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Review

Risk-based methods for fish and terrestrial animal disease surveillance

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ABSTRACT

Over recent years there have been considerable methodological developments in the field of animal disease surveillance. The principles of risk analysis were conceptually applied to surveillance in order to further develop approaches and tools (scenario tree modelling) to design risk-based surveillance (RBS) programmes. In the terrestrial animal context, examples of risk-based surveillance have demonstrated the substantial potential for cost saving, and a similar benefit is expected also for aquatic animals. RBS approaches are currently largely absent for aquatic animal diseases. A major constraint in developing RBS designs in the aquatic context is the lack of published data to assist in the design of RBS: this applies to data on (i) the relative risk of farm sites becoming infected due to the presence or absence of a given risk factor; (ii) the sensitivity of diagnostic tests (specificity is often addressed by follow-up investigation and re-testing and therefore less of a concern); (iii) data on the variability of prevalence of infection for fish within a holding unit, between holding units and at farm level. Another constraint is that some of the most basic data for planning surveillance are missing, e.g. data on farm location and animal movements. In Europe, registration or authorisation of fish farms has only recently become a requirement under EU Directive 2006/88. Additionally, the definition of the epidemiological unit (at site or area level) in the context of aquaculture is a challenge due to the often high level of connectedness (mainly via water) of aquaculture facilities with the aquatic environment. This paper provides a review of the principles, methods and examples of RBS in terrestrial, farmed and wild animals. It discusses the special challenges associated with surveillance for aquatic animal diseases (e.g. accessibility of animals for inspection and sampling, complexity of rearing systems) and provides an overview of current developments relevant for the design of RBS for fish diseases. Suggestions are provided on how the current constraints to applying RBS to fish diseases can be overcome.

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Abbreviations: APB, aquaculture production business; EHN, Epizootic haematopoietic necrosis; EU, European Union; IPN, infectious pancreas necrosis; ISA, infectious salmon anaemia; KHV, Koi herpes virus; MS, member state; RBS, risk-based surveillance; STM, scenario tree modelling; SSC, surveillance system component; VHS, viral haemorrhagic septicaemia.

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1. Introduction

Before planning surveillance activities, the objective of a surveillance system or component should be considered as this is most influential in determining a suitable design. The objective of surveillance is closely related to disease mitigation (Häsler et al., 2011), i.e. disease control. Mitigation can be in one of the three stages: Stage I, 'sustainment', where the mitigation objective is to sustain a free or low-prevalence status. In this stage, the objective of surveillance is to demonstrate that a certain threshold of occurrence is not passed. This also includes the objective of early warning and freedom from infection. The estimation of the level of occurrence of a pathogen or disease relates to Stage II of mitigation, which was proposed to be called 'investigation' (Häsler et al., 2011). In this stage, the objective of surveillance is to obtain epidemiological information to inform a decision on interventions. If the latter are implemented, mitigation moves into Stage III 'implementation'. In this stage, the objective of surveillance is to inform the choice, timing, and scale of interventions and to document the progress of reduction of occurrence of the pathogen or disease. This is often measured in the form of incidence, prevalence or number of reported cases over time.

According to the European Centre for Disease Control (Anonymous, 2008), "**surveillance**" is defined as

> the ongoing collection, validation, analysis, interpretation and dissemination of health and disease data that are needed to inform key stakeholders to permit them to plan and implement more effective, evidence-based public health policies and strategies relevant to disease mitigation and to demonstrate the absence of disease or infection or food borne hazards.

This definition shall also be used here. Additionally, the term "**surveillance system**" shall be used, which is defined by the Office International des Epizooties (World Organisation for Animal Health OIE, 2012b) as

> a method of surveillance that may involve one or more component activities that generates information on the health, disease or zoonosis status of animal populations.

Each **surveillance system component** (SSC) consists of an independent surveillance protocol that focuses on a particular data source (Martin et al., 2007b). The surveillance **approach** chosen for a SSC can be either passive or active. A passive approach to surveillance generally involves minimal input from the central unit or competent authority to encourage reporting. Statutory case reporting is the most broadly used passive surveillance (Doherr and Audige, 2001). In active surveillance, the central unit or competent authority is securing sampling and reporting by its own activities (Salman, 2003). The selection of the surveillance approach is a key feature of its design because it is linked to the quality of the information obtained. The surveillance **design** describes all activities and methods used in the implementation, analysis and communication of SSCs.

Over the last years, the use of risk assessment methods led to the emergence of new surveillance approaches, referred to as **risk-based** surveillance. Risk-based surveillance has been defined by Stärk et al. (2006) as:

> a surveillance programme in the design of which exposure and risk assessment methods have been applied together with traditional design approaches in order to assure appropriate and cost-effective data collection.

In this approach, factors can be taken into account that have been shown to be associated with the risk of infection or disease. This is similar to so-called **targeted surveillance** which was defined as

> focusing the sampling on high-risk populations in which specific commonly known risk factors exist. (Salman, 2003)

The definition of targeted surveillance provided by the OIE Terrestrial Animal Health Code is similar (World Organisation for Animal Health OIE, 2012b) indicating an approach focusing on

> selected sections of the population in which disease is more likely to be introduced or found.

The EC directive 2006/88 uses the term targeted surveillance in a different way to mean (a) routine inspections, (b) sampling of animals to be tested for a specified pathogen and (c) notification of suspicion of a listed disease or abnormal mortality (annex IV).

In addition, risk-based surveillance can also use risk assessment in the selection of pathogens to be included in surveillance (McKenzie et al., 2007).

Surveillance systems are sometimes also classified according to the source of data and the way diagnosis is reached, e.g. abattoir surveillance, or syndromic surveillance, or laboratory surveillance. These classifications are of minor relevance here and therefore not elaborated further because we are considering surveillance regardless of data source.

This review was designed in order to outline methods for risk-based surveillance, and to provide an overview of the work already done and the relevant literature available both in relation to terrestrial animals as well as aquatic animals. The focus was on farmed animals but – where relevant – work on wild animals was also included. The second objective of the review was to examine advantages and possible problems of the application of RBS to aquatic animal health. Literature was searched using defined search terms for both terrestrial and aquatic animals (list of search terms available from authors on request). Electronically accessible literature was included as well as additional sources available to the authors (mainly national reports).

2. Principles, methods and examples of risk-based surveillance in terrestrial, farmed and wild animals

Surveillance can be classified on the way the units under observation are chosen. Random sampling implies choosing such that each unit has the same chance of being selected (Thrusfield, 2005). However, at population level, when the disease occurrence is rare, this type of data collection is limited in terms of financial and operational feasibility, because the lower the prevalence in a specific population, the larger the sample size that is required (Salman, 2003). Therefore, for rare disease events, non-random sampling is more efficient. In non-random sampling, the population is structured into risk strata, typically using risk factor information (Thrusfield, 2005). The probability of a unit being selected between strata differs; however, the probability within a stratum is equal. This concept is implemented in risk-based surveillance (RBS).

RBS takes into account the probability of a hazard, its consequences, management, and perception to detect cases in a population or sub-population. A broad range of factors can be used as long as they are associated with disease occurrence, i.e. they are risk factors. Examples are spatial factors (e.g. climate, population density, and trade), host factors (e.g. age, species), management factors (e.g. bio-security, antimicrobial usage) and other factors (e.g. history of cases or risky practices) (Wells et al., 2009). Spatial risk factors have been very widely used to produce risk maps. These have been used to inform surveillance design, particularly for vector-borne diseases (e.g. Khormi and Kumar, 2011).

The expected advantage of using RBS is an increased efficiency (Stärk et al., 2006). This is expected to become visible in the costs related to obtaining a defined level of confidence, particularly in relation to rare event and for surveillance conducted to demonstrate freedom from disease or infection. This economic criterion is the most important advantage of RBS. Presi et al. (2008) documented the advantages of RBS over conventional surveillance for residue monitoring. His paper quantified the benefits of RBS designs in terms of detection efficiency for rare events. Hadorn et al. (2009) used economic analysis for an optimised design of bluetongue surveillance.

However, RBS has also disadvantages. Most importantly, there needs to be evidence indicating the suitability of at least one risk factor. For this, specific studies have to be conducted to quantify the association between disease occurrence and the factor under consideration. It has also been highlighted that crude (unadjusted) relative risk or odds ratio estimates should be used (Willeberg et al., 2012), while the published literature mostly reports adjusted estimates. A second disadvantage of RBS is that an observed prevalence cannot be easily extrapolated to the general population but is valid only for the exposed population (Williams et al., 2009). This issue has been addressed by Cannon (2009).

Since the emergence of RBS approaches, methods have been developed that facilitate their design and also the analysis of related data. Risk assessment methods are now broadly used, including both qualitative and quantitative approaches (Anonymous, 2011). A semi-quantitative scoring approach was applied to develop risk-based designs for the monitoring of antimicrobial resistance (Presi et al., 2009). Also, risk-factor studies continue to be conducted and now use increasingly advanced analytical tools that allow the quantification of risk factors in strata, for example, by using cluster analysis (Pfeiffer et al., 2008).

Most importantly for RBS, scenario tree modelling continues to be applied to a broad range of surveillance questions (see Table 1). Scenario tree modelling was developed by Martin et al. (2007a, 2007b) to demonstrate freedom from infection in specified populations. The method aims to combine data obtained through structured representative surveys with non-representative data, which for example have been obtained from farmer observations, slaughterhouse sampling, laboratory records or research projects. Previously, data obtained through non-representative methods may have been used for a qualitative assessment by a panel of experts to assess the likelihood of a claim for disease free status of a country. Scenario tree modelling provides a method to quantify and combine multiple and diverse data sources. In addition, scenario tree modelling provides a tool for calculating the overall confidence level that a country or area is free from disease through using data obtained via surveillance applied to different target groups/populations and using tests with different test performances.

The method has been widely applied to optimise surveillance designs in various contexts. Hadorn and Stark (2008) developed a protocol for optimising surveillance for rare and emerging infectious diseases. Their protocol also uses scenario tree modelling and they presented an example for bovine tuberculosis. However, the application of this method for infections that are not rare (e.g. brucellosis, see Hadorn et al., 2008) required further methodological development. Scenario tree modelling has also become a key approach to the evaluation of surveillance systems (e.g. Hernandez-Jover et al., 2011). Particularly where several SSC are used, scenario tree modelling can assess the combined performance regardless of whether RBS approaches were used or not (e.g. Knight-Jones et al., 2010; Wahlstrom et al., 2010, 2011).

A review of the use of models in relation to surveillance has recently been conducted by Willeberg et al. (2011). They distinguish between models used for the planning, the evaluation and the interpretation of surveillance data. Regarding the latter, models were often used to demonstrate absence of disease or infection.

Examples of RBS used in terrestrial animals are given in Table 1.

There are very few examples of the application of RBS to non-domesticated animals (e.g. deer, wild boar), both farmed and wild. There are some examples related to pathogens that are also relevant for livestock and where wildlife acts as a reservoir, such as Trichinellosis and avian influenza. When demonstrating freedom from infection, wildlife also has to be included. This has been demonstrated for a few examples, such as tuberculosis or Echinococcosis (Wahlstrom et al., 2010, 2011).

Risk-based concepts are increasingly also used in food safety surveillance, in meat inspection and in priority setting for interventions for disease control. These topics have, however, not been the focus of this review.

3. Surveillance for fish diseases

The purposes of surveillance in aquatic animals are in principle the same as for terrestrial animals. However, special challenges for surveillance planning do occur due to the fact that the animals are kept in water, are kept in often complex rearing system (hatchery, freshwater or marine site), the size of the fish population on farm, and

Table 1
Examples of risk-based surveillance used in terrestrial animals. All references referring to surveillance systems matching the definition of risk-based surveillance were included regardless whether they made reference to this term or not.

Pathogen, agent, disease	Species	Risk factors	Reference
Poliovirus	Human	Region Age Population immunity	Watkins et al. (2009)
Bovine spongiform encephalopathy (BSE), prions	Cattle	Age Feeding practices (use of meat-and-bone meal), intensive feeding	Doherr et al. (2000), Morignat et al. (2002), Prattley et al. (2007), and World Organisation for Animal Health OIE (2012b)
Enzootic bovine leukosis, infectious bovine rhinotracheitis	Cattle	Importation	Blickenstorfer et al. (2011)
Avian influenza	Poultry, wild bird	Contact to wild birds, husbandry system	Snow et al. (2007), Alba et al. (2010), Knight-Jones et al. (2010), Tracey (2010), Welby et al. (2010), Christensen et al. (2011), and Martin et al. (2011)
Trichinella spp.	Pigs, wild boar	Husbandry system (outdoor access, wildlife contact) Feeding practice (swill)	Alban et al. (2008), Zimmer et al. (2008), Theodoropoulos et al. (2009), Schuppers et al. (2010), and Alban et al. (2011)
Salmonella	Pigs	Spatial risk factors	Benschop et al. (2008, 2010)
Rabies	Racoon	Environmental factors (habitat, topography)	Rees et al. (2011)
General	Cattle, poultry	Animal movements, movement networks	Van Kerkhove et al. (2009) and Noremark et al. (2011)

accessibility for inspecting and sampling animals. Furthermore, some basic information relevant to planning, such as expected prevalence in infected populations and diagnostic test performance, is often limited available.

The sections below provide some general principles of pathogen exposure, transmission and surveillance of selected diseases (VHS, ISA, EHN and infection with *Gyrodactylus salaris*) in farmed fish.

3.1. Pathogen exposure and transmission

A schematic illustration of pathways of pathogen introduction into freshwater fish farms is provided in Fig. 1.

The most important pathway of pathogen introduction into freshwater fish farms is probably via introduction of infected (mostly subclinically infected) live fish directly onto farm (Langdon et al., 1988; Oidtmann et al., 2011). Fish to fish transmission is enhanced by close proximity of infected to susceptible host, making fish farms (where host density is higher than found in wild populations) generally a suitable environment for pathogen establishment. A sufficient pathogen concentration (sufficient to cause infection in a susceptible host) needs to be present in the water for a sufficient time period (Oidtmann et al., 2011). Where pathogen dilution is high and the number of fish infected is relatively low (such as is often the case in the wild) establishment of a pathogen tends to be less likely or the consequences (at a populations level) less severe. Environmental factors play a crucial role in whether infection leads to clinical disease (see Section 3.2). Live fish movements are also an important pathway of pathogen transmission for marine salmon farms, although probably less relevant compared to many freshwater sites, due to different stock management practices (smolt are moved into net cages at the beginning of the saltwater growing period and introduction of new fish groups to an existing population is uncommon).

True vertical transmission (via infected eggs rather than contaminated egg surface) appears not to occur for most of notifiable fish diseases (e.g. VHS, IHN, ISA (Batts et al., 1991; Jørgensen, 1992; Rimstad, 2011)). For these pathogens, introduction via eggs would be via contaminated egg surface. Appropriate egg disinfection will in most cases prevent pathogen transmission although this may not always be the case (Wolf, 1988; Goldes and Mead, 1995). True vertical transmission is known from other important diseases of fish such as IPN and channel catfish virus (Wolf, 1988).

Freshwater fish farms often use surface water (not disinfected) for rearing fish. Use of untreated surface water carries the risk of exposure of farmed fish to pathogens introduced into (e.g. via restocking or release of ornamental fish) or established in (e.g. infected wild fish populations) the water source (Oidtmann et al., 2011). Examples are river water for freshwater farms abstracting river water and lake water in freshwater netcage aquaculture. The likelihood that a farm becomes infected via this route is only to a limited extent under the control of the fish farmer.

Exposure via water is probably an even more important pathway for pathogen introduction into marine farm sites. Transmission through short seaway distances between farming sites has been supported in studies for Infectious salmon anaemia virus (ISAV) (Vagsholm et al., 1994; Jarp and Karlsen, 1997; McClure et al., 2005a; Gustafson et al., 2007; Aldrin et al., 2010, 2011; Lyngstad et al., 2011a).

Proximity to fish processing facilities is considered to be a risk for disease introduction (Vagsholm et al., 1994; Jarp and Karlsen, 1997; McClure et al., 2005a; Diserens et al., 2011; Jansen et al., 2011; Oidtmann et al., 2011). If infected fish are processed, pathogen release may occur through unsafe storage, disposal or discharge of solid or liquid waste. Of particular concern is on-farm processing, if fish are sourced from outside the farm. Some farms receive live fish for processing. The likelihood of pathogen introduction

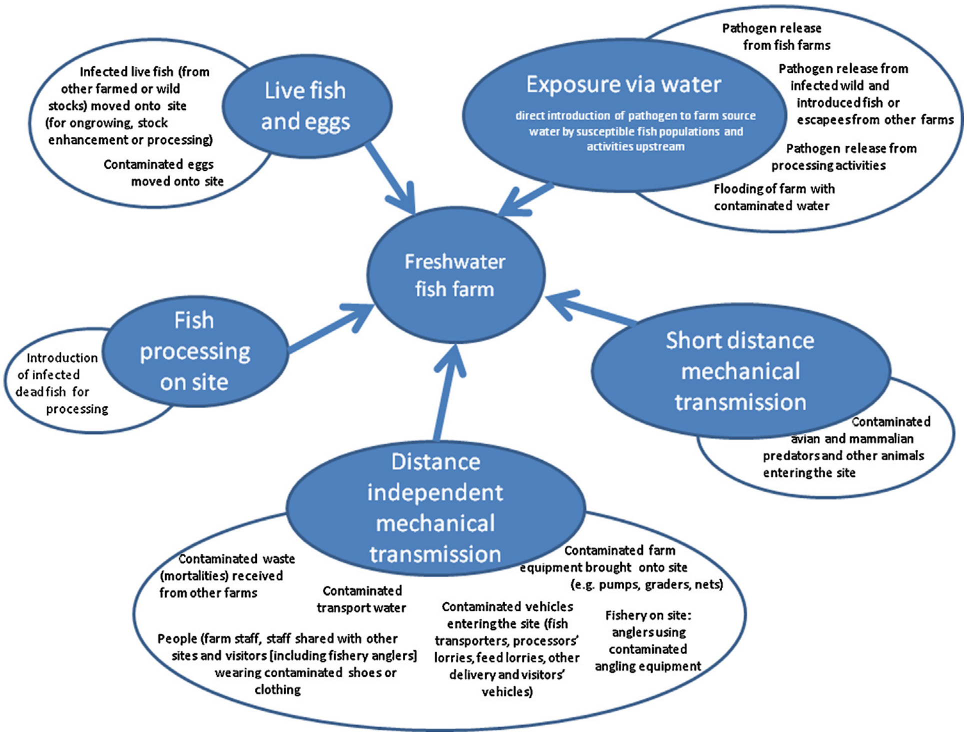

Fig. 1. Pathways of pathogen introduction to a freshwater fish farm. Freshwater fish farms may be exposed to multiple routes of pathogen introduction, which can be grouped into risk themes (risk themes shown as filled oval shapes surrounding the freshwater fish farm oval in the centre). Examples of pathways falling into each risk theme are listed in the white ovals attached to the respective risk theme oval. More detail on each risk theme is provided in the text.

and establishment via live fish for processing depends on factors discussed above for live fish movements.

The other pathways of pathogen introduction are via mechanical transmission (via fomites or live vectors (Peters and Neukirch, 1986; Hattenberger-Baudouy et al., 1988; Murray et al., 2002; McClure et al., 2005a; Lyngstad et al., 2008)). The likelihood of these pathways leading to pathogen introduction and establishment on a farm site depends to a great extent on the biosecurity practices applied by the farm and farm contacts. Depending on size, freshwater farms can fence the farm site (including bird netting) to reduce the risk of pathogen introduction via live vectors. Other possible biosecurity measures include disinfection of vehicles and equipment etc.

On most occasions, the visible impact is mainly on the farmed and not on wild fish. However, some pathogens can be well controlled in a farming environment, but not in the wild, and as a result are mainly affecting wild fish populations (e.g. sea lice and *G. salaris*) (Bakke et al., 1990; Johnsen and Jensen, 1991; Krkosek et al., 2005; Costello, 2009).

3.2. Likelihood of disease expression: interaction pathogen–host–environment

Interaction between pathogen, host and environment probably plays an even more relevant role in aquatic animals compared to farmed terrestrial species. Aquatic animals are ectothermic ("cold-blooded"), meaning that their body temperature is largely the same as the ambient water temperature. At the same time, the functionality of the fish (host) immune system varies with water temperature, with immune response delayed at low temperatures (low for the respective species) (Watts et al., 2001; Bowden et al., 2007), or impaired when stressed due to high temperatures (high for the respective species; the preferred temperature range varies with species).

Similarly, pathogen survival and amplification (in the environment and host respectively) is temperature dependent. Overall, temperature is probably the most relevant environmental factor in the pathogen–host–environment triad. Examples of the infectivity and impact of notifiable fish diseases varying with temperature are infection with VHSV (clinical infection rarely above 15 °C (Hill and Williams, 1984; Neukirch, 1984)) and koi herpesvirus disease (infections are usually not clinical at temperatures below 16 °C (Hedrick et al., 2000; Perelberg et al., 2003; Haenen et al., 2004; Sano et al., 2004)). In addition to temperature itself, the occurrence of diseases is also seen to be linked to periods with seasonal increasing and/or decreasing water temperature, a change in environment that may act as stress inducers. However, other water quality features can be relevant for both pathogen survival in the water and host response (e.g. poor water quality, including low oxygen levels, causing stress and therefore impaired host immune response; salinity and presence of microorganisms are relevant for pathogen survival in the water).

The dependency of host immune response as well as pathogen amplification and survival on temperature means that aquatic animal disease detection is most likely during periods where environmental conditions are suitable for clinical manifestation of disease (which is often seasonal). Similarly, certain pathogens are adapted to a certain salinity which will guide surveillance to salt or freshwater sites.

3.3. Population

Fish aquaculture production can be of single species (as is often the case in Atlantic salmon or rainbow trout production) or mixed species (as is often the case in farms producing for restocking). Where multiple species are farmed, fish on a farm can theoretically be divided into strata by species. However, whether or not the fish are kept in separate units by species may vary (e.g. cyprinid aquaculture often mixes multiple species in farm ponds). Separation by size/age (and therefore ease to specifically sample specific size/age classes) largely depends on farm management (e.g. frequency of grading; availability of separate holding units). Therefore targeting certain strata during sampling may require further selection of fish netted from a given holding unit. Within each holding unit, the number of individual fish is often several thousands. Aquatic animal populations are often considered infinite for the purpose of sample size calculations.

In pond aquaculture, ponds can be several hectares in size and such rearing systems are often extensive, meaning that fish are not observed on a daily basis and only a small subset of fish would be visible at any given time. Similarly, net cages (freshwater or seawater; often several metres deep and wide) provide limited visibility of the animals and may use divers for inspection. In bigger marine production systems inspection is usually done by divers or underwater cameras. Difficulties in access to and visibility of aquaculture fish mean that disease problems may not be noticed immediately. Even when fish displaying clinical signs are noticed, sampling these animals can be difficult because they have to be caught from amongst thousands of other fish. Moribund or newly dead fish will often be available for sampling and good routines for collecting such fish are therefore essential for good surveillance.

3.4. Diagnostic test sensitivity of tests applied for surveillance

Lethal sampling is commonly used for routine diagnostics in aquatic animals and diagnostic tests are usually based on the direct detection of the pathogen in a tissue sample rather than non-lethal methods to test for presence of antibodies against a specific pathogen (an appropriate diagnostic target in clinically normal animals). For fish with high individual economic values, non-lethal sampling may be performed. Reasons for the different developments in diagnostic testing are mainly based on the value of the individual animal, which is usually low for aquatic animals, but may be substantial in terrestrial animals, making lethal sampling inappropriate.

The diagnostic test sensitivity of many diagnostic tests for the notifiable aquatic animal diseases is unknown. The tests are suitable to diagnose clinical outbreaks (where the pathogen load in samples tissues is high); however, the diagnostic test sensitivity for screening of clinically little or unaffected populations is likely to be low. Diagnostic test performance data are available for ISA (McClure et al., 2005b; Nerette et al., 2005a, 2005b, 2008; Abayneh et al., 2010; Caraguel et al., 2012).

Sensitivity of screening tests for pathogens can be further reduced by pooling samples. The OIE manual of diagnostic tests for aquatic animals generally permits pooling of samples for diagnosis of many listed diseases (e.g. ten fish can be pooled for viral haemorrhagic septicaemia testing). Pooling allows the number of fish sampled in a population to increase for the same cost as individual sampling. However, pooling negative fish with positive ones may dilute the concentration of a pathogen below the minimum detection level of the diagnostic test, negating the potential benefits of testing more fish. To determine whether pooling is worthwhile, the minimum detection limit of the diagnostic test, the average and range of concentration of the agent in the tissue sampled is needed but generally not known.

Methods for the detection of aquatic animal pathogen DNA or RNA in environmental samples have been the subject of a number of studies (see Longshaw et al., 2012). Such methods would possibly provide opportunities to obtain data on pathogen prevalence at the pond or rearing unit level. However, data on pathogen quantities shed by infected fish into the water are very limited, and high dilution in water poses substantial challenges for pathogen detection (Longshaw et al., 2012). Results from a study on ISA showed that increase in pathogen shedding to relevant levels (that may be detectable in water samples) virtually concurs with the onset of mortalities (Gregory et al., 2009). More work is required before the use of environmental samples provides clear benefits over the use of testing animals.

3.5. Design prevalence

The OIE code for terrestrial animals provides design prevalences and detailed guidance for surveillance specific to several of the listed diseases. In the aquatic animal health code, general recommendations are provided, rather than specific guidance for the individual listed diseases (World Organisation for Animal Health OIE, 2012a). This may be due to the fact that for many of the aquatic animal diseases, basic data – such as prevalence in infected populations – are often not available, since testing usually implies pooling (Anonymous, 2012; World Organisation for Animal Health OIE, 2013) and therefore prevalence data cannot easily be generated. In the absence of specific requirements for specific diseases, the design prevalence needs to be set applying the guidance provided in the Aquatic Code and in a guide developed for aquatic animal health surveillance (Corsin et al., 2009):

- At the individual animal level, the design prevalence should be 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 transmitted slowly; and
  - over 5% for more contagious infections.
- 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) may be up to 2%.

3.6. Sample sizes

Sample size calculations should take into account diagnostic test performance. However, for many diseases test characteristics are not available and the assumption of a perfect test is often used in the sample size estimation. In the EC draft decision SANCO/6049/2009 the recommended sampling regime to achieve freedom from VHSV comprises two 150 fish samples taken at separate times, with an observation period of 2 years. A 150 fish sample is sufficient to detect disease at a design prevalence of 2% using a perfect test at the 95% confidence level (and assuming no clustering within the population of interest). Since tests are not perfect and some level of clustering is always present the confidence generated by a 150 animal sample is less than 95%.

3.7. Random sampling

Many farmed terrestrial animals are identified by an individual number (e.g. cattle, pigs). This means that a sampling frame can be drawn up and a random sample selected chosen using random numbers/random number tables. In aquatic animals, animals are not marked individually, more resembling the situation in poultry farming. A sampling frame can therefore not been drawn up in the same way.

Cameron (2002) discussed the problems of random sampling of aquatic animal populations comprehensively. Apart from absence of individual marking of animals, other reasons for difficulties in applying random sampling are that the animals are usually in large and relatively homogeneous populations (thousands of animals in a pond) and the animals are highly mobile (apart from molluscs).

A variety of methods, partly making use of management practices applied in aquatic animal farming, can be employed to obtain a sample, which is as close to true random sampling as can be achieved within the given constraints. For example, systematic sampling can be applied during grading or transfer of fish within a farm, where the animals are effectively 'lined up'. A sample could be taken at set time intervals or after every *x*th animal. Other opportunities arise during vaccination (by injection); when animals are stocked; or during harvest. If an entire population is harvested, every animal would be available for sampling and a random sampling method could be set up. However, all these methods have their practical constraints: the management activities are usually stressful for both animals and farm staff. Accommodating for interference due to sampling may be difficult. Furthermore, the management activities can take several hours (or days) and would keep (veterinary) staff on site for possibly extensive time periods.

A number of other factors may make sampling during such management activities not the method of choice: the population transferred or harvested may not represent the target population; the animals could represent the wrong age or exposure group. Furthermore, the time of year could be unsuitable to detect the disease and it may be difficult to time the visit of veterinary services to coincide with the management activities.

For the reasons given above, the method most frequently employed on aquatic animal farms is capture sampling. Capture sampling is likely to introduce some bias into the sample and it is important to be aware of the direction of bias. If fish are caught with a dip net, the healthier fish are more likely to escape. Attracting fish by using feed may over-represent dominant and healthy animals. In some cases, bias towards weak animals may be deliberate (sampling to detect disease). The purpose of sampling will therefore need to be considered.

Sampling of wild aquatic animal populations represents another range of challenges, which are not further discussed here.

4. Risk-based surveillance for fish diseases

Currently there are no references in the peer reviewed scientific literature presenting a RBS scheme in aquatic animals. Council Directive 2006/88/EC on animal health requirements for aquaculture animals and products thereof, and on the prevention and control of certain diseases in aquatic animals (Anonymous, 2006), requires that surveillance to maintain the disease status is risk-based. The frequency of inspections should take account of the likelihood that the fish farm may contract and spread disease, thus the risk must be assessed for each aquaculture production business. Triggered by the new European legislation, a number of authors have suggested methods for prioritising fish farms or sampling based on risk.

Oidtmann et al. (2011) presented a semi-quantitative model for ranking fish farms for pathogen introduction and spread. The model is suited to risk rank freshwater salmonid farms which are declared free of a defined pathogen. Following expert consultations through which risk routes for pathogen introduction onto farm and spread from farm were identified, the authors identified 5 main risk themes as relevant to risk ranking: (1) live fish and egg movements; (2) exposure/spread via water; (3) processing plant on site; (4) geographical factors (flood risk); and (5) mechanical transmission. Within each theme up to 12 individual risk factors contribute to the score of the individual theme. Scores for risk of introduction and spread are calculated separately and then combined to an overall score. Using VHS (genotype 1a) as a case study, expert consultation was used to assign weights to the relevance of the individual risk themes for this disease. The most important risk theme was live fish and egg movements (receiving a weighting of 0.63 (out of 1)), followed by exposure/spread via water and mechanical transmission (both 0.13); processing plant on site (0.07) and geographical factors (0.04). The authors suggest that the live fish movement element provides a good approximation of a likelihood estimate for risk of pathogen introduction via this route, since the number of live fish movements onto a farm, including the sources of these movements, should be known. Earlier versions of the model have been presented at international conferences (Oidtmann et al., 2009a, 2009b).

In the non peer-review domain, a number of authors have presented work for risk ranking of individual farms:

Kleingeld (2010) developed a model for risk ranking farms based on 5 risk themes: (1) live fish movements (including eggs), (2) biosecurity, (3) Exposure/spread via water; (4) proximity to other fish farms; and (5) farm management. The themes are weighed: the most important theme is live fish movements (including eggs) with a relative weight of 0.5. This is followed by biosecurity (0.2), exposure/spread via water (0.2); (4) proximity to other fish farms (0.05); and (5) farm management (0.05). Scores are calculated separately for risk of introduction and spread and then combined. A comprehensive range of data was collected from farms to risk categorise them. An alternative approach to the method suggested by Commission Decision 2008/896 (Commission of the European Union 2008) for combining risk of introduction with risk of spread is suggested, where risk of introduction is given higher relevance for the overall score compared to risk of spread.

Diserens et al. (2011) suggested the following risk factors for introduction of VHS or IHN onto fish farms in Switzerland: (1) Species kept (susceptible, vector species, non susceptible); (2) type of water supply; (3) live fish movements, (4) other fish farms in the vicinity, (5) fish processing; (6) biosecurity. The authors suggest the same risk factors for disease spread, except water supply changed to water effluent; and flooding and the type of ponds are additional risk factors. The risk factors were identified through an internal expert consultation process. Each of these individual risk factors is scored on a scale from 0 to 4 and the total score calculated by summing up all scores. No weighting is applied for the relevance of the individual risk themes. Within each of these principal risk groups, several individual risk factors may be included.

Several other publications can be found that present information on principal pathways of (or risk factors for) pathogen introduction and spread:

An international panel of fish health experts identified and weighted risk factors perceived important to the emergence and spread of the viral genotype, VHSV IVb, within and from the Great Lakes region of the US and Canada (VHSV Expert Panel Working Group, 2010). The experts were asked to identify and rank factors essential for predicting a watershed's (catchment) risk for acquiring VHSV IVb. Genotype VHSV IVb affects mainly wild fish. Through a group process designed for subjective probability assessment, the factors identified by the expert panel (listed in the order of relevance) included (1) hydrologic connectivity and proximity to known VHSV-positive areas with fish movements; (2) live fish transfer without testing (live bait); (3) linear distance to known positive regions; (4.a) live fish transfers for aquaculture or restocking (without testing) and (4.b) frozen fish transfer (without testing; bait); (5) uncontrolled exposure to fomites associated with boat and equipment or fish wastes from known VHSV-positive areas, (6) the presence of known VHSV-susceptible species, (7) water temperatures conducive for disease, (8) insufficient regulatory infrastructure for fish health oversight.

Although the context for which the expert group evaluated risk factors was for a disease mainly affecting wild fish (not farmed) and risk for introduction at catchment level (rather than farm), the principal pathways of pathogen transmission are similar to those presented by the farm targeted risk ranking models described above.

There is a wealth of publications that report on pathways of pathogen introduction and spread, of which we have referenced a small selection in Table 2. However, when risk ranking farms, it is relevant not only to be aware whether or not a certain transmission pathway may apply, but also its relative importance in general and the extent to which it applies to a respective farm. In the absence of studies that provide quantitative data on the relative importance of the various risk pathways, researchers have used expert opinion. Furthermore, in models developed to explain the introduction or occurrence of infection on fish farms, the relevance of risk pathways is also quantified.

5. Examples of scenario tree modelling and other methods used in the context of aquatic animal surveillance

Work on use of STM in the area of aquatic animal disease has so far only been presented at conferences. Oidtmann et al. (2008) used scenario tree modelling to identify requirements to achieve 95% confidence in freedom from infection with *G. salaris* in England and Wales. The authors assumed that sampling of rainbow trout farms was the only surveillance system component available and assumed non-risk-based sampling. They concluded that the sampling effort would be substantial and that risk-based surveillance should be explored to reduce the sampling effort. Their analysis highlighted significant gaps in the data needed to design surveillance programmes to demonstrate freedom from infection – for *G. salaris* and for other notifiable aquatic animal pathogens. Lyngstad et al. (2011) used STM to evaluate the sensitivity of surveillance

Table 2
Examples of studies investigating risk factors or principal risk pathways of pathogen spread of specific fish diseases.

Disease	Risk pathway	Reference
Viral haemorrhagic septicaemia	• Birds • Shared river-system • Hydrologic connectivity; live fish movements	Peters and Neukirch (1986) Jensen et al. (2009) VHSV Expert Panel Working Group (2010)
Infection with koi herpes virus	• Live fish movements	Taylor et al. (2010b)
Infectious salmon anaemia	• Short seaway distance between sites; hydrographic links; neighbouring hazards (e.g. transport route)	Vagsholm et al. (1994), Jarp and Karlsen (1997), Gustafson et al. (2007), Scheel et al. (2007), Aldrin et al. (2011), Lyngstad et al. (2011a), and Mardones et al. (2011)
Epizootic haematopoietic necrosis	• Birds • Live fish movements • Imports of live fish • Via water	Whittington et al. (1994) Langdon et al. (1988) Peeler et al. (2009) Whittington et al. (1994)
Infection with G. salaris	• Inflow of fresh water in to fjords – risk factor for inter-river dispersal, geographic risk factors	Jansen et al. (2007)

for VHS in Norway. The authors identified (1) region (based on proximity to area previously reported as VHSV positive), (2) distance to slaughter and processing plant, (3) species, (4) production stage, (5) production density, and (6) biosecurity level as category nodes.

Jansen et al. (2011) applied STM to estimate the probability of freedom from salmonid alphavirus in farmed Atlantic salmon in a defined non-endemic area in Norway. She concluded that the probability of freedom was 99%, given only one positive site with less than 1% infected fish.

6. Surveillance in wild fish populations

Monitoring and surveillance for aquatic animal disease is undertaken by governments to demonstrate disease freedom or progress in disease control, and generally remains unpublished. Some exceptions from this are the work by Raynard et al. (2001) in Scotland following the outbreak of infectious salmon anaemia in 1998 and 1999, surveys were undertaken to investigate the prevalence of the virus in wild freshwater salmon. Furthermore, Norway has a national surveillance programme for *G. salaris* in wild salmonids (*and* a surveillance programme on salmonid brood stock for cultivation purposes) as part of an early warning system for the detection of spread. The results are published in annual reports from the Norwegian Veterinary Institute.

However, most published surveys have been undertaken under the auspices of research projects rather than statutory investigations. In many instances these have been instigated because of concerns about the impact of these diseases on wild populations and potential influence of aquaculture and other anthropogenic activities. Examples include investigations of renal myxosporidiosis in wild salmonids in the UK (Feist et al., 2002; Peeler et al., 2008) and Switzerland (Wahli et al., 2002, 2007). The potential impact of bacterial kidney disease in both Europe and North America in wild salmonids has been a long standing concern. Studies of *Renibacterium salmoninarum* (the causative agent) revealed a low prevalence in wild salmonid fish (brown trout, Atlantic salmon, grayling) populations in the UK (Chambers et al., 2008). By contrast surveys of North American salmonid species using an antigen ELISA found much higher prevalences in a number of native salmonid species (Mitchum et al., 1979; Meyers et al., 1993). A number of surveys of VHSV in wild marine fish have been undertaken in the North Sea (Dixon et al., 2003; Skall et al., 2005) and more recently in freshwater in North America (reviewed by Faisal et al. (2012)).

Surveillance undertaken for regulatory purposes has generally used methods to directly identify the agent (e.g. culture). Research projects are more likely to have used serological tests. Very high levels of antibodies to *R. salmoninarum* have been observed in Alaskan wild stocks of trout (rainbow and steelhead), char and grayling indicating that these species may be resistant hosts and an important reservoir of the disease (Meyers et al., 1993). The geographic distribution of koi herpesvirus (KHV) in England and Wales was assessed using an antibody ELISA that allowed non-lethal sampling (Taylor et al., 2010a).

In general, few papers have used advanced statistical or modelling approaches in the analysis of surveillance data. However, analysis of data from a large-scale survey of Atlantic salmon in Scotland modelled the within and between river prevalences to correct the bias that arose from low sample sizes (in some rivers) and pooling (Raynard et al., 2001). Peeler et al. (2008) used multi-level modelling approaches to analyse prevalence data of renal myxosporidiosis and hepatitis in wild brown trout, and concluded that site level factors (compared with factors at the level of the fish or river) exerted most influence. Some studies have attempted to use surveillance data to assess the impact of disease at a population level. Johnsen and Jensen (1986) compared Atlantic salmon catch rates and parr densities in Norwegian rivers where *G. salaris* was known to be present and absent. Although no formal statistical associations were provided, the data clearly demonstrated a decline in infected compared with uninfected salmon populations. Several longitudinal studies have been conducted to establish statistical associations between pathogen prevalence/abundance and impact.

7. Discussion

Over recent years there have been considerable methodological developments in the field of animal disease surveillance. Although there are some genuine differences between farming of terrestrial animals and aquaculture, the key design concepts and challenges are equally valid across industries. Work by Cameron and Baldock (1998a, 1998b) provided an improved methodology to demonstrate freedom from infection or disease. The principles of risk analysis were conceptually applied to surveillance (Stärk et al., 2006). Martin et al. (2007b) developed an approach and tools (scenario tree modelling) that allowed RBS to be practically applied. This has allowed the evaluation of surveillance systems to shift from assessing inputs (i.e. number of animals sampled) to outputs (i.e. confidence in freedom at a specified design prevalence) (More et al., 2009). There are now many examples of the use of STM in the animal health literature, although mainly for terrestrial animals. Martin et al. (2007b) has been the most cited paper published in Preventive Veterinary Medicine over the last 5 years. Despite the differences between terrestrial and aquatic animal farming, the key challenges such as adjusting for clustering and diagnostic test limitations are similar. An enhanced use of novel methods in the design of surveillance systems targeted at aquaculture would therefore be beneficial. The core concepts of improved surveillance designs are transferable between industries without restrictions.

Whereas there are several examples of RBS approaches being applied in the assessment or design of a number of terrestrial animal diseases such as trichinella, brucellosis, enzootic bovine leucosis, and avian influenza (Hadorn et al., 2002; Snow et al., 2007; Alban et al., 2008), examples for aquatic animal diseases are fewer, and are currently being developed. A number of publications or presentations at conferences provided suggestions for methodological approaches for risk categorisation of farms (Oidtmann et al., 2009a, 2011; Kleingeld, 2010; Diserens et al., 2011), or suggested scenario tree modelling approaches for the evaluation of surveillance systems (Oidtmann et al., 2008; Lyngstad et al., 2011b).

There are a number of constraints that currently limit progress in developing RBS designs in the aquatic context. The first considerable constraint is the paucity of published data to assist in the design of RBS: this applies to data on (i) the relative risk of farm sites becoming infected due to the presence or absence of a given risk factor; (ii) the sensitivity of diagnostic tests; (iii) data on prevalence of infection for fish within a holding unit, between holding units and at farm level (these would be required for different stages in the establishment and spread of the disease and may require data relevant to the geographic region for which RBS is being planned). Studies that have described pathways of aquatic animal pathogen transmission tend to provide evidence that a certain route can lead to pathogen transmission rather than provide data that would allow to determine the relative risk for farms with and without the factor. Likely reasons for the lack of studies required to assist with the design of RBS are costs and possibly lack of incentive in countries where the pathogen is already present. Studies into risk factors for pathogen introduction into fish farms are very complex, since fish farms are more exposed to the environment than terrestrial farms.

The second constraint to the development of RBS in the aquatic context in the EU is that the most basic data for planning surveillance were missing. In the terrestrial field, the capturing of a range of data has been in place for some time; e.g. data on farm location and animal movements. In the aquatic field, farm registration or authorisation has only recently become a requirement under EU Directive 2006/88. Several EU member states did not have a central register with all names and addresses of aquaculture production businesses (Bang Jensen et al., 2011). Given that data, required at the most basic level for planning any kind of surveillance, were not complete in several EU member states, it is not surprising that there are even fewer MS that have collected more detailed farm data on the exposure of farms to recognised risk pathways. It is possible that the costs involved in collecting data to undertake RBS outweigh the financial advantages that may accrue from a more efficient system. This may deter countries from embarking down the road of RBS. This aspect is worth considering as part of the development of risk-based surveillance design.

Finally, the definition of the epidemiological unit (at site or area level) in the context of aquaculture may be a challenge. Several types of terrestrial farming businesses are sufficiently contained to allow regarding them as a self-contained epidemiological units (e.g. indoor housed pigs, cows, or poultry). However, due to the often high level of connectedness (mainly via water) of aquaculture facilities with the aquatic environment, the definition of the epidemiological unit is complex. For example, EU legislation allows shellfish farming areas instead of farms to be risk ranked which acknowledges the close connectedness between sites. Similarly, there may be situations where multiple fish farms should be considered as a single epidemiological unit.

8. Conclusions and recommendations

European Council Directive 2006/88/EC on animal health requirements for aquaculture animals and products thereof, and on the prevention and control of certain diseases in aquatic animals (Anonymous, 2006), requires that surveillance to maintain the disease status is risk-based. However, there are currently no clear recommendations for suitable methods. The purpose of this review was to provide an overview of current developments relevant for the design of RBS for fish diseases.

Considerable advances have been made in the methodological development of animal disease surveillance. Some progress has also been made in the development of risk-based approaches for surveillance of fish diseases. However, the paucity of published data to assist in the design of RBS present a major constraint in developing RBS designs in the aquatic context. The areas where data are required include the relative risk of farm sites becoming infected due to the presence or absence of a given risk factor; the sensitivity of diagnostic tests; and data on prevalence of infection for fish within a holding unit, between holding units and at farm level. In the absence of suitable published data, a method frequently used to generate the required information is expert consultation (Burgman et al., 2006). Given that the information required to inform RBS for the listed aquatic animal pathogens is largely missing, expert consultation appears to be the only feasible approach at present to allow the design of RBS programmes to progress. To improve the information for future RBS designs, it is strongly recommended that studies are undertaken to fill the existing data gaps. This will have benefits not only for efficient surveillance but for disease control in general.

The expected advantage of risk-based surveillance is increased efficiency. Costs will initially arise due to the need to obtain farm level data required to plan risk-based surveillance in the first place. Potential savings due to reduced sampling effort need to be weighed against the upfront costs for farm data collection. The latter could be substantially reduced if a farmer self-reporting system was developed.

In the terrestrial context, examples of risk-based surveillance have demonstrated the massive potential for cost saving (for example: Alban et al., 2008, 2011; Hadorn et al., 2009; Baptista et al., 2011; Calvo-Artavia et al., 2013), and a similar potential is assumed also for aquatic animals.

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