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Scientific Opinion on factors relevant for listing infectious diseases of aquatic animals

Opinion of the Panel on Animal Health and Welfare of the Norwegian Scientific Committee for Food Safety

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Norwegian Scientific Committee for Food Safety (VKM)
Po 4404 Nydalen
N – 0403 Oslo
Norway

Phone: +47 21 62 28 00
Email: vkm@vkm.no

www.vkm.no
www.english.vkm.no

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Scientific Opinion on factors relevant for listing infectious diseases of aquatic animals

Authors that prepared the draft Opinion

Espen Rimstad (chair), Øivind Bergh, Edgar Brun, Brit Hjeltnes and Stein Mortensen

(Authors in alphabetical order after chair of the working group)

Assessed and approved

This Opinion has been assessed and approved by the Panel on Animal Health and Welfare. Members of the panel are: Brit Hjeltnes (chair), Øivind Bergh, Edgar Brun, Knut Egil Bøe, Carlos Goncalo Afonso Rolhas Fernandes das Neves, Jacques Godfroid, Roar Gudding, Kristian Hoel, Cecilie Mejdell, Stein Mortensen and Espen Rimstad

(Panel members in alphabetical order after chair of the panel)

Acknowledgments

The Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) has appointed a working group consisting of both VKM members and external experts to answer the request from the Norwegian Food Safety Authority (NFSA). The project leader from the VKM secretariat has been Angelika Agdestein. The members of the working group, Espen Rimstad, Øivind Bergh, Edgar Brun, Brit Hjeltnes, and Stein Mortensen, are acknowledged for their valuable work on this Opinion. In order that the answer to the request from the NFSA included relevant, present-day practical insights, VKM invited fish health experts from 12 Norwegian fish farming companies of different sizes and located in different areas to present their current experiences from the field regarding monitoring the health status in aquaculture facilities. Representatives from two companies attended a project group meeting as hearing experts to present their insights on the topic. VKM would therefore like to thank the hearing experts, Tor Eirik Homme and Karl Fredrik Ottem, for their valuable contributions to the answer to ToR 9.

Competence of VKM experts

Persons working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

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Summary

There is often a long time delay from when an emerging or new infectious disease of aquatic animals is first identified to when the Norwegian Food Safety Authority (NFSA) has completed their evaluation regarding whether national listing (List 3) should be recommended. Listing is important because it enables interventions to be used in the field. The time lag associated with listing of diseases is mainly due to a lack of knowledge on the disease-causing agent and its epidemiology. Furthermore, some diseases remain listed, but no intervention measures are applied.

In the opinion of the NFSA, the process of diseases being added to the List could be shortened. However, this requires the establishment of efficient routines for inclusion of emerging or new infectious diseases of aquatic animals on List 3 and exclusion of already listed diseases. In this context, “aquatic animals” includes only fish, molluscs, and crustaceans.

The NFSA requested the Norwegian Scientific Committee of Food Safety (VKM) to assess which factors should be considered to determine whether an infectious disease should be included on List 3. This list appears as Annex I in Regulation 17 June 2008 on the placing on the market of aquaculture animals and products thereof, and on the prevention and control of certain diseases in aquatic animals (omsetnings- og sykdomsforskriften for akvatiske dyr). In addition, the NFSA requested VKM to assess which factors of a disease and an infectious agent are significant regarding the effect of subsequent interventions. Nine detailed Terms of Reference (ToR) in the request from the NFSA were addressed by VKM.

The report from the VKM has been prepared by a working group consisting of five members of the VKM Panel on Animal Health and Welfare. The final Opinion was assessed and approved by all members of this panel.

The VKM highlights the following principal factors that should be evaluated when considering whether a disease should be included on List 3:

a) the infectious nature of the disease and the efficiency of transmission of the associated pathogen

b) the consequences of the disease for farmed aquatic animals and/or wild aquatic animal populations

c) the possibilities for disease control

Knowledge regarding the infectious nature of the disease is particularly challenging for new and emerging diseases. In order to meet this challenge, field observations to assess infectivity or contagiousness are valuable, especially before experimental trials are able to

provide conclusive results. In the long-term, prevention would have a more positive impact than treatment, as the latter is mainly associated with damage control.

Transmission efficiency is influenced by many factors, including, but not limited to: the course of the disease, the persistence of the infection in the host animals, the range and proximity of susceptible species, the existence and proximity of reservoirs, the environmental stability of the infectious agent, the management and other characteristics of the affected aquaculture facilities, and interactions between farmed aquatic animals and wild populations.

The relevant consequences of the disease to consider are increased mortality, reduced welfare and production losses in farmed populations, and reduced welfare and other impacts on wild populations.

The possibilities for disease control will depend upon the success of hygiene and sanitation measures, and of vaccination. Vaccination would, however, not be applicable in either molluscs or crustaceans.

Key words: VKM, Scientific Opinion, Expert Opinion, Norwegian Scientific Committee for Food Safety, Norwegian Food Safety Authority, aquatic animals, fish, molluscs, crustaceans, List 3, national disease list, criteria for listing diseases, disease properties

Sammendrag på norsk

Det tar ofte lang tid fra en ny smittsom sykdom blir oppdaget i akvakulturnæringen til Mattilsynet har vurdert om sykdommen bør listeføres på Liste 3 - Nasjonale sykdommer. Når en sykdom er listeført, gir det et grunnlag for å iverksette tiltak i felt. Det at listeføringsprosessen tar lang tid skyldes først og fremst manglende kunnskap om agens. Enkelte sykdommer har blitt stående på sykdomslisten selv om det ikke har vært hensiktsmessig å iverksette offentlige kontrolltiltak.

Mattilsynet mener at det går an å korte ned på behandlingstiden, men dette forutsetter etablering av effektive rutiner for å vurdere om en ny sykdom hos akvatiske dyr skal føres på Liste 3, eventuelt om det er grunnlag for å ta sykdommer ut av listen. Med akvatiske dyr mener vi fisk, bløtdyr og krepsdyr.

Mattilsynet ber VKM vurdere hva myndighetene bør legge vekt på før de avgjør om smittsomme sykdommer hos fisk, bløtdyr og krepsdyr skal listeføres på Liste 3 i Vedlegg I i omsetnings- og sykdomsforskriften for akvatiske dyr. I tillegg ber Mattilsynet VKM vurdere hvilke faktorer og egenskaper ved sykdommer og smittestoffer som har betydning for effekten av offentlige tiltak. Mattilsynet har i oppdraget formulert ni konkrete spørsmål til VKM.

Rapporten er blitt utarbeidet av en prosjektgruppe bestående av fem medlemmer fra Faggruppen for dyrehelse og dyrevelferd i VKM. Den endelige vurderingen ble gjennomgått og godkjent av alle medlemmer av denne faggruppen.

VKM identifiserer følgende overordnede faktorer som bør vektlegges når listeføring av en sykdom skal vurderes:

a) sykdommens infeksiøse natur og spredningspotensial

b) konsekvenser for oppdretts- og villpopulasjoner av akvatiske dyr

c) muligheter for kontroll

Ved utbrudd av nye sykdommer er mangel på kunnskap om spredningspotensial spesielt utfordrende. I slike tilfeller bør feltobservasjoner danne grunnlag for epidemiologiske vurderinger, spesielt i perioden før det foreligger konklusjoner fra eksperimentelle studier. I det lange løp vil man være bedre tjent med å ha fokus på forebygging fremfor behandling, da sistnevnte i forbindelse med fisk hovedsakelig dreier seg om å drive skadebegrensning.

Spredningspotensialet av sykdommer blir påvirket av sykdommens forløp, infeksjonens persistens, mottakelige arter og reservoarer, og stabiliteten av agens i miljøet. Videre vil blant annet utforming av oppdrettsanlegg, driftsrutiner, samt interaksjoner mellom oppdretts- og villpopulasjoner være viktige faktorer av betydning for spredningspotensialet.

Konsekvenser av sykdommen som er relevante å vurdere innbefatter i hovedsak økt dødelighet, redusert dyrevelferd og i oppdrettspopulasjoner også produksjonstap.

Muligheter for sykdomskontroll forutsetter god effekt av hygieniske tiltak og sanering eller av vaksiner. Vaksinasjon er ikke relevant for bløtdyr og krepsdyr.

Abbreviations and glossary

Abbreviations

AGD – amoebic gill disease

BKD – bacterial kidney disease

HSMI – heart and skeletal muscle inflammation

ISA/ISAV – infectious salmon anaemia/infectious salmon anaemia virus

IPN/IPNV – infectious pancreatic necrosis /infectious pancreatic necrosis virus

NFSA – Norwegian Food Safety Authority (Mattilsynet)

OIE – World Organisation for Animal Health

PD – pancreas disease

qPCR – quantitative real-time polymerase chain reaction

RAS – recirculating aquaculture system

SAV – salmonid alphavirus

VHSV – viral haemorrhagic septicaemia virus

VKM – Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet)

Glossary

Alevins: salmonid fry still attached to the yolk sac.

Biosecurity: a set of measures designed to reduce the risk of introduction and spread of disease agents.

Emerging disease: a disease caused by a known pathogenic agent spreading to a new geographic area or species, or caused by a newly recognised or suspected pathogenic agent.

Fallowing: leaving sites empty of all animals before restocking at the end of a production period.

Fry: recently hatched fish.

Recirculating aquaculture systems (RAS): systems in which the water in the fish tanks flows to a treatment unit before being returned to the tank.

Smolt: juvenile anadromous salmonids physiologically ready to enter seawater.

Background as provided by the Norwegian Food Safety Authority

A long period of time may elapse from detection of an emerging or novel infectious disease in aquatic animals until implementation of a governmental action or response. Actions include implementing interventions and deciding whether the disease should be included on the national list (List 3).

For some diseases that are currently listed, it would not be appropriate to implement interventions.

The delay from detection until listing is due to multiple factors, but foremost is lack of knowledge on the agent causing the disease. Nevertheless, in the opinion of the Norwegian Food Safety Authority (NFSA) it should be possible to reduce the procedural duration.

However, this would require the establishment of more efficient routines for proposing the inclusion of emerging or novel aquatic animal diseases on List 3, or for excluding diseases that are already listed.

As more knowledge is needed to improve the current routines, the NFSA requests the Norwegian Scientific Committee of Food Safety (VKM) to determine and assess those factors relevant for listing of infectious aquatic animal diseases on List 3 – National diseases.

For the purposes of this Opinion, aquatic animals are defined as including only fish, molluscs, and crustaceans.

Terms of reference as provided by the Norwegian Food Safety Authority

The Norwegian Food Safety Authority (NFSA) requests the Norwegian Scientific Committee for Food Safety (VKM) to determine and assess those factors that the authorities should take into consideration when determining whether an infectious disease of fish, molluscs, or crustaceans should be listed in the List 3 of Annex I in Regulation 17 June 2008 on the placing on the market of aquaculture animals and products thereof, and on the prevention and control of certain diseases in aquatic animals.

In addition, the NFSA requests the VKM to assess which factors and characteristics of a disease and an infectious agent are significant when considering the potential effect of implementing preventive interventions or measures.

The NFSA particularly request the VKM to answer the following questions:

1. Which factors and properties should be considered and taken into account in the risk assessment of a disease or an infectious agent on the health situation in an aquaculture facility?

2. Which factors and properties are significant for the control of a disease or an infectious agent in a fish farm or mollusc farming area?

3. Which factors and properties of a disease or an infectious agent:

    a. are significant for achieving and maintaining areas that are free of that disease or of that infectious agent?

    b. indicate that it would be expedient to achieve and maintain areas free of the infectious agent, given the feasibility of achieving and maintaining such areas?

    c. indicate that it would be expedient to achieve and maintain areas free of the disease, given the feasibility of achieving and maintaining such areas? Areas free of disease are, in this context, defined as areas free from outbreaks of the disease, but not necessarily free of the infectious agent.

4. Which information should be available for the authorities to assess whether:

    a. a disease is infectious?

    b. a disease can be diagnosed based on the presence of infectious agent or of pathological findings?

5. Which factors and properties of a disease or an infectious agent are significant for the risk of introduction of that infection due to transport or sale of live aquatic animals, given that:

    a. there is suspicion or confirmation of the presence of an infectious agent in these aquatic animals in a fish farm or mollusc farming area, but without clinical signs of disease.

    b. there is a suspicion or confirmation of the presence of an infectious agent in these aquatic animals in parts of a fish farm or a mollusc farming area, but where the animals to be moved or sold are clinically healthy and without present or prior clinical signs of disease?

    c. disease has been confirmed in a fish farm for these aquatic animals or in a mollusc farming area, but the disease outbreak has terminated and the animals to be moved or sold are currently healthy.

6. Which factors and properties of a disease or an infectious agent are significant for risk of infection when aquatic animals are used for breeding and reproduction, given that:

    a. there is suspicion or confirmation of the presence of an infectious agent in these aquatic animals in a fish farm or mollusc farming area, but without clinical signs of disease.

    b. there is a suspicion or a confirmation of the presence of an infectious agent in these aquatic animals in parts of a fish farm or a mollusc farming area, but where the animals to be used for breeding and reproduction are clinically healthy and without present or prior clinical signs of disease?

    c. a disease has been confirmed in a fish farm for these aquatic animals or mollusc farming area, but the disease outbreak has terminated and the animals to be used for breeding and reproduction are currently healthy.

7. Which factors and properties of a disease or an infectious agent are significant for reducing the risk of spread given that:

    a. all the animals in the farm or mollusc farming area are slaughtered immediately?

    b. only units with aquatic animals with clinical signs of disease are slaughtered?

    c. individual farms are fallowed?

    d. all the farms in a zone are fallowed in a coordinated manner?

8. Which factors or properties are significant for a disease or an infectious agent to pose a threat to wild aquatic animals if left uncontrolled or contained at a low level?
9. Are there any other factors or properties of a disease or an infectious agent that have not been mentioned in Questions 1 to 8, and that should be taken into consideration when assessing whether a disease should be listed?

Introduction

Scientific literature or guidelines directly related to the assessment of criteria for listing of diseases are scarce, almost non-existent. This has represented a methodological challenge in preparing the present Opinion.

Surveillance and epidemiological considerations in aquaculture

The World Organization for Animal Health (OIE) sets standards and lists many diseases of aquatic animals as being of major importance for safe trade. National authorities can define other diseases as well that are of particular importance within their own specific country. The Norwegian Food Safety Authority (NFSA) is responsible for surveillance of the health status of aquaculture animals in Norway. This includes detection of new and exotic diseases, assessment of changes in prevalence of endemic diseases, and ascertaining areas as being free from specific diseases.

New and previously unknown infectious diseases have frequently been detected in Norwegian farming of Atlantic salmon (*Salmo salar* L.) and rainbow trout (*Oncorhynchus mykiss*) (Norwegian Veterinary Institute, 2015). The infrastructure of the industry, with high animal density and extensive movement of fish along the Norwegian coast, has contributed to the rapid spread and establishment of infectious diseases in farmed salmonid populations. Horizontal transmission between fish and movement of live fish are the two means by which most of these infectious diseases are spread.

One prerequisite in prevention of spread of infectious disease is an efficient surveillance system. This requires standardization of criteria for disease diagnostics and characterization. Uncertainty and differences in diagnostic criteria will reduce diagnostic sensitivity and specificity, and could result in diminishing the impact of subsequent measures that are taken or could instigate unnecessary actions being taken in the event of false positives. New diseases pose a particular challenge in this regard.

In Norwegian fish farming, disease surveillance performed by competent authorities and mandatory fish health services provides documented information on the disease status of the industry. Results from research also contribute. Surveillance is mostly risk-based and has been shown to be able to identify infectious diseases. The discovery of new and emerging diseases often relies on diagnostic investigations. Through research and the fish health services, the industry has gained substantial insights on risk factors related to introduction and spread of infectious agents. Criteria for assessment of the consequences of such spread, other than those directly related to economy, are less well established. However, criteria to ensure that any actions implemented are relevant and socio-economically sound should be developed and adopted.

Listing of diseases in aquatic animals

Fish diseases are listed internationally or nationally to establish standards in the control of fish diseases. The OIE publishes health standards for international trade in animal and animal products. In the EU/EEA area, Lists 1 and 2 were originally declared by the European Commission (Council Directive 91/67/EEC, Annex A) and are included in Part II of Annex IV of the Council Directive 2006/88/EU (fiskehelsedirektivet). The Norwegian national List 3 has been adopted by the Ministry of Trade, Industry and Fisheries in the Annex I in Regulation 17 June 2008 on the placing on the market of aquaculture animals and products thereof, and on the prevention and control of certain diseases in aquatic animals (FOR-2008-06-17-819, omsetnings- og sykdomsforskriften, Norwegian Ministry of Trade, Industry and Fisheries).

Freedom from disease

The concept of freedom from Lists 1 and 2 diseases is based on implementation and fulfilment of relevant international standards (OIE Aquatic Animal Health Code 2015, EU). The freedom from disease concept is a probability of freedom where the level of probability is dependent on historical data, the sensitivity of the surveillance system, and the probability of a new introduction to a population currently declared free. However, it is not possible to provide absolute certainty of the absence of disease.

In surveillance and disease control, the sensitivities of the surveillance system and testing regime are important for determining whether a population can be declared positive or negative with regards to a specific infectious agent.

One part of this regime is the characteristics of the laboratory tests used; another part is choice and number of tissues and animals sampled. A positive laboratory result indicates that the sample is positive, and one or more positive samples may demonstrate the population as being positive. Negative results, however, have to be considered more carefully, as presence can be demonstrated, but absence cannot. A negative result may reflect an uneven distribution of the agent, or agent-specific DNA/RNA, or antigens, in the tissue sampled. It could also be due to sub-optimal sampling of tissue or animals, or that the prevalence in the population is below the detection limit, given the number of animals sampled. In order to increase confidence in a negative result being indicative of absence, the population should be followed over time and the sampling strategy carefully designed according to the characteristics of the agent being investigated.

Susceptibility of aquatic animals to infectious diseases

An aquatic animal species is recognized as susceptible to a particular pathogen if infection has been demonstrated to occur by natural exposure or by experimental exposure mimicking natural transmission. Infection can be defined as invasion and multiplication of agents that are not normally present within the body and may result in clinical disease or sub-clinical infection. Mechanical vectors, i.e. species that may carry the pathogenic agent without replication of the agent occurring, are also regarded as being susceptible.

Clinical disease following infection is a result of a multitude of factors that are not always present in experimental challenges. For example, outbreaks of infectious pancreatic necrosis (IPN), heart and skeletal muscle inflammation (HSMI), and pancreas disease (PD) in Atlantic salmon are well documented in Norway, and all three diseases have had a severe impact on Norwegian aquaculture. However, consistently demonstrating significant mortality in experimental challenge trials of these diseases has been difficult to achieve (Bowden et al., 2003; Cano et al., 2015; Wessel et al., 2015).

Susceptibility may be affected by many possible factors, e.g. developmental stage and size of fish. In general, young fish tend to be especially vulnerable to infectious diseases.

It is important to determine the susceptibility of different species and populations in order to ascertain the possible impact on a population at risk and to assess whether a particular population can serve as a reservoir. When cases or natural outbreaks are lacking, then experimental challenges are important tools for assessing susceptibility. In Chapter 1.5 of the OIE Aquatic Animal Health Code, approaches are outlined for assessing susceptibility to specified pathogens.

Infectious agents and host specificity

As a prerequisite to causing disease, infectious agents (viruses, bacteria, parasites, fungi) must infect a host. Furthermore, in order that the agent is transmitted to other susceptible individuals, the agents must either be shed, or the host animal eaten, or the pathogen taken up by another host or a vector. Some infectious agents can also be passed vertically, from parent to offspring. Infectious agents cause diseases through a large variety of pathogenic mechanisms. The outcome of the agent-host encounter is essentially the product of three elements: a) the load and virulence of the infecting agent; b) the susceptibility, genetics, and immunity of the host; and c) influences from environmental factors, including management of an aquaculture facility, that may exacerbate or ameliorate the infection.

Different groups of pathogens have different characteristics. Viruses are either pathogenic or non-pathogenic, whereas bacteria, fungi, oomycetes and some parasites may either be opportunistic pathogens (able to multiply outside the host) or obligate pathogens (unable to multiply outside the host in its natural environment). Pathogenic agents may be characterized by their infectivity and virulence. Infectivity is the ability of a pathogen to establish an infection, whereas virulence and pathogenicity both refer to the capacity of the agent to infect and persist in its host, often resulting in clinical signs. Virus infection of a cell requires interaction between surface components of the virus particle with a component of the host cell. This interaction is specific and is an important determinant of the species specificity of a virus. Many agents are able to infect several host species; this is particularly true for viruses infecting fish.

Ocean-based aquaculture is an open system where farmed fish may incubate and transmit infectious agents to and from wild fish.

Most of the viral pathogens that are often found in the seawater phase of Norwegian commercial Atlantic salmon farming occur in freshwater farming in other geographical areas and under other farming management systems. This can be explained by the lack of influence that water salinity has on the intracellular environment, within which viruses and other intracellular agents reside in the host. Thus, most fish viruses of anadromous fish are able to establish infections regardless of whether the host is in fresh water or seawater.

Most fish pathogenic bacteria are opportunists, typically residing in the environment. Two examples of obligate pathogenic bacteria are *Renibacterium salmoninarum*, to which salmonids are susceptible, especially Pacific salmon, and *Francisella noatunensis*, to which several fish species are susceptible, including Atlantic cod, Atlantic salmon and tilapia.

There are some examples of pathogenic bacteria being introduced into new areas. One example from Norwegian aquaculture is that of *Aeromonas salmonicida* subsp. *salmonicida*, causing furunculosis in salmonids (Håstein and Poppe, 1986).

Pathogenic fungi are ecologically similar to opportunistic bacteria, i.e., they are able to grow outside the host. There are also examples of pathogenic oomycetes that have been introduced to Norway. The most important is *Aphanomyces astaci*, a water mould causing crayfish plague in crayfish. It was introduced to Italy in the 1850s, either in imported crayfish from North America or in ballast water. Since then it has spread throughout Europe, reaching Norway in 1971 (Vrålstad et al., 2014).

Parasites are a heterogeneous group of organisms, which, for simplicity, can be divided into endoparasites (internal parasites) and ectoparasites (on the host surfaces). In a crowded net pen situation, parasitic infections may become epidemics. The gills are preferred organs for many ectoparasites, one example of which is *Paramoeba perurans*, the cause of amoebic gill disease (AGD). Infestations may also be found on the skin surface; examples are *Gyrodactylus salaris* and salmon lice, *Lepeophtheirus salmonis.* Many parasites have complex life cycles that are not compatible with modern aquaculture, and thus transmission can be interrupted by implementation of interventions aimed at vulnerable life cycle stages. For example, problems with the myxozoan parasite *Myxobolus cerebralis* (Sarker et al., 2015), were almost completely abrogated when use of earthern ponds was abandoned, as the life cycle of these parasites is also dependent on another host, tubificid worms, that thrive in earthen ponds.

Infectious agents may be characterised in greater detail than just at the level of species. **Biotypes** are classified by their phenotypic characteristics. This encompasses various characteristics, such as phenotype, morphology, species specificity, behaviour in cell culture etc. **Serotypes** are classified by their antigens using well-characterized antibodies/antisera. For bacteria, the term serovar may be used. **Genotypes** are classified by gene sequences, and related genotype variants may be divided into genogroups. A **subtype** is a term that encompasses any of the above-mentioned characteristics.

Interactions between wild and farmed populations of aquatic animals

One of the major factors constraining fish production is the requirement to minimize potential pathogen exchange with wild salmonids. The current focus is particularly on the transfer of sea lice. The public generally assume that wild fish are ‘healthy’, although it is not clear what the concept includes; in contrast, farmed fish are often popularly viewed as ‘diseased’ (Bergh, 2007). Knowledge on the importance of epidemics among wild fish is limited, especially regarding viral and bacterial diseases. There are several reasons for this, but one important one is that wild fish with diseases are easily predated, but also that there have been few investigations specifically targeting diseases of wild fish (Bergh, 2007). In contrast to the popular view, the available data indicate that diseases among wild fish are common, that epidemics may be of ecological and economic importance, and that diseases occurring among wild fish, as well as those being cultivated, may be associated with reduced welfare. One well-studied example is the salmonid-salmon louse interaction, while two other parasitic pathogens, the monogenean ectoparasite *Gyrodactylus salaris* and the protozoan *Ichthyophonus hoferi,* are known to cause diseases that impact wild fish populations.

Viral haemorrhagic septicaemia virus (VHSV) has been studied in wild fish from many different fish families in several regions of the world, including Norwegian coastal waters (Johansen et al., 2011; Johansen et al., 2013; Sandlund et al., 2014). A large outbreak of VHS affecting many wild fish species is currently occurring in North America, centred on the Great Lakes region of USA and Canada (Al-Hussinee et al., 2011).

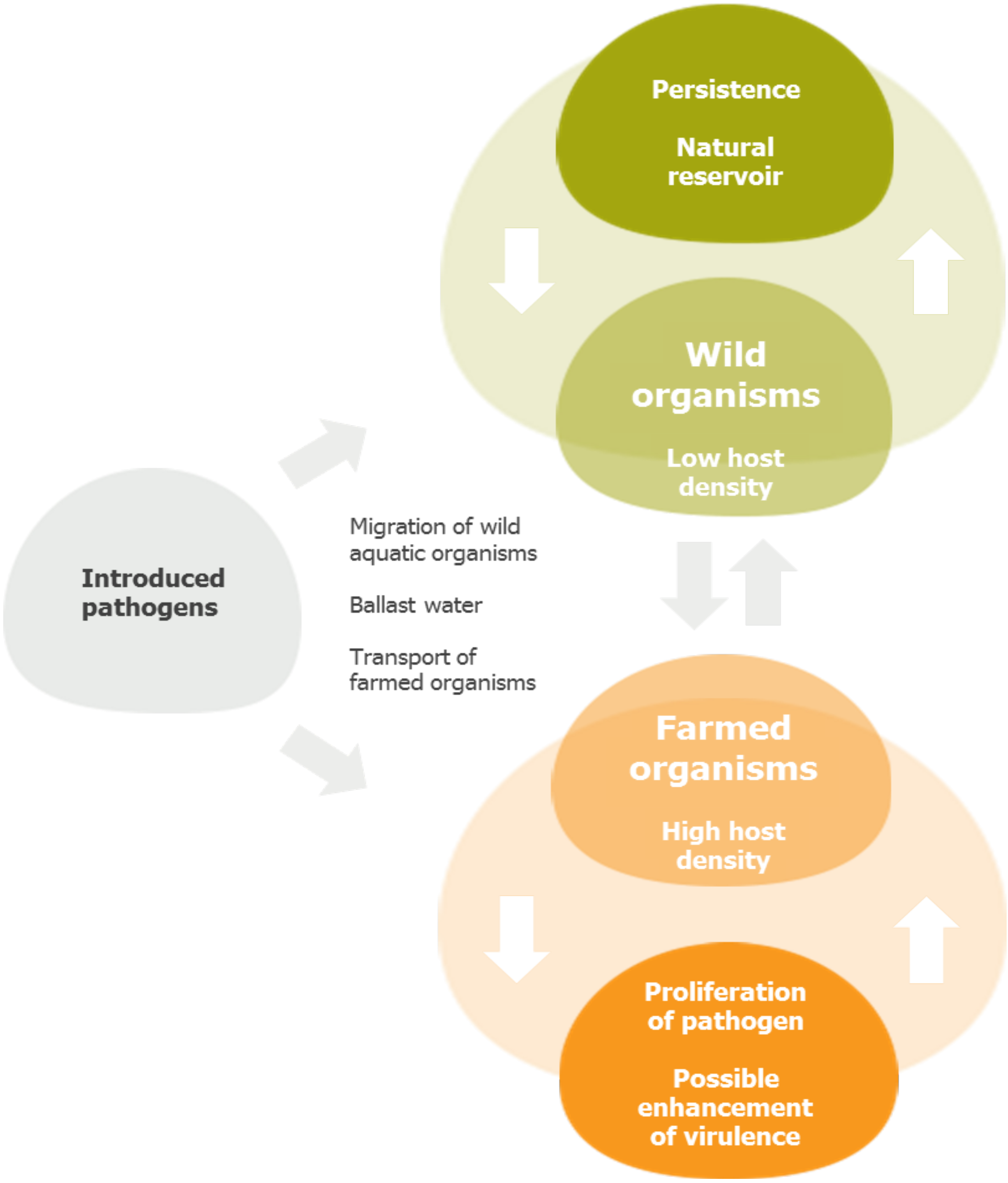

Figure 1: A general overview of the pathogen exchange between farmed and wild aquatic organisms. The figure displays general patterns of movements of pathogens between farmed and wild aquatic organisms in marine environments. Wild organisms are natural reservoirs of all known pathogens, but the reservoirs are not evenly distributed in time and space. Human influences, such as transport of wild and farmed organisms or movement of ballast water, can introduce pathogens to new areas. Natural migration of wild host organisms may also result in the introduction of their associated pathogens to new areas and new hosts. Such introductions may cause serious events, as they can disturb the equilibrium among indigenous host-pathogen relationships due to lack of resistance or because the new intruders lack natural enemies.

Diseases of molluscs and crustaceans

Surveillance and control of diseases in molluscs and crustaceans represent different challenges to those in fish. Only crustaceans belonging to the order Decapoda and molluscs belonging to the class Cephalopoda are included in the Norwegian Animal Welfare Act (LOV-2009-06-19-97, dyrevelferdsloven, Norwegian Ministry of Agriculture and Food), whereas other crustaceans and molluscs are not. Diseases that have caused major problems in marine shellfish farming internationally have generated knowledge and experience on preventive measures that can be used for the protection of the health of shellfish.

Mollusc cultivation is performed in open environments, such as cultivation units where they are suspended in the water, or on ramps, or in bottom cultures. Wild populations often occur within or near the cultivation areas, making differentiation between wild and cultivated stocks difficult. Molluscs feed by filtering particles from the water and are thereby exposed to potential pathogens present in their environments.

Invertebrates have no adaptive immune system and therefore no immunological memory. This excludes vaccination as an option for prevention and control of disease. However, immunostimulants may be a promising option in the future (Karunasagar et al., 2014). Medical treatment would only be feasible in land-based plants, but would not be a relevant strategy with regard to the prevailing diseases experienced by the shellfish industry. As control of diseases already present in mollusc populations is virtually impossible, strategies to prevent introduction and spread of pathogens are central. However, such implementation has proven difficult in European bivalve cultivation, where production is based on transport of different life stages between distinct production areas. The spread of diseases is often associated with the transport of live animals. Dispatch centres, processing large quantities of live bivalves for human consumption, may thus also be crucial points for the introduction and subsequent spread of pathogens.

The production of bivalve molluscs is relatively extensive, usually with a low frequency of farm inspection, resulting in low disease surveillance sensitivity. However, frequent surveillance in hatcheries and land-based plants with juvenile stages would be feasible and would improve surveillance for these production systems.

The shell covering the soft parts of molluscs prevents external detection of signs of disease. Furthermore, the soft parts of dead molluscs disintegrate rapidly, complicating diagnostics and identification of causes of mortality.

Recognising events of abnormal mortality in molluscs is demanding, as considerable changes in their environments, often resulting in high mortality, may occur. Both in commercial hatchery production and in nature, entire batches of larvae, post-larvae, or juveniles may die off, and it is considered normal that only a small proportion of juveniles survive to reach commercial size. Assessment of mortality requires experience with the particular species and area and/or farm, and is a result of a combination of passive and active surveillance. Mortality in molluscs is typically acute and limited in time, and weekly surveillance intervals have been commonly used, in accordance with §12 of the Regulation for the operation of aquaculture establishments (FOR-2008-06-17-822, akvakulturdriftsforskriften, Norwegian Ministry of Trade, Industry and Fisheries).

In general, the same challenges are encountered with crustaceans as with molluscs. Knowledge and experience regarding marine crustaceans is limited, as farming is restricted to a few plants rearing European lobsters (*Homarus gammarus*). Greater experience has been gained with freshwater crayfish, but is limited to a couple of diseases in European crayfish (*Astacus astacus*), mainly in wild stocks.

Answers to the terms of reference

ToR 1

Which factors and properties should be considered and taken into account in the risk assessment of a disease or an infectious agent for the health situation in an aquaculture facility?

The first priority should be to obtain clarification regarding the infectious nature of the disease or agent and establish a clear case definition. Four further important factors that should be considered are: 1) the consequences of the disease or agent for the farmed population 2) the efficiency of transmission; 3) the potential impact on wild populations; and 4) the possibilities for disease control.

Aspects of these various factors are described in detail under different sub-headings below. Molluscs and crustaceans are considered in their own section.

The impact on the farmed population can be estimated by measuring mortality and production losses, as well as by considering fish welfare.

The efficiency of transmission depends on various factors for which data may or may not be available. These include, but are not limited to: the environmental stability of the infectious agent (i.e. ability to stay infectious in the environment), the available transmission routes for the specific agent, the characteristics of the aquaculture facility, and the properties of the fish populations at risk.

The possibilities for disease control depend on having sufficient knowledge about the particular infectious agent. The information required includes its distribution, its physical and chemical (environmental) stability, the availability of effective management measures, the practicality of relevant contingency plans, and the existence of commercially available vaccines, or the possibility of development of such vaccines.

1.1 Transmission efficiency

The infectious nature of the disease

Having sufficient knowledge on the infectious nature of new diseases is particularly challenging. Field observations are important for predicting the epidemic potential of a new disease. Experimental challenges may not be necessary to determine this, but may be suitable to determine whether a disease is contagious or infectious.

Initial investigations of a previously unknown disease should focus on properties, such as mortality, production losses, transmissibility, and pattern of spread. This will require epidemiologic field investigations, identification of the aetiological agent, case definition, and development of diagnostic tests. The causative relationship between agent and disease should be established, as simply detecting a specific agent in a diseased animal does not necessarily prove this relationship. Knowledge on pathogenesis and shedding of infectious agents is also important.

Course of the disease

The consequences of an acute disease are perceived immediately, in contrast to those of a slowly developing, chronic disease. However, from a long-term perspective, the impact of a chronic disease may be worse. Mortality, reduced fish welfare, and production losses are important factors for some of which numerical data can be gathered when assessing the consequences of a disease. Persistence of infection and the dynamics of shedding of the infectious agent in the population at risk and in wild populations are also important factors.

Persistence of infection

An infection becomes persistent within a host organism when the host fails to eliminate the infectious agent. Persistent infections result from modulation of the pathogen's gene expression and of the immune response of the host animal. Such infections may be latent or productive. Stress factors, such as transportation, handling, and hormonal fluctuations may trigger latent infections to become productive. In molluscs and crustaceans, infections will typically become persistent in animals surviving an infection.

Susceptible species and reservoirs of the infectious agent

The **host range** refers to all those species that a pathogen is able to infect. **Species specificity** is another term for the host range. For intracellular pathogens, interactions of surface proteins with cellular receptors are, in most cases, the major determinant for species specificity. A **natural reservoir** is a population in which the pathogen can survive, and be shed from, for an extended period of time. The presence of natural reservoirs relevant for salmonid farming in Norway should never be excluded. However, the probability that an infectious disease will be eradicated is greater when reservoirs outside farms are of negligible size.

Physical and chemical (environmental) stability of the infectious agent

Even for several well-characterised fish pathogens there are only limited data available regarding environmental stability. Knowledge about the effects of temperature, pH, ultraviolet (UV) irradiation, salinity, desiccation, and the effects of common disinfectants should be established and used as guidelines. For agents that are not easy to cultivate under laboratory conditions, it may be difficult to obtain these data.

Properties of the aquaculture facility and management factors

The type of facility, i.e. freshwater or marine facility, land-based or open cages, flow-through or recirculating aquaculture systems (RAS), hatchery, growing, or brood stock facility, will impact on the risk of spread of an infectious agent. The location of the facility is also important, i.e., whether it is situated close to other facilities or remotely sited, hydrographic and -dynamic conditions, water catchment areas, etc. Fish are particularly vulnerable to diseases during and shortly after stressful events, such as routine handling for grading and parasitic treatment.

Interactions with, and properties of, other populations

This is elaborated in the answer to ToR 8.

1.2 Consequences for the farmed population

Increased mortality

Mortality is a central measurable feature when assessing the consequences of a disease. The method by which mortality is measured should be standardised so that results are comparable between aquaculture facilities. Sufficient resources should be made available to identify the causes of increased or unexpected mortality as soon as possible after the increase is noticed. Establishing whether the cause of mortality is infectious or contagious is pivotal to management decisions. Experimental challenge may provide useful information.

Mortality in aquaculture varies between fish species, developmental stages, environmental conditions, and technological development and management of production. For instance, the mortality of larvae of marine fish species would be expected to be higher than for salmonid alevins. This is because larvae of marine species hatch at an earlier developmental stage. A general mortality threshold or specified acceptable proportion for mortality for all cultivated fish species may therefore not be suitable in disease assessment. Fluctuations or increases in mortality are often more informative.

New fish species are regularly being brought into aquaculture. However, there are wide variations in both biological knowledge and the technical platforms used in the aquaculture industries. The threshold for "normal" or “acceptable” mortality for a new species must be established and based upon “best management practice”. Mortality must be used with caution as a measurement of the infectivity and pathogenicity of a disease, and awareness of its limitations as a measure of both these characteristics should be understood.

The recommendation from the NFSA is that a mortality >0.5 ‰ per day for fish below 0.5 kg and 0.25 ‰ per day for fish above 0.5 kg can be used as a rule of thumb threshold for increased/unacceptable mortality in Norwegian salmonid farming. This threshold is well established within the industry and functions according to intention for salmonids. Higher mortality rates require further investigation and explanation. Similar guidelines could be suitable also for other fish species, taking into account both species characteristics and best management practice.

Reduced welfare

Reduced fish welfare should be considered when assessing the impact of an infectious disease. Neither survival nor unaffected production is the same as good welfare. Reduced fish welfare, for example, ulcers, blindness, or experiencing pain, may not necessarily cause mortality or production losses. Moreover, both the intensity and duration of the condition should be taken into account. Animal welfare is defined as the state of each individual, but often has to be measured at the farm level. Average values can mask individual suffering.

Good welfare indictors should reflect the actual state of welfare, be reliable, and be feasible to measure. Validated welfare indicators in fish are currently lacking, but some indicators have been suggested. Such indicators may be directly evaluated from the fish, whereas as others may involve measurements of environmental factors (Rosten et al., 2007). Systems for overall welfare assessments of farmed fish have also been developed. One salmon welfare index model (Pettersen et al., 2014) uses the condition of the eyes, heart, abdominal organs, gills, opercula, skeletal muscles, vaccine-related pathology, aberrant fish, necropsy of the dead fish, and active euthanasia. Another model mainly makes use of environmental measurements, and is intended for welfare assessment and control by farmers themselves. In this model the factors to be considered include: water temperature, salinity, oxygen saturation, water current, stocking density, lighting, daily mortality rate, appetite, sea lice infestation ratio, condition factor, emaciation state, vertebral deformation, maturation stage, smoltification state, and fin and skin condition (Stien et al., 2013). A system for on-farm surveillance of environmental parameters, including an evaluation of the findings regarding fish welfare, has been developed (Welfaremeter, http://www.imr.no/welfaremeter/index.htm).

Production losses

Examples of production losses that are not necessarily related to increased mortality are reduced growth and quality downgrading. Such conditions affect productivity biomass and profitability. In some disease situations, mortalities may be low, but production losses due to reduced growth may be significant. One example of downgrading is quality reduction due to melanisation of white muscle.

1.3 Possibilities for disease control

Possibilities for disease control through biosecurity measures, including vaccination, are elaborated in the answer to ToR 2.

1.4 Special considerations for molluscs and crustaceans

In general, the same factors must be considered for molluscs and crustaceans as considered for fish. However, it is important to be aware that therapeutic medication and vaccination are not applicable. The biology, including the life cycles, of such animals is also of central importance.

Mortality should be linked to a particular infection, where the virulence of the pathogen should be known. Knowledge on causality is, in most cases, based on epidemiological and experimental studies. However, disease development is often influenced by environmental factors like salinity and temperature, and by the physiological and reproductive status of the host animals. Therefore, the course of a disease in a population or area after detection of a specific pathogen may be difficult to predict. Basic knowledge about the relevant pathogens is crucial, as it is for fish pathogens.

Knowledge of the host range of the specific pathogens being considered is important. If there are wild populations of molluscs or crustaceans in or near the farming areas, the possible establishment of reservoirs must be considered. Such reservoir populations may lead to re-infection of new batches of farmed animals, as well as further amplification and spread of an infectious agent.

When assessing the risk of spread of a disease, the transmission efficiency of the relevant pathogens should be considered. Spread is linked to the stability of the pathogen in the environment or in the pelagic life stage of the host. It is important to note that the host ranges and life cycles of some parasites of bivalves are not fully known (like *Marteilia* spp. in mussels and oysters). This makes assessment of spread difficult, if not impossible.

ToR 2

Which factors and properties are significant for the control of a disease or an infectious agent in a fish farm or mollusc farming area?

Three factors, transmission efficiency, the available possibilities for control of the disease or infectious agent, and diagnostic criteria are significant for control. These are considered separately below.

2.1 Transmission efficiency

Preventing shedding and interrupting transmission are both crucial in the control of a disease. This requires detailed knowledge about the factors described in greater detail in the answer to ToR 1, such as physical and chemical (environmental) stability of the infectious agent, pathogenesis of the disease, persistence of infection, routes of transmission, and duration of shedding. This information should be made readily available for the known and common diseases of aquaculture. The host range of the infectious agent and reservoirs of infectious agents in wild populations are particularly important. Although aquaculture populations often function as sentinels, an agent may be present in wild aquatic animals without obvious effects on farmed populations.

2.2 Possibilities for disease control

Biosecurity

Intensive aquaculture farming can lead to accumulation of infectious agents. Therefore, biosecurity measures that minimize the risk of introduction and spread of infectious agents should be in place, as well as routine to ensure correct implementation of such measures (Gudding et al., 2015; Lillehaug et al., 2015).

Fallowing ensures that fish aquaculture facilities are emptied, cleaned, and disinfected between cohorts of fish. Regular fallowing can therefore limit or moderate the accumulation of infectious agents. Coordinated fallowing should be based on knowledge and experience regarding spread and environmental stability of the infectious agent. Reservoirs in wild fish may reduce the intended effects of fallowing. Wild reservoirs are of special importance in mollusc farming areas, as fallowing would require emptying an area of host animals.

In order to be effective, disinfectants must be used properly. The efficiency of disinfection is influenced by: concentration of active agent, duration of exposure, temperature, and access of the disinfectant to the area to be cleaned. The effect of disinfectants is reduced by biological material. Therefore, surfaces covered with layers of biological material, or cracks and crevices should be mechanically cleaned prior to application of the disinfectant.

Vaccination

For any specific disease, the possibility of control by vaccination, and whether vaccines are available, or could be developed, should be assessed. There are several examples of successful disease control through vaccination in Norwegian salmonid aquaculture; examples are vaccination against furunculosis, vibriosis, and cold-water vibriosis. Vaccination success depends upon protection efficiency of the vaccine and also the cost, including for delivery. Vaccines with only a moderate protective effect may nevertheless be useful by reducing mortality, shedding, production loss, and the duration of outbreaks.

Vaccination is not applicable in molluscs and crustaceans. However, immunostimulation may become a useful tool in the future for moderating diseases in crustaceans (Karunasagar et al., 2014).

2.3 Diagnostic criteria

Defined criteria for specific disease diagnosis, i.e., case definitions, are vital for disease control. Criteria should be determined by national reference laboratories and be available for other diagnostic laboratories. Pathological and, preferably, agent-specific criteria should be included. In many cases the presence of an infectious agent may be strongly suspected, but the pathogen is not immediately identified and characterized.

The characteristics of particular disease agents may change over time and, in order to reflect this, the setting of criteria should be dynamic and incorporate guidelines for regular re-evaluation.

ToR 3

Which factors and properties of a disease or an infectious agent:

a. ...are significant for achieving and maintaining areas that are free of that disease or of that infectious agent?

3a.1 Transmission efficiency

Many of the aspects outlined in the answers to the preceding questions also apply here. It must, however, be emphasised that knowledge on patterns of shedding, environmental stability, and pathogenesis is crucial for interrupting transmission. Additionally, in order to achieve and maintain areas free of disease and of infectious agent, it is important to map the routes of transmission within the aquaculture industry. These are partially determined by the structure of the industry, i.e. transport and import of live material, the use of well boats, sea current speed and direction, farm density, fish density, susceptibility of farmed species, and more. A geographically widespread agent has better chances for further spread than an agent with restricted local occurrence.

Reservoir of infectious agent

Reservoirs within farmed or wild aquatic animals will hinder the possibility of achieving and maintaining disease-free or pathogen-free areas.

3a.2 Possibilities for disease control

Vaccination

Vaccines that protects against a disease may be a significant advantage for keeping areas free of that disease. However, if vaccinated individuals are sub-clinically infected they may also shed the agent. Thus, vaccination may keep an area free of disease, but not free of the infectious agent. Information regarding whether a vaccine can be used to keep an area free of infectious agent or only free of disease should be provided for commercially available vaccines. Vaccination is not applicable in molluscs and crustaceans.

3a.3 Diagnostic criteria

This is elaborated in the answer to ToR 2.

b. **...indicate that it would be expedient to achieve and maintain areas free of the infectious agent, given the feasibility of achieving and maintaining such areas?**

3b.1 Transmission efficiency

Transmission efficiency is influenced by several factors including: the speed and direction of sea currents, farm density, fish density, susceptibility of farmed species, reservoirs of the infectious agent, and more. The size and geographical distribution of farmed salmonids will, in most cases, mean that the aquaculture industry itself is the most comprehensive reservoir for pathogens of salmonid fish. However, reservoirs of infectious agents in wild populations are more difficult, or impossible, to control, and may be particularly important as sources for introduction or re-infection. Challenge experiments may provide information on the possible species range of certain infectious agents.

When eggs and fish are transported through, or imported to, an area there will be a risk for introduction of any pathogens that they harbour. Therefore live material brought into the area should only originate from zones with equal or better disease status. Equipment that may be shared with facilities outside the area, such as well boats and equipment used for delousing and AGD treatment, may represent an additional risk.

c. **...indicate that it would be expedient to achieve and maintain areas free of the disease, given the feasibility of achieving and maintaining such areas? Areas free of disease are in this context defined as areas free from outbreaks of the disease, but not necessarily free of the infectious agent.**

3c.1 Development of disease.

Clinical disease is a result of a multitude of factors related to the agent, host, and environment, including management. Susceptibility to disease may, for example, be dependent on developmental stage and size of the fish and not only related to factors and properties of the disease or infectious agent. Many infectious agents do not always cause disease.

Susceptible species and reservoirs of the infectious agent

Determination of susceptibility to disease is important in order to be able to assess whether it is expedient to achieve and maintain areas free of the disease. For example, if rainbow trout were introduced into an area with an ongoing outbreak of infectious salmon anaemia (ISA), the rainbow trout would not become diseased, but they would become infected and would contribute to propagation of the virus and thus exacerbate the risk of further spread.

Furthermore, many of the agents that cause disease in aquaculture are capable of adapting to, and causing diseases in, new host species.

In conclusion, apart from diseases where vaccination is an efficient preventive measure, there are no particular factors and properties of a disease or an infectious agent that would make it expedient to achieve and maintain areas free of the disease, given that the area is not free of the infectious agent.

ToR 4

Which information should be available for the authorities to assess whether:

a. ...a disease is infectious?

Epidemiological field data should be collected and analysed in order to obtain insights on the transmissibility of a particular disease. The results should be verified by experimental challenge. However, it should be noted that for a number of well-characterised infectious diseases of salmon, experimental challenge models have often failed to reproduce the mortality observed in field. Experimental challenge studies may also be hampered by difficulties in cultivating and purifying disease-causing agents, making it necessary to use field isolates for the challenge experiments.

b. ...a disease can be diagnosed based on the presence of infectious agent or of pathological findings?

It is important that diagnostic systems are reliable, rapid, have adequate sensitivity and specificity, and are subject to regular quality control. This requires that the criteria used for disease characterisation and identification are standardized. Uncertainty and differences in the interpretation of the diagnostic criteria will reduce diagnostic sensitivity and specificity.

For new diseases, macro- and histopathological examination, together with clinical signs, will be important for early disease characterisation, although may be of low diagnostic specificity. There is considerable experience with this approach in Norway.

Other techniques used in combination with histopathological examination, i.e. gross observation and detection of agent-specific antigens or nucleic acids, will substantially improve the specificity. It may be possible to make the diagnosis without detecting the agent itself.

Screening for the presence of pathogens by qPCR is another health management practice that often focuses on detection of specific pathogens in sub-clinically infected fish. It is beneficial to detect a pathogen prior to, or early in, disease development. The laboratory criteria used for detection of agents should be validated for detecting virulent variants of the agent. For instance, detection of non-virulent ISAV-HPR0 should be clearly distinguished from detection of virulent ISAV-HPRΔ.

Whether detection of a well-known pathogen in sub-clinically infected animals justifies the same response as detection of clinical disease should be assessed on a case-by-case basis, and based on the drawbacks of the particular infection.

ToR 5

Which factors and properties of a disease or an infectious agent are significant for the risk of introduction of that infection due to transport or sale of live aquatic animals given that:

a. ...there is suspicion or confirmation of the presence of an infectious agent in these aquatic animals in a fish farm or mollusc farming area, but without clinical signs of disease.

Transport of live animals inevitably increases the possibility of disease introduction. All aquatic animals are susceptible to opportunistic infections that may establish when immunity has been decreased, which often occurs as a result of stress. Transport of live animals is a relevant stressor and therefore increases the probability for disease development. ToR 5 is interpreted as referring to animals to be moved that are suspected to be, or confirmed as, infected. The use of the term "suspicion" in the present report should be viewed according to the definition applied by the national reference laboratories.

5a.1 Transmission efficiency

Transmission routes and duration of shedding of infectious agents are discussed under the answers to ToR 3. Transport of live animals imposes stress on these animals and thus increases the possibility of reactivation, and subsequent shedding, of an infectious agent. Thus, careful handling during transport is a necessary precaution to minimize stress.

Persistence of infection

Many infections in farmed fish may become persistent. This persistence may continue for a period, or may even be lifelong. Lifelong persistence is particularly true for several viral and bacterial infections in fish, when considering a population and not the particular individual. However, the various infections differ in this respect. This means that it could be meaningless to differentiate between populations in which animals show clinical signs of disease and populations that do not.

Physical and chemical (environmental) stability of the infectious agent

This is elaborated in the answer to ToR 1.

Properties of the aquaculture facility and management factors

Many infectious agents that are present in aquaculture do not always cause disease. Non-infectious factors, such as management, density of farms, infectious pressure, subtype of the agent etc. are determinative of the outcome of the infection. Many infections can be considered as ubiquitous in Norwegian farming areas and are likely to be already present in the area to where the aquatic animals are to be moved.

Interactions with and properties of other populations

Persistence of infectious agents in wild populations is important, but often not well understood. See answer to ToR 8 for details.

Susceptible species and reservoirs of the infectious agent

If there is suspicion of the presence of an infectious agent in the animals to be moved, confirmation of the infectious status should be obtained. The diagnostic procedure should be clear and robust, although this may slow down the speed of obtaining a diagnostic confirmation. There should be continuous discussion regarding whether “diagnostics” should include clinical and pathological diagnostics, or should be restricted to detection of a specific agent. Which is most appropriate will depend upon agent and disease properties.

Susceptibility to the disease of the aquatic animal species to be moved and of the farmed species in the recipient area is of importance. Some fish species may be prone to both infection and development of a particular disease, while other species may only develop sub-clinical infection and act only as carriers of the infection. One example is infectious salmon anaemia, to which Atlantic salmon are susceptible to both infection and disease, whereas, under farming conditions, rainbow trout are only susceptible to infection. Another example is viral haemorrhagic septicaemia, to which susceptibility to disease is lower in Atlantic salmon than in rainbow trout (King et al., 2001).

OIE lists the susceptibilities of different aquatic animal species to various agents (OIE Aquatic Animal Health Code, Section 10 and 11), compiled in a table by the EU commission (Part II of Annex IV to Council Directive 2006/88/EC). However, these lists should be interpreted with caution, and the reference literature used should be thoroughly examined. The OIE lists aim to cover diseases that are notifiable, and thus many infectious agents that are present in Norwegian aquaculture do not appear on these lists. The susceptibility to infection and disease development for aquatic animal species that have not been traditionally farmed, for instance various species of cleaner fish such as wrasse, have not been studied particularly well. Due to the lack of information, transport of such fish should probably only be undertaken after an appropriate level of risk assessment.

5a.2 Molluscs and crustaceans

Molluscs are frequently reared in environments where wild molluscs are numerous. Eradication of an introduced pathogenic agent in such a situation will be practically impossible. If the farmed molluscs are removed, wild specimens will still be present in the farming environment, and will act as reservoirs. The lack of an acquired immune response in invertebrates means that these animals become persistently infected. In most cases, pathogens introduced in a stock, population, or production area will become established in the molluscs, even if the animal do not display clinical signs of disease. In principle, when an infectious agent is present in an area, then healthy animals must be considered as carriers or reservoirs.

b...there is a suspicion or a confirmation of the presence of an infectious agent in these aquatic animals in parts of a fish farm or a mollusc farming area, but where the animals to be moved are clinically healthy and without present or prior clinical signs of disease?

The only difference from ToR 5a, is that it is not the animals to be moved that are infected, but other groups of animals from the same farm/farming area. If this is just a suspicion, the first priority is to try to obtain confirmation about the infectious status of the suspected animals.

The same factors as in ToR 5a will be valid. Even if the animals to be moved are healthy, they are originating from an area where they may have been infected. The risk of them being infected carriers that may transport the infection with them will depend upon the factors mentioned in ToR 5a.

c...a disease has been confirmed in a farm for these aquatic animals or in a mollusc farming area, but the disease outbreak has terminated and the animals to be moved or sold are currently healthy.

The only difference from ToR 5b, is that the infection is presumably confirmed (not just a suspicion) and has resulted in a disease outbreak in the area that has now terminated. The first priority is to try to obtain confirmation about the infectious status of the relevant animals.

The same factors as in ToR 5a and b will be valid. If a fish population has been infected, the population should generally be regarded as being persistently infected.

ToR 6

Which factors and properties of a disease or an infectious agent are significant for risk of infection when aquatic animals are used for breeding and reproduction given that:

a. ...there is suspicion or confirmation of the presence of an infectious agent in these aquatic animals in a fish farm or mollusc farming area, but without clinical signs of disease.

Many of the factors mentioned in the answers to the previous ToRs are also valid and relevant here. Of particular importance are:

6a.1 Transmission efficiency

Whether vertical transmission is known to occur for the specific infectious agent is of particular importance for this question. In the 11th edition of the Aquatic Animal Health Code (2008), OIE uses the following definition of vertical transmission: “Vertical transmission means the transmission of a pathogen from a parent aquatic animal to its progeny via its sexual products”. This definition includes vertical transmission where the pathogen is inside the egg after fertilization (e.g., infectious pancreatic necrosis (IPN) and bacterial kidney disease (BKD)), and transmission due to the pathogen being attached to the outside of the fertilized egg.

Pathogens present and able to attach to the outside of the egg originate from infected brood stock or from contamination. The probability of contamination will be influenced by various factors, such as the load of agent in brood fish and the infected organ or tissue; if the infectious agent is in blood or in the surface mucus, the risk of contamination of sexual products will be higher than if the agent is restricted to internal organs. The pathogen load in an individual will further depend on the infectious phase, and shedding of agents may be high before clinical signs become apparent. If infection is present, the presence or absence of clinical signs is of minor importance regarding the risk of vertical transmission.

Under aquaculture conditions, the eggs from salmonids are disinfected by use of iodophore (100 ppm, 10 min) following fertilization. This disinfection regime is often repeated at the eyed egg stage, before the eggs are shipped to hatcheries. Disinfection will not be effective against agents inside the egg and vertical transmission cannot be prevented unless the brood stock is free of the infection. The likelihood that an infectious agent is transferred vertically by attachment to the outside of the fertilized egg depends upon a variety of factors. Strict biosecurity measures are essential to ensure a specific pathogen-free environment, and disruptions or inadequate performance of routines, such as the mandatory disinfection process (§11 of the Regulation on the operation of aquaculture establishments, akvakulturdriftsforskriften, Norwegian Ministry of Trade, Industry and Fisheries), will enable further spread of a pathogen in the population during reproduction. Some fish pathogens, such as *Flavobacterium psychrophilum*, are known to form strong biofilms on the surfaces of the salmonid eggs (Sundell and Wiklund, 2011). The recommended disinfection procedures may be insufficient to prevent vertical transmission of this bacterium.

Physical and chemical (environmental) stability of the infectious agent

The equivalent responses under ToR 1 are relevant here. Even for several well-known fish pathogens only rudimentary data are currently available regarding environmental stability. Basic knowledge about the effects of temperature, pH, UV, salinity, desiccation, and the effects of common disinfectants should be established and used as guidelines. In order to obtain these data, it is generally necessary that the agent can be cultivated in the laboratory.

Properties of the aquaculture facility and management factors

Factors such as management, density of farms, infection pressure, variant of the agent, are determinative for the risk for vertical transmission. Regularly biosecurity audits by the competent authorities of sites and harvest vessels can be important to ensure good management practice.

b. ...there is a suspicion or a confirmation of the presence of an infectious agent in these aquatic animals in parts of a fish farm or a mollusc farming area, but where the animals to be used for breeding and reproduction are clinically healthy and without present or prior clinical signs of disease?

This question differs from ToR 6a, in that the animals to be used for breeding and reproduction are historically healthy individuals, but nevertheless belong to the same farm/farming area where infection is suspected or demonstrated to be present. All susceptible individuals in such a farm should be regarded as being infected provided that the farm has not been divided into areas with strict biosecure separation prior to infection. Historic freedom, based on adequate surveillance, i.e., testing and clinical signs, is only valid if the risk of introduction over time has been documented to be negligible. All aquatic animals in an open farming area should be regarded as infected, even if infection has only been confirmed in animals in sub-areas.

The same factors as mentioned in the answer to ToR 6a will also be valid.

c. ...a disease has been confirmed in a fish farm for these aquatic animals or mollusc farming area, but the disease outbreak has terminated and the animals to be used for breeding and reproduction are currently healthy.

The only difference from ToR 6b, is that the infection has resulted in a disease outbreak in the area that has now terminated.

For many infections in fish, survivors become sub-clinically infected carriers for an extended period of time, and even lifelong, i.e. IPNV. The characteristics of the pathogen and the hosts' response that result in such persistence are mostly unknown, and can only be a matter of speculation. The lack of class-switch in the antibody response in fish, which is known to be important for affinity maturation in the mammalian antibody response, is an example of such speculation. In a population of animals surviving an outbreak of an infectious disease, it should be assumed that some animals will be carriers. Generally, the pathogen load is low in healthy carriers and therefore difficult to detect by laboratory testing. The load of infectious agent will normally increase during stress or hormonal fluctuations, both of which will be relevant during sexual maturation and stripping. The probability of finding fish that are positive for the infectious agent may then increase, as will the probability of transmission during the reproduction process.

As mentioned in answers to preceding ToRs, crustaceans and molluscs have no adaptive immune system and therefore no immunological memory. Survivors should be regarded as lifelong carriers.

The same factors as mentioned in ToR 6a and 6b will be valid.

ToR 7

Which factors and properties of a disease or an infectious agent are significant for reducing the risk of spread given that:

a. **...all the animals in the farm or mollusc farming area are slaughtered immediately?**

7a.1 Transmission efficiency

Physical and chemical (environmental) stability of the infectious agent

The physical and chemical stability of infectious agent and factors affecting the transport of infectious agents through the water column will affect the risk of spread. See answer to ToR 1 for further details.

Properties of the aquaculture facility and management factors

In addition to properties of the disease and the infectious agent, other factors, such as how the fish are slaughtered and transport of the fish in connection with the slaughtering, processing, or destruction, are very important regarding the risk of spread. Well boats operate in national and international markets and interchangeably carry smolts and fish for slaughter. Disinfection between shipments, which is mandatory, reduces the risk of spread. The use of shared waiting pens in slaughterhouses, instead of on-shore waiting tanks from which the effluent water is disinfected, is a possible risk factor for spread.

Susceptible species and reservoirs of the infectious agent

Host specificity and proximity to susceptible wild and farmed hosts are important in terms of possible likelihood of spread and establishment of new reservoirs of infection in farmed or wild populations. Population size and the potential for interactions with these populations will also be important factors. The properties of the fish farm (marine/fresh water, open or closed system, RAS) and size of the farm are likely to influence the interactions. In marine fish farming, establishment of a reservoir in the farmed population has been shown to be the most important factor for several viral diseases (e.g., SAV, ISA). However, for other diseases, such as typical furunculosis, the reservoirs are probably much more complicated. For molluscs, there will often be a close interaction between farmed populations and wild populations.

7a.2 Diagnosis and disease development

Rapid diagnosis, decision-making, and slaughtering after the population has been infected are important factors in the prevention of further spread of an infection. Capabilities regarding detection and diagnosis of a disease will be important factors to consider in reducing the period between introduction of infection and slaughter. Disease development and production of infectious agent will influence the spread of the infectious agent before slaughter. Temperature and stress are two important factors that will also affect this. Chronic diseases can sometimes be difficult to detect and the spread of infectious agents may have occurred over an extended period before the disease is diagnosed. For many viral diseases maximal production of infectious virus particles occurs before disease onset (e.g., SAV). Some vaccines can decrease virus excretion, despite having only limited efficacy regarding reducing mortality.

b. ...only units with aquatic animals with clinical signs of disease are slaughtered?

If animals in an aquaculture facility are diagnosed with a disease, the whole facility must be regarded as infected. This applies even if animals in the units are subsequently tested as being negative for the disease. If the units have separate water supplies and are physically separated, slaughter of units of animals with clinical signs may reduce the risk of spreading.

There are indications that some individual fish that survive certain virus infections can be cleared of the virus. However, as a rule, an infection that starts in a cage will spread to the whole farm over time, and fish in various stages of the disease will be found. These fish shed virus, and, as previously noted, some recently infected fish may shed more virus than clinically diseased fish.

c. ...individual farms are fallowed?

This is a more stringent reaction than those outlined in 7a and 7b. When this approach is used, the aim should be to eradicate the infectious agent from the farming area. Success of this strategy will depend upon the host range and the environmental stability of the infectious agent.

Physical and chemical (environmental) stability, as well as susceptible species and reservoirs of the infectious agent, are of importance. In order to eradicate an infectious agent and reduce the possibilities for spreading, the duration of fallowing is important. This will depend on the environmental stability of the infectious agent, which can vary. Temperature, salinity, and geographical features of the site are factors that should be considered as having a possible impact. Many fish pathogens survive for longer at low temperatures and in environments with a high organic load.

The possibilities for spread will depend on the proximity and interactions with other farmed or wild populations.

d. ...all the farms in a zone are fallowed in a coordinated manner?

This approach has proven to be very effective in the eradication and prevention of spread of many fish pathogens. The strategy has been used to combat ISA in the Faroe Islands, Chile, Norway, and Scotland. In addition, it has been used in the eradication of VHS in Danish freshwater aquaculture. In coordinated fallowing, both the infected population and the possibly infected farmed populations in a defined area are eliminated. The outcome will depend on the efficacy of fallowing and the possibility of reservoirs of infection establishing in the wild fauna.

ToR 8

Which factors or properties are significant for a disease or an infectious agent to pose a threat to wild aquatic animals if left uncontrolled or contained at a low level?

A threat is regarded as the potential ability of the disease or infectious agent to have a negative influence on the population size and/or the potential for survival, including reproduction, of wild populations. Infection among wild fish is common, but the consequences are mostly unknown. This is due to lack of data, especially regarding viral and bacterial infections, and few investigations specifically target diseases of wild fish. Wild fish that are weakened due to disease are more easily predated. In general, the higher the load of infectious agents spread from a farming site, the greater the risk. The consequences will depend upon the accessibility of wild host organisms to the specific agents, which may vary with season, location etc.

8.1 Transmission efficiency

Transmission efficiency, as previously outlined, depends upon the infectivity of the pathogen, its environmental stability, as well as host range and reservoir. For wild animals, transmission efficiency is also influenced by migration of the hosts.

Susceptible species and reservoirs of the infectious agent

A key factor regarding wild aquatic animals is the range of susceptible species and reservoirs of the infectious agent. If the reservoir is in wild animals, the presence of the disease and the infectious agent, or the infectious agent alone, must be considered as a natural phenomenon. Apart from eradicating host populations, the means to control such a pathogen are very limited. It should be noted that disease is not necessarily related to the presence of a reservoir. If, however, the reservoir is in farmed animals, it is possible to apply several control measures in order to avoid or minimise transfer to wild animals. These measures could include: vaccination, treatment of affected animals/populations, control of sources of juveniles, and restricted transport of biological material.

Interactions with, and properties of, other populations

Pathogens associated with animals that are farmed and then released into the wild may constitute a great risk for further transfer to wild organisms. For example, the release of Baltic salmon infested with *Gyrodactylus salaris* in Norway had a significant impact on several wild salmon stocks (Scholz, 1999). As released organisms may have extensive contact with wild populations, stricter control measures are recommended for these organisms compared with those reared exclusively for farming.

Pathogens associated with animals kept for food produced for in farms may constitute a smaller risk for transfer of disease to wild or to other cultured populations. Nevertheless, there are several examples of important epidemics caused by pathogens introduced in this way. The most important case in Norway may be the re-introduction of *Aeromonas salmonicida* subsp *salmonicida,* the causative agent of furunculosis, to farms, and possibly further to wild salmon stocks (Håstein and Poppe, 1986). It is also important to keep in mind that escapees from farms producing fish for food might come into even closer contact with wild organisms and thereby increase the risk of transfer of pathogens.

Molluscs are often farmed in areas where there are wild stocks of the same species. Pathogenic agents will therefore be readily transmitted between farmed and wild animals.

Physical and chemical (environmental) stability of the infectious agent

This is elaborated in the answer to ToR 1.

8.2 Consequences for the population

The consequences of the disease on the wild population, including mortality, reduced welfare, degradation of fish quality, or a negative impact on reproduction, are all highly relevant factors. These are further elaborated, although with specific focus on farmed populations, in the answer to ToR 1. Data on disease consequences in wild populations are scarce.

ToR 9

Are there any other factors or properties of a disease or an infectious agent that have not been mentioned in Questions 1 to 8, and that should be taken into consideration when assessing whether a disease should be listed?

In the event of an outbreak of a previously unknown disease, there will be a lack of basic knowledge regarding, for example, the epidemiology, the short-term and long-term consequences, and many other important factors. This lack of knowledge could justify that for new diseases there is a compensatory lower threshold for intervention and listing by fish health authorities.

An important factor that should be noted is the adaptation of pathogenic organisms to the farming environment. Such adaptation is a continuous process and the large number of available hosts will select for those agents that spread most easily. There is frequently an association between virulence, rapid propagation, and shedding. The large number of susceptible hosts, as occurs on farms, may thus gradually select for those pathogens that are more virulent. An equivalent situation has been observed for some agents in poultry farming.

The Norwegian fish health hearing experts were requested by the VKM Working Group to present their opinions on the responses to the questions from the NFSA regarding the health status at aquaculture facilities. The experts highlighted regional differences in competence and judicial assessment within the supervisory and regulatory authorities as being particularly challenging. It would be advantageous to establish a systematic approach to the use of judicial assessment. Furthermore, databases of causes of death and real-time descriptions of disease outbreaks, updated on a weekly basis, could also prove to be valuable.

Conclusions

The VKM highlights the following principal factors for evaluation when considering whether a disease should be listed:

- the infectious nature of the disease, including transmission efficiency
- the consequences of the disease for farmed and wild aquatic animal populations
- the feasible possibilities for disease control

Knowledge about the infectious nature of a disease is particularly challenging for previously unknown diseases. Field observations are therefore particularly valuable in the early phases of such outbreaks and should be considered as a valid means for assessing the epidemiology of the infection at this stage. The knowledge gap associated with new diseases may justify a compensatory lower threshold for intervention and listing by fish health authorities. In the long run, prevention would have a more positive impact than treatment; for fish, the latter option is mainly associated with damage control. The infectious nature of the disease should be clarified at an early stage by experimental challenge experiments that simulate the natural situation.

New species used in commercial fish farming, for example cleaner fish such as wrasse, constitute a challenge regarding lack of knowledge on the relevant infectious agents, diagnostic criteria, transmission, and reservoirs.

Transmission efficiency will be influenced by the course of the disease, the persistence of the infection in fish hosts, the range of susceptible species and reservoirs, as well as the environmental stability of the infectious agent, the properties and management of the implicated aquaculture facilities, and interactions between farmed and wild populations. It is not possible to single out which of these factors that will be most important for a particular agent, and this may vary on a case-by-case basis, even for the same agent.

The relevant consequences of the disease to consider in the aquatic animal populations may be related to increased mortality or negative impacts on animal welfare, in addition to production losses in farmed populations. Economic consequences alone would not be suitable for evaluating whether a disease should be listed, as the market price levels fluctuate constantly. It is generally more difficult to assess the consequences of disease in wild populations than in farmed fish This is further complicated for molluscs by the animals being farmed in areas where there are wild stocks of the same species.

Possibilities for disease control will mostly depend upon the success of hygiene and sanitary measures, and, if available, upon vaccination. Whether hygiene and sanitary measures are effective will be affected by the range and proximity of reservoirs, routes of transmission, and the environmental stability of the infectious agent. Vaccination is not possible in molluscs and crustaceans.

It is important to keep in mind that once infection has been confirmed in a population, this population should be regarded as permanently harbouring this particular agent, regardless of future clinical status and lack of detection of the agent.

Uncertainties and data gaps

Literature search and assessment of criteria

Scientific literature or guidelines directly related to the assessment of criteria for listing of diseases are scarce, almost non-existent. This has represented a methodological challenge in writing this Opinion.

Infectious agents

There are considerable data gaps related to the environmental stability of infectious agents, as well as of the reservoirs and life cycles of pathogens of molluscs. Improvements in our knowledge on these factors could affect some of the statements of the present report related to transmission of infectious agents.

Welfare parameters

Assessment of the welfare of farmed fish is still frequently assessed solely on the basis of mortality rates and production parameters, both of which are very coarse indicators. However, a welfare assessment should include an assessment of morphology and health conditions, measurements of physiological stress and behaviour, and, ideally, indicate the affective state of the fish. With more knowledge within this field, currently unknown consequences of outbreaks of infectious diseases in aquatic animals might be recognised.

Very little is known regarding decapod crustaceans. In contrast to vertebrates and decapod crustaceans, bivalves are not thought of as sentient animals and are not protected by the Norwegian Animal Welfare Act.

The intrinsic amorality of nature means that it is difficult to assess the consequences of the spread of infectious agents on the welfare of wild fish, and wildlife in general. For example, what is bad for one species may be considered as beneficial for its predator.

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