methods are used, making sample size calculation more complex.

For targeted sampling to detect disease in wild populations, other factors have to be taken into account, such as the selection of the most susceptible species or development stage, season, high-risk locations, etc. Survey reports should therefore indicate: i) the sampling site and population, ii) the population size, iii) the fish species and strata (if applicable) under study, iv) the method of sampling and the sampling points (or transects) at the site, v) sample size (and size of pools, if employed) and method of calculation, and vi) the method of diagnosis employed and its accuracy in terms of sensitivity and specificity. Unfortunately, in many reports of fish S&M, some of these data are missing, makeing the results unreliable or difficult to interpret.

Common errors and missing data in S&M programs

More than 600 survey reports were examined from more than 30 different scientific journals. [ARC2]Among the surveys on freshwater resident fish populations, one of the most complete was published by Peribañez et al (1997), where even an estimation of the number of fish in each group is defined. Multistage sampling at different times was performed with a clear calculation and justification of the sample size needed to estimate prevalence of a PKD in trout farms. However, sensitivity nor specificity of the diagnostic tests were considered for sample size calculation. Reports of surveys on anadromous and cathadromous populations commonly omitted information on the diagnostic accuracy, population size, sampling methodology and distribution of sampling sites. In this scenario, we found an excellent report by Barker and Cone (2000), where the prevalence of two parasites in wild eels was estimated. The target area for each location was shown, and an interesting observation was made about multiple recaptures of wild eels (these data would potentially allow estimation of size of target population). However, as in other studies, a lack of information on diagnostic accuracy and population size was observed. In a different scenario (coastal resident fish communities) there are few reports related to S&M. However, in most cases they were actually focused to the detection of a certain agent, or even to compare diagnostic methods. The study by Ragias et al (2004) on infection of sea bass with parasites *Caligus,* provides an example of epidemiological method, and gives a clear and concise description of the method for sample collection; however, they included no indication on sample size (30), what would be enough to estimate unknown prevalences with accepted errors of 18%[ARC3]. In the case of oceanic pelagic fish, the populations are not in direct contact with farmed fish, but they may be a source of infection through two ways: forming part of diet of farmed fish or having contact with anadromous/cathadromous fish which can act as temporary carriers. There are lots of species that live in this environment and global migration for some species are reported related with oceanic currents. Interactions between species are almost unknown, but predator behaviour is likely in most of them, favouring pathogen transfer. The time between the collection of samples to laboratory processing can be long, so adequate transport protocols must to be carried out, to avoid a decrease of sensitivity. The 14-month survey of Mortensen *et al* (1999) that reported six new fish species demonstrated to be carriers of VHSV virus, in Baltic Sea, may be considered as a model, with clearly defined haul stations and sample size. Sample size was adjusted by logistic reasons, and perhaps more detection ability would have been possible including fewer fish in each pooled sample. Dopazo *et al* (2002) carried out a study in wild halibut in the Flemish Cap zone, using a total sample size of 80 fish, which is sufficient to detect infection if the prevalence is above 3.7%.

How to design and present a S&M program: a hypothetical case

An oceanic scenario has been used in the following example as it is one of the most complicated situations for S&M due to the reasons cited above. If the survey is to determine the prevalence of a certain agent in a certain fish species, and in a certain location, the following aspects should be first taking into account: **1) The agent:** This must be the first concern. The selection of the tissues to be analyzed depends directly on the type of agent, and only when a general diagnosis is performed, kidney and spleen are most frequently employed (at least for bacteria and virus), which unfortunately may miss some agents. In addition, the type of agent is important to decide “when” and “where” the diagnosis must be performed. In the case of viruses, the samples can be frozen and transported to the lab a few weeks after collection. On the other hand, for bacterial diagnosis, isolation must be performed on the site. So, in our example, we will study the prevalence of a specific neurotropic virus. Therefore, the sampled tissue could be the brain, and the samples can be frozen and transported to the diagnostic laboratory after the survey voyage. **2) The location and sample sites:** Prior knowledge of the area where that fish population inhabits is necessary. A varietyof methods may be used for the selection of representative sampling sites. In this example, transects are used. The location is open ocean and has and area of 50 square miles, that could be

subdivided into 50 transects. **3) The population and sampling size:** If available, from previous studies, the size of the population should be taken into account for calculation of the sample size. If, as is commonly the case, this information is not available, an 'infinite' population size may be assumed (greater than 5 000-10 000 fish). The population size of the fish species under study is around 25 000 individuals, and assuming worst situation (unknown prevalence) we can use an expected prevalence of 0.5, and accepted error of 0.05[ARC5]. Then we must apply adequate formulae and we will get a required sample size of 385 fish (95% level of confidence).

Information is also needed on the diagnostic method used. In selecting an appropriate method, the type of agent is the first concern, and previous reports on the accuracy of each method for the specific agent should also be considered. Aspects to consider should therefore be: **1) The agent:** Depending on the nature of the pathogen, the method of diagnosis might be different. In our case, the agent under study is a virus; therefore, a molecular technique could be chosen for diagnosis, although more data would be necessary to reject other methods. **2) Type of infection:** The load of the agent in the individuals is different between acute infections and carrier states, and therefore it must be considered to select the method of diagnosis. In this sense, if fish in the population are considered to be asymptomatic carriers, diagnostic methods [ARC6]capable of detecting very small amounts of pathogenic agents should be used. In our case, the fish under study belong to a wild population; therefore, a molecular technique must be preferred. Some additional considerations should be made about use of pooled samples, because this method could reduce sensitivity of diagnosis (dilution effect) and makes more difficult further calculations. **3) Diagnostic accuracy:** Sensitivity and specificity of the diagnosis is related not only to the method chosen but also to the complete protocol, considering the type of agent, fish species and population. The level of detection and the reliability of the method has been reported for most of the available techniques. However, sensitivity and specificity in terms of epidemiology should be determined previously. We have chosen PCR technology, which is supposed to detect between 0.1 to 1 pfu of virus from tissues.[ARC7] The lack of reliable information about diagnostic accuracy makes impossible previous calculations of needed sample size, or further estimation of true prevalence.

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