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SCIENTIFIC OPINION

Viral haemorrhagic septicaemia virus, infectious haematopoietic necrosis virus and HPR-deleted infectious salmon anaemia virus – Risk of introduction in free areas through fertilised eggs and gametes

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The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts.

Abstract

EFSA assessed the risk of introducing viral haemorrhagic septicaemia virus (VHSV), infectious haematopoietic necrosis virus (IHNV) and highly polymorphic region-deleted infectious salmon anaemia virus (HPR-deleted ISAV) into free areas through movement of fertilised eggs and gametes. No evidence of true vertical transmission of these viruses was found. However, viral contamination of the surface of fertilised eggs and gametes cannot be ruled out. Assuming full compliance with the recommendations in the new draft 4.Z chapter of the Aquatic Animal Health Code of the World Organisation for Animal Health, including individual testing of broodfish at stripping, the median probability of introduction following importation of fertilised eggs was 0.36% for VHSV and IHNV, and 0.4% for HPR-deleted ISAV. The median probabilities were reduced to 0.02% and 0.01%, respectively, if fertilised eggs were disinfected twice (as green and as eyed eggs) at origin. Additional disinfection upon arrival in the importing establishment reduced the median probability to 0.02% for all viruses with one disinfection at the origin, and to virtually zero when two disinfections were applied at the origin. The assessment also evaluated probability of introduction when only population testing before stripping is applied. Two disinfections at the origin plus one at destination also resulted in virtually zero probability of introduction. For gametes, individual testing of broodfish at stripping, along with the other risk mitigation measures recommended in the new chapter resulted in a probability of introduction ranging from 0% to 0.3%. Implementation of the measures included in the new draft 4.Z chapter is recommended to prevent the introduction of VHSV, IHNV and HPR-deleted ISAV when moving gametes and fertilised eggs from non-free to free areas. In addition, it is recommended that fertilised eggs are disinfected twice before being traded and, to further reduce the risk, also disinfected at the establishment receiving the imported materials.

KEYWORDS

disinfection of eggs, fertilised eggs, gametes, IHNV, ISAV, risk mitigation measures, risk of introduction, salmonids, vertical transmission, VHSV

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SUMMARY

Background and terms of reference

The Aquatic Animals Commission of the World Organisation for Animal Health (WOAH) is discussing a new draft chapter (4.Z) for the Aquatic Animal Health Code on general recommendations for the safe trade of gametes and fertilised eggs of fish from non-disease-free areas. The chapter applies to pathogens which are not transmitted vertically and proposes that these products can be safely traded given compliance with the listed risk mitigation measures (e.g. disinfection of fertilised eggs, observations of the listed provisions for certification of the health status of broodstock at the aquaculture establishment of origin, individual testing of broodstock after stripping, biosecurity measures applicable to the collection and incubation centres, conditions applicable to the collection and storage of milt and preparation of milt samples). The European Commission asked EFSA for a scientific opinion on viral haemorrhagic septicaemia virus (VHSV), infectious haematopoietic necrosis virus (IHNV) and highly polymorphic region -deleted infectious salmon anaemia virus (HPR-deleted ISAV), all listed in Commission Implementing Regulation (EU) 2018/1882, and specifically, in the light of the above, EFSA was mandated to:

- conduct a risk assessment of VHSV, IHNV and HPR-deleted ISAV introduction in areas declared free of those diseases via movement of gametes and/or fertilised eggs from a non-free area taking into consideration the risk of vertical transmission of those viruses and the effectiveness of specific mitigation measures implemented (ToR1);
- identify the critical points implemented at the establishment of origin of the gametes and the fertilised eggs to early detect these viruses and to prevent their further spread within the establishments and their introduction into free areas (ToR2);
- describe the implementation of how the identified risk mitigating measures are currently applied by different stakeholders, including real examples on best practices or practical difficulties (ToR3);
- consider and describe the uncertainty around ToR1, ToR2 and ToR3.

Introduction to the pathogens

1. VHSV, family *Rhabdoviridae*, genus *Novirhabdovirus*, comprises four genotypes and nine subtypes. Several fish species are susceptible to the infection, with rainbow trout (genus *Oncorhynchus*) being the most susceptible. The virus is present in wild marine fish in the northern hemisphere; all areas, except for those officially declared free, should be considered infected.
2. IHNV, family *Rhabdoviridae*, genus *Novirhabdovirus*, mainly affects salmonid species belonging particularly to the genus *Oncorhynchus*. The most susceptible stage is fry, followed by the spawning stage when the fish can release the virus through ovarian fluid and milt. Since the virus is present in several areas globally, all areas should be considered infected, except for those officially declared free.
3. HPR-deleted ISAV, family *Orthomyxoviridae*, genus *Isavirus*, causes a severe and lethal disease in farmed Atlantic salmon. The disease has rarely been detected in fresh-water reared Atlantic salmon, and never in naturally farmed rainbow trout. The virus variants with a full-length HPR (HPR0 or non-deleted) are the non-pathogenic progenitors to HPR-deleted ISAV and are present in most areas worldwide where Atlantic salmon are farmed.

Production systems

Two principal broodstock production systems are considered in this EFSA opinion:

The fresh-water-sea-fresh-water production system: common for Atlantic salmon and sometimes for rainbow trout. After grow-out of broodstock in sea cages, selected broodfish are transferred to on-land fresh-water facilities for gamete collection and egg fertilisation. After incubation and hatching of the eggs, the progeny is grown to parr, smoltified and subsequently transferred to sea cages to grow new generations of broodfish.

The fresh-water-only production system: typical for rainbow trout, with broodstock maintained entirely in fresh-water environments, where fertilisation and disinfection occur in situ.

Data and methodology

ToR1: EFSA outsourced two systematic literature reviews (SLR1 and SLR2) that were reviewed and complemented by EFSA's experts and then summarised into main conclusions.

- SLR1 focused on the risk of vertical transmission of infection with the three viruses from parents to progeny in the listed species. Both true vertical transmission (which cannot be prevented by disinfection treatments/measures) and egg surface-associated transmission (which can be prevented by disinfection) were considered.
- SLR2 focused on the risk mitigation measures to prevent the spread or introduction of the three viruses in question via the movement of gametes or fertilised eggs from a non-free to a free area.

The outcome of SLR2, along with an overview of the measures provided in the WOAH draft Chapter 4.Z, were used to identify the following key risk mitigation measures (RMM) for the safe trade of gametes and fertilised eggs: **testing**

broodfish to certify absence of the viruses (RMM1); **disinfection of fertilised eggs** (RMM2), applied once to green eggs (RMM2a) and/or applied to eyed eggs (RMM2b); and additional disinfection of the eggs at arrival in the importing facility (RMM3).

An expert knowledge elicitation (EKE) to assess the effectiveness of the identified RMMs was conducted in two steps: elicitation of individual judgements followed by collective discussion to reach a consensus. The results of the expert elicitation were used to estimate a combined probability of virus introduction.

For RMM1 the assessments were performed under two scenarios. **Scenario 1**, in which broodfish are individually tested at stripping as suggested in the new WOAH chapter, was considered to only be feasible for implementation in current large-scale Fresh-water-Sea-Fresh-water production systems. **Scenario 2**, in which the health status of the population of origin of the broodstock is assessed through testing a sample of the fish population in the same epidemiological unit, was also included to reflect the reality of current fresh-water systems in Europe.

ToR2: The EFSA experts identified, through group discussion, a series of **critical points** that could be effectively controlled in the establishments of origin of gametes and fertilised eggs to ensure early detection of VHSV, IHNV and HPR-deleted ISAV or, if already present, to prevent their further spread.

ToR3: The EFSA experts developed a structured **survey** to gather relevant practical information on the implementation of risk mitigation measures identified under ToR1, as well as of the critical control points identified in ToR2. The survey was administered to 18 fish health practitioners whose countries of practice cover 20 European countries (not all EU MS).

ToR4: Uncertainty was captured quantitatively for ToR1 by asking experts to give their estimates as a probability range during the EKE and expressing the final results as probability distributions. In ToR2 and ToR3 the uncertainty analysis consisted in the identification of the sources of uncertainty influencing the assessment through discussion among EFSA's experts and the evaluation of the results of the survey, and the certainty on certain conclusions was quantified through group discussion.

Assessment and Conclusions

ToR1

There is no evidence of true vertical transmission (i.e. vertical transmission which cannot be prevented by disinfections of fertilised eggs) of VHSV, IHNV and HPR-deleted ISAV. However, these viruses have been detected in the reproductive fluids of infected broodfish and in the surrounding aquatic environment and can contaminate the surface of fertilised eggs and gametes. Therefore, RMM are necessary to reduce the risk of virus introduction via movement of contaminated gametes or fertilised eggs originating from non-free areas.

The results of this assessment presented below regarding the risk of introduction of VHSV, IHNV and HPR-deleted ISAV in areas declared free via movement of gametes and fertilised eggs from non-free areas, are based on the following assumptions: the disinfection of fertilised eggs is compliant with the disinfection protocol set out in WOAH Aquatic Animals Code Article 4.5.2 (or other protocol demonstrated to have similar effectiveness at the establishment of origin); establishments of origin intended for exportation comply with biosecurity measures that ensure the effectiveness of these measures, and prevent contamination of eggs after disinfection.

All probabilities reported for fertilised eggs refer to the introduction associated with the importation of a batch of eggs in the worst-case scenario (at least one fish in the group of broodstock from which eggs were harvested in a batch was infected with the respective viruses, and infection introduction in the establishment has gone unnoticed).

Fertilised eggs – The final distributions for the probability of introduction due to external contamination of fertilised eggs (egg surface-associated transmission) with VHSV, IHNV and HPR-deleted ISAV were:

- For VHSV and IHNV, the median probability in **Scenario 1** was 0.36% (with an upper 95% percentile of 1.33% and maximum of 2%), and for HPR-deleted ISAV the median probability was 0.4% (with an upper 95% percentile of 1.1% and maximum of 1.5%). The median probabilities are reduced to 0.02% and 0.01%, respectively, if fertilised eggs are disinfected twice at the origin (as green eggs and then as eyed eggs), with maximum probabilities of 0.4% for VHSV and IHNV introduction, and 0.11% for HPR-deleted ISAV.
- If the provisions of the WOAH draft chapter are applied at the origin, and the imported fertilised eggs are disinfected upon arrival at the importing establishment, the median probability of introduction is reduced to 0.02% for all viruses with one disinfection at the origin (maximum 0.2% for VHSV and IHNV, and 0.13% for HPR-deleted ISAV), and to virtually zero when two disinfections are applied at the origin (maximum 0.04% for VHSV and IHNV, and 0.01% for HPR-deleted ISAV).

In the specific scenario of fresh-water production where broodstock are not necessarily culled and not individually tested at stripping – **Scenario 2** – and RMMs considered were population testing with negative results coupled with disinfection of fertilised eggs, the probability estimates were:

- For VHSV and IHNV, the median probabilities of introduction were 1% (upper 95% percentile at 3.4%, maximum 6.6%) and 0.85% for HPR-deleted ISAV (upper 95% percentile at 3.23%, maximum 6.6%) if eggs are disinfected once at the origin.

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- Disinfection upon arrival would further reduce the median probability of introduction of VHSV and IHNV to 0.05% (maximum 0.66%) and of HPR-deleted ISAV to 0.05% (maximum 0.66%), in the scenario of one disinfection at the origin. Two disinfections at the origin plus disinfection at the destination result in median probabilities of introduction virtually zero (maximum 0.12% for VHSV and IHNV, and 0.13% for HPR-deleted ISAV).

Milt – There are no currently available disinfection procedures directly applicable to milt, however, eggs fertilised using the imported milt can be disinfected. Under Scenario 1, if broodfish are individually tested at the establishment of origin, the risk of introduction through importation of milt ranges between 0.1% and 0.5% for VHSV or IHNV, and 0.1%–0.8% for HPR-deleted ISAV. The risk drops to 0%–0.3% if eggs fertilised using the imported milt are properly disinfected in accordance with the protocol set out in WOAH Aquatic Animals Code Article 4.5.2 or equivalent. Under Scenario 2, applying population testing, the risk of infected milt ranges from 5% to 33%, and the disinfection of eggs fertilised with this milt reduces transmission risk to 0.05%–6.6%. To prevent potential release of virus into free areas, effluent water from the hatchery using the imported milt can be disinfected before release using ultraviolet (UV) light, ozone, heat or chlorine with 99.9% efficacy.

All the conclusions on milt are applicable to **unfertilised eggs**, including that there are no disinfection procedure available. However, unfertilised eggs are fragile and generally unsuitable for transport.

ToR2 and ToR3

Ensuring **compliance with critical control points** is essential for the effectiveness of the RMMs recommended in ToR1. EFSA's experts agreed on the proposed list of control points aligned with WOAH draft Chapter 4.Z and grouped them under four main categories: **health and surveillance, conditions of the establishment, biosecurity procedures** and **diagnostics**. It was concluded that **regular health checks** and surveillance are crucial. Countries producing large numbers of Atlantic salmon conduct 12 health visits/year while other European countries, mainly producing rainbow trout, in general follow the minimum requirements laid down in Annex VI to Commission Delegated Regulation (EU) 2020/689. **Individual testing of broodfish** at stripping is only being carried out in countries producing a large number of salmonids but unrealistic to be implemented in traditional rainbow trout production in fresh-water because of the reuse of broodfish and resource constraints. In this scenario, broodstock spend their entire lifespan in pathogen-free water sources and **population testing** can replace individual testing at stripping. The **egg-producing establishments** must verify that their water source is pathogen-free and that all the biosecurity measures are implemented. The establishments must develop **customised biosecurity plans approved by the competent authorities**. Documentation and quality control of implementation should be part of the biosecurity plan. There was consensus from the fish health practitioners on the list of measures to be implemented in the biosecurity plans. A remaining bottleneck is the risk of disease introduction associated with the sea phase of broodstock in production systems that include a seawater phase. In this type of systems, the conclusion on the importance of individual testing of broodstock is ratified. The 'all-in–all-out' approach is recommended for exporting broodstock farms. **Disinfection of eggs** in accordance with the WOAH protocol is generally practised at least once but also commonly reported to be applied at both stages (green and eyed stages) before leaving the establishment of origin. Disinfection at the destination is not always applied, but its implementation was considered feasible. Health certification relies on accurate and reproducible laboratory testing for VHSV, IHNV and HPR-deleted ISAV. Only **ISO 17025-accredited laboratories** should be used. All national reference laboratories in Europe operate under these guidelines.

ToR4 – There was high certainty (99%–100%, almost certain) about the effectiveness of RMMs (i.e. eggs and milt disinfection at establishment) in preventing introduction of the viruses into free areas in all scenarios evaluated, as assessed in ToR1. The main source of uncertainty on ToR2 and 3 is the degree of compliance of establishments with identified critical control points. Compliance varies with establishment size, production type, trading activity and national legislation. While confidence in the health status of broodstock relies on compliance with the control points, risks can be mitigated through proper application of RMMs based on disinfection of eggs. Disinfection at the receiving establishments for milts or fertilised eggs increases the effectiveness of RMMs in preventing introduction of the viruses into free areas to almost certain in all scenarios evaluated.

Recommendations

ToR1 – Proper implementation of the measures included in the draft of new Chapter 4.Z of the WOAH Aquatic Animal Health Code is recommended to prevent the introduction of VHSV, IHNV and HPR-deleted ISAV when moving fertilised eggs and/or gametes from non-free to free areas. Those measures include determining population health status with ≥ 95% confidence, individual testing of broodstock, using approved collection/incubation centres for gametes and fertilised eggs and individual testing of broodstock at these centres, destroying biological materials if there are positive results and disinfection of fertilised eggs before trading. As individual testing is practised only in fresh-water-sea-fresh-water systems, population testing is recommended in fresh-water-only systems. With full biosecurity compliance, population testing yields a very low risk of introduction (0.05% vs. 0.02% for individual testing). It is recommended that fertilised eggs are disinfected twice (once as green eggs and again after hardening, as eyed eggs) before being traded. Disinfection at the receiving establishment is also recommended. For gametes, the same measures apply. Disinfection of eggs after fertilisation using imported milt is recommended at destination since no disinfection can be applied to milt. In this scenario

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it is further recommended to disinfect the hatching unit effluents before releasing them into the environment to avoid environmental spread.

ToR2 and ToR3 – Effective enforcement and monitoring of critical control points are recommended to ensure the effectiveness of the RMMS and low probabilities of virus transmission/introduction. Health visits should be planned, carried out and documented to ensure systematic control of the health status of the broodstock population. It is recommended to complement the health inspections by CA with visits by trained fish health personnel. Strict adherence to WOAH or equivalent disinfection protocols is also recommended. Biosecurity standard operating procedures and plans, including traceability systems, should be reviewed and updated annually. Third-party audits of the implementation of the biosecurity plan should be embedded in the plan itself. Ongoing staff training that are required should also be maintained and updated. Health certification must rely on ISO 17025-accredited labs.

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1 | INTRODUCTION

1.1 | Background and Terms of Reference as provided by the requestor

Article 4(3) Regulation (EU) 2016/429[1] provides for a definition of aquatic animals, and considers all life stages of certain aquatic animals, including eggs, sperm and gametes of aquaculture animals under the scope of the definition.

When regulating the movements of aquaculture animals intended for Member States, zones or compartments which have been declared disease-free or which are subject to an eradication programme (Article 197 of Regulation (EU) 2016/429), aquaculture animals of listed species must originate from third countries, Member States, zones or compartments thereof which have been declared free of those diseases. There are no specific rules, EU derogations or possibilities for national derogations for eggs, sperm and gametes.

Diseases of relevance for the purposes of this scientific opinion are three diseases listed in Commission Implementing Regulation (EU) 2018/1882[2] (namely viral haemorrhagic septicaemia (VHS), infectious haematopoietic necrosis (IHN) and infection with HPR-deleted infectious salmon anaemia virus (HPR-deleted ISAV)).

Requirements for a Member State, zone or compartment thereof to achieve disease free status for certain listed diseases of aquatic animals, including VHS, IHN and HPR-Deleted ISA are laid down in Annex VI to Commission Delegated Regulation (EU) 2020/689.[3]

As regards listed species, they are listed in the Annex to Implementing Regulation (EU) 2018/1882 for VHS, IHN and HPR-deleted ISAV.

The Aquatic Animals Commission of the World Organisation for Animal Health (WOAH) is discussing a new draft chapter for the Aquatic Animal Health Code on 'Control of pathogenic agents in traded gametes and fertilised eggs of fish'.[4,5]

Article 4.Z.2. of the new draft chapter explains its scope: to describe general recommendations for safe trade in gametes and fertilised eggs of fish from an area other than a free country, free zone or free compartment.

The Aquatic Animals Commission made an assessment[6] of the suitability of the provisions of draft new Chapter 4.Z. for WOAH listed diseases of fish considering risk factors as the mode of transmission of the pathogen, relevance of trade in gametes or fertilised eggs or presence of an accepted egg disinfection protocol. Of particular importance is the risk of vertical transmission: if there is vertical transmission, draft new Chapter 4.Z will not be applicable to this disease.

It should also be noted that only fertilised eggs of salmonids have a disinfection protocol in the Aquatic Animal Health Code (Chapter 4. 4[7]) i.e. no such disinfection protocol exists for the disinfection of gametes (including milt) or unfertilised eggs. However, the trade of fertilised eggs or gametes from any species may be subject to this.

Following the above referred assessment of the Aquatic Animals Commission, draft new Chapter 4.Z will only be relevant at this stage for VHS, IHN and (HPR-deleted ISAV).

The draft new WOAH chapter proposes different risk mitigation measures to make possible the safe trade of gametes and fertilised eggs of fish with regard to the diseases mentioned above. The risk mitigating measures are laid down in the following draft Articles:

- Specific measures required for trade of gametes and fertilised eggs of fish (draft Article 4.Z.3.);
- Health status of broodstock at the aquaculture establishment of origin (draft Article 4.Z.4.);
- Collection and incubation centres (draft Article 4.Z.5.);
- Testing of broodstock at the collection and incubation centre (draft Article 4.Z.6.);
- Conditions applicable to the collection and storage of milt and preparation of milt samples (draft Article 4.Z.7.).

In the light of the above, in accordance with Article 29 of Regulation (EC) No 178/2002,[8] the Commission requests EFSA to provide a scientific opinion covering the following issues:

1. Conduct a risk assessment of VHS, IHN and HPR-deleted ISAV introduction in areas declared free of those diseases related to the introduction of eggs, sperm and gametes of listed species originating from areas which are not

[1]Regulation (EU) 2016/429 of the European Parliament and of the Council of 9 March 2016 on transmissible animal diseases and amending and repealing certain acts in the area of animal health (Animal Health Law).

[2]Commission Implementing Regulation (EU) 2018/1882 of 3 December 2018 on the application of certain disease prevention and control rules to categories of listed diseases and establishing a list of species and groups of species posing a considerable risk for the spread of those listed diseases.

[3]Commission Delegated Regulation (EU) 2020/689 of 17 December 2019 supplementing Regulation (EU) 2016/429 of the European Parliament and of the Council as regards rules for surveillance, eradication programmes, and disease-free status for certain listed and emerging diseases.

[4]Last version of the draft chapter can be found at - https://www.woah.org/app/uploads/2024/04/a-aac-feb-2024-report.pdf.

[5]Note that the term milt is included in the term gametes.

[6]Page 30 of the Report of the Meeting of WOAH Aquatic Animal Health Standards Commission (14 to 21 February 2024) https://www.woah.org/app/uploads/2024/04/a-aac-feb-2024-report.pdf.

[7]Chapter 4.4 on *Recommendations for surface disinfection of salmonid eggs* only provides a recommended protocol for the disinfection of newly fertilised salmonid eggs https://www.woah.org/en/what-we-do/standards/codes-and-manuals/aquatic-code-onlinehttps://www.woah.org/en/what-we-do/standards/codes-and-manuals/aquatic-code-online-access/?id=169&L=1&htmfile=chapitre_disinfection_eggs.htmaccess/?id=169&L=1&htmfile=chapitre_disinfection_eggs.htm.

[8]Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1.

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free of those diseases when they are obtained subject to the conditions provided for in the draft new Chapter 4.Z. of the WOAH's Aquatic Code.

The following aspects are of particular relevance for this risk assessment:

- The risk of vertical transmission of diseases referred to above, including the potential differences in transmission by eggs, sperm and gametes in listed species.
- The assessment of all available risk mitigating measures, including those proposed in Chapter 4.Z. of the WOAH's Aquatic Code and their ranking in accordance with their effectiveness to prevent the introduction of the diseases referred to above.

2. Identify the critical points to be controlled at the establishments where the eggs, sperm and gametes of aquaculture animals are produced to ensure early detection of the diseases referred to in point 1 or, when detected, to prevent their further spread within the establishments where the eggs, sperm and gametes of aquaculture animals are produced and their subsequent introduction into a Member State, zone or compartment thereof declared free of those diseases.
3. Describe the implementation of these effective risk mitigating measures, if applied currently by competent authorities or operators, including practical experiences, best practices or practical difficulties.
4. Consider and describe the uncertainty related to any of the above.

1.2 | Interpretation of the Terms of Reference

In addressing the terms of reference, this scientific opinion will consider the following:

1. The risk of transmission to free areas through the importation of gametes and/or fertilised eggs is considered within the specific context of **their importation for reproduction and/or restocking**; importation of eggs for consumption as food is outside the scope of this mandate and not assessed. It also only covers the specific scenario of reproduction in captivity.
2. The assessments will consider primarily **salmonids** (order Salmoniformes, family Salmonidae), given the scope and the availability of evidence.

The following specific definitions have been adopted:

3. **Gametes** are the haploid reproductive cells, and the term is used to refer to **milts** (sperm) and **unfertilised eggs** (ova). **Fertilised eggs** are the diploid cells resulting from fertilisation of eggs by sperm. This scientific opinion will cover gametes and fertilised eggs.
4. **Vertical transmission** is defined as the transfer of virus particles from parents to progeny. **True vertical transmission** cannot be prevented by disinfection of the fertilised eggs. All other vertical transmission (which can be prevented by disinfection treatments/measures) is considered '**egg surface-associated transfer'**. Both true vertical transmission and egg surface-associated transfer of infection from parents to progeny are here considered.
5. **RMMs** are measures which can be applied to mitigate or reduce the risk of the virus presence in gametes and fertilised eggs within the scope defined above. These include measures to ensure the health status of the broodstock, measures to ensure biosecurity in the aquaculture establishment of origin, as well as measures applied during the collection and storage of gametes and fertilised eggs, including disinfection procedures.
6. In accordance with Chapter 2.3.4 of the Manual of Diagnostic Tests for Aquatic Animals of WOAH, **infection with ISAV** means infection with the pathogenic agent HPR-deleted ISAV or the non-pathogenic HPR0 (non-deleted HPR) ISAV of the genus *Isavirus* of the family Orthomyxoviridae. The HPR-deleted ISAV is associated with the generalised disease in Atlantic salmon (*Salmo salar*), which is a lethal condition characterised by severe anaemia, and variable haemorrhages and necrosis in several organs. However, understanding that earlier references will not distinguish between HPR-deleted ISAV and HPR0, 'infection with ISAV' is used as a generic term to refer to either. When referring only to infection with HPR-deleted ISAV, this must be explicitly mentioned.
7. In accordance with Chapter 2.3.5 of the Manual of Diagnostic Tests for Aquatic Animals of WOAH, **infection with IHNV** means infection with the pathogenic agent salmonid novirhabdovirus (commonly known as IHNV) of the genus *Novirhabdovirus* and family Rhabdoviridae.
8. In accordance with Chapter 2.3.10 of the Manual of Diagnostic Tests for Aquatic Animals of WOAH, **infection with VHSV** means infection with the pathogenic agent VHSV, of the genus *Novirhabdovirus* and family Rhabdoviridae

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1.2.1 | ToR1 – Risk of introduction of VHSV, IHNV and HPR-deleted ISAV from non-disease-free areas into officially disease-free areas through fertilised eggs and gametes

ToR 1 was interpreted by EFSA's experts as an assessment of the risk of introduction of three viral pathogens namely VHSV, IHNV or HPR-deleted ISAV, via the movement of gametes or fertilised eggs from a non-free to a free area taking into consideration whether those viruses could be transmitted vertically and the effectiveness of specific measures implemented to mitigate that risk.

The assessment specifically considers the presence and transmission of the viruses, determined by a positive result to one of the recommended tests in the WOAH Manual of Diagnostic Tests for Aquatic Animals, rather than the diseases caused by these viruses.

Still in accordance with the scope and definitions above, considering the extent of evidence and the currently existing trading practices, only fish species in the salmonid family are explicitly considered. Where evidence in any other species is found, it will be reviewed and reported, but results should not be extrapolated to other species without careful consideration. For most of the listed non-salmonid fish species, disinfection of fertilised eggs is possible. The appropriate disinfectant, concentration, and timing are, however, highly dependent on the fish species, egg stage, water salinity and targeted pathogen, and often require preliminary testing to ensure egg safety and effective neutralisation of the pathogens in question.

The above considered information on existing evidence on the risk of **vertical transmission** of VHSV, IHNV or HPR-deleted ISAV, including potential difference in vertical transmission by gametes and fertilised eggs, will be collected from various sources. The evidence retrieved will be narratively described. In addition, evidence will be collected to obtain an **overview** of the available RMMs to prevent VHSV, IHNV or HPR-deleted ISAV spread or introduction via the movement of gametes and fertilised eggs and of **the effectiveness** of such measures. An internal working group assessment will be conducted to evaluate the effectiveness of the risk mitigating measures.

1.2.2 | ToR2 – Critical points to be controlled in the establishment of origin

ToR2 was interpreted by EFSA's experts as a comprehensive identification of the critical control points, implemented at the establishment level where the gametes and fertilised eggs of aquaculture animals are collected, that could potentially affect the effectiveness of the RMMs to detect and prevent spread of VHSV, IHNV or HPR-deleted ISAV via gametes and fertilised eggs which were identified under ToR 1. Evidence available in the scientific literature and other sources will be collected and described.

1.2.3 | ToR3 – Implementation of effective RMMs

ToR3 was interpreted by EFSA's experts as a detailed description of how the identified RMMs for the safe trade of gametes and fertilised eggs, with regard to the three pathogens, are currently being implemented by different stakeholders (competent authorities and operators of establishments of the types listed in the WOAH draft Chapter 4.Z). The aim was to explore real examples of how these measures are applied, highlighting the successes or challenges that have been encountered, identifying the most effective strategies that have proven successful in mitigating risks and the difficulties that may arise. It was done by conducting a survey which was sent to relevant Member States where those measures are applied.

1.2.4 | ToR4 – Uncertainty

ToR4 was understood by EFSA's experts as a comprehensive description of the uncertainty that can arise from any of the above ToRs.

1.3 | Introduction to the pathogens

1.3.1 | Infection with VHSV

Infection with VHSV means infection with the pathogenic agent viral haemorrhagic septicaemia virus of the Genus *Novirhabdovirus* in the family Rhabdoviridae. Using G-gene nucleotide sequences, VHSV isolates are grouped into four main genotypes (I, II, III and IV) and nine subtypes (Ia–Ie and IVa–IVd), which mostly align with distinct geographical regions. The infection is characterised by the occurrence of the following clinical signs: rapid onset of mortality, lethargy, darkening of the skin, exophthalmia, anaemia (pale gills), haemorrhaging at the base of the fins or in the gills, eyes or skin, abnormal swimming such as flashing and spiralling, and a distended abdomen due to fluid accumulation in the peritoneal cavity. Mortality varies, depending on many environmental and physiological conditions. In general it is highest in water temperatures between 9°C and 12°C. Rainbow trout is the most susceptible species to VHSV infection with genotype Ia, the

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most common and frequently reported genotype in fresh-water in Europe. VHSV genotype III has caused disease in farmed turbot and wrasse and genotype IVa in sea-farmed Atlantic salmon, turbot, and olive flounder (WOAH Aquatic Manual Chapter 2.3.10). The other VHSV genotypes have been reported mainly in wild fish such as Atlantic herring and sprat. Infection with VHSV has been reported from countries in Europe, North America and northern Asia (Skall et al., 2005). Some countries, zones and compartments in these regions have officially been declared free from infection with VHSV. The disease has never been reported from the southern hemisphere. According to the Animal Disease Information System (ADIS) held by the European Commission, VHSV is or was recently present in a number of European countries (Austria, Belgium, Croatia, Czechia, Estonia, Finland, France, Germany, Iceland, Italy, the Netherlands, Poland, Romania, Slovakia, Slovenia and Switzerland) as well as Asian countries (China, Iran, Japan and South Korea) (Ahmadivand et al., 2016; Ahmadivand et al., 2021; Oh et al., 2024; Zhang et al., 2019), and North America (USA, Canada) (Hedrick et al., 2003). As the virus is considered widespread in wild fish species in the sea/ocean (North Sea, Atlantic Ocean etc.) (Kim & Faisal, 2011), all countries and territories should be considered infected, except those 'officially declared free'.

1.3.2 | Infection with IHNV

Infection with infectious haematopoietic necrosis virus means infection with the pathogenic agent salmonid novirhabdovirus (commonly known as IHNV) of the Genus *Novirhabdovirus* in the family Rhabdoviridae. Phylogenetic analysis of G-gene nucleotide sequences has grouped IHNV isolates into five main genogroups (U, M, L, E and J), based on geographic location rather than host species. IHNV predominantly infects salmonid species, particularly of the genus *Oncorhynchus*, with fry being the most highly susceptible stage. As fish age, their resistance to infection generally increases, but it declines during the spawning stage. Spawning adult salmon can carry high levels of the virus and release it through ovarian fluid and milt, even without showing signs of disease. The severity of IHNV outbreaks, ranging from acute to chronic, depends on factors such as fish species, rearing environment, temperature and the specific virus strain. Fish with acute IHNV infection may display abnormal swimming, lethargy, darkened skin, swollen abdomens, exophthalmia, distended abdomen and external haemorrhaging. Cases of infection with IHNV have been reported from Europe, Asia-Pacific, Africa and the Americas. Some countries, zones and compartments in these regions have officially been declared free from infection with IHNV. IHNV is endemic among wild salmonids in USA and Canada (WOAH Aquatic Manual Chapter 2.3.5.)

IHNV is present in a number of European countries (Austria, Belgium, Croatia, Czechia, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, the Netherlands, North Macedonia, Poland, Slovenia and Switzerland) as well as Asian countries (Xu et al., 2019) (China, Georgia, Iran and South Korea) and North America (Canada and the USA) (Black et al., 2016; Breyta et al., 2017). As the virus can be considered to be present in wild salmonids all countries/territory should be considered infected, except those 'officially declared free'.

1.3.3 | Infection with HPR-deleted ISAV

Infection with infectious salmon anaemia virus (ISAV) means infection with the pathogenic agent HPR-deleted ISAV or the non-pathogenic HPR0 ISAV (non-deleted HPR) of the Genus *Isavirus* of the Family *Orthomyxoviridae*. According to the new nomenclature of ICTV the taxonomic name is *Isavirus salaris*. HPR-deleted ISAV may cause a severe and lethal disease, ISA in Atlantic salmon (*Salmo salar*), characterised by severe anaemia, variable haemorrhaging and necrosis in several organs. ISA has only been observed naturally in farmed Atlantic salmon at marine grow-out sites. The disease has rarely been reported Atlantic salmon reared in fresh-water and has not been observed in naturally farmed rainbow trout, however, ISAV RNA has been detected in the internal organs of rainbow trout farmed in close proximity to Atlantic salmon suffering an ISA outbreak (Alarcón et al., 2021).

In Atlantic salmon, the disease progression varies, it may be acute with daily mortality 0.5%–5% (Hammell & Dohoo, 2005), but more commonly the progression is slow with daily mortality (0.05%–0.1%) typically affecting a few cages. Without intervention cumulative mortality can become high over months. HPR0 ISAV has not been linked to clinical signs of disease in Atlantic salmon but is the ancestor of all HPR-deleted ISAV variants (EFSA AHAW Panel, 2012). ISAV was first reported in Norway in the mid-1980s and has since been detected in Canada, Scotland (1998), the Faroe Islands (2000), the USA (Maine in 2001), Chile (2007) and Iceland (2021) (WOAH Aquatic Manual Chapter 2.3.4). The HPR0 variant has been found in all regions with HPR-deleted ISAV.

1.4 | Current legal framework

The legal framework currently in place is outlined in Sections 1.4.1 and 1.4.2, detailing EU legislation and the WOAH framework, respectively.

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1.4.1 | EU legislation

The EU Animal Health Law (Regulation (EU) 2016/429), provides the legal basis for regulating the movements of aquaculture animals within the EU. Under Article 197, operators must only move aquaculture animals to disease-free areas or areas under eradication programmes if those animals of listed species originate from non-EU countries, Member States, zones or compartments that have also been declared free of those diseases. The European Commission is empowered to adopt delegated acts allowing derogations from the movement requirements, if they do not pose a significant risk of spreading disease. These exceptions may take into account various factors, including the species, categories and life stages of the aquaculture animals involved; the type of establishment from which the animals originate and their intended destination; the specific intended use of the aquaculture animals; the characteristics of the destination and any RMMs that have been applied, such as treatments, processing methods or other precautions at the place of origin or destination. There are no specific rules, EU derogations or possibilities for national derogations for fertilised eggs and gametes as these are treated as live animals.

The three diseases that are of relevance for this scientific opinion (namely VHS, IHN and HPR-deleted ISAV), are listed and categorised in Commission Implementing Regulation (EU) 2018/1882 and subsequent amendments and integrations, which complements Regulation (EU) 2016/429. The species they affect are outlined and prevention and control measures specified.

For each of the three diseases the following three categories are assigned:

1. **Category C**: listed diseases which are of relevance to some Member States and for which measures are needed to prevent them from spreading to parts of the EU that are officially disease-free or that have eradication programmes for the listed disease concerned.

   Optional eradication programmes:

   - Rules for obtaining disease-free status.
   - Compartmentalisation.
   - Disease control.

2. **Category D**: listed diseases for which measures are needed to prevent them from spreading on account of their entry into the EU or movements between Member States.

   Rules for movements:

   - Within the EU.
   - On entry/exit from the EU.

Also applicable to categories A, B and C.

3. **Category E**: listed diseases for which there is a need for surveillance within the EU.

   Rules for notification and surveillance.

   Applicable also to categories A, B, C.

1.4.1.1 | *Granting disease-free status under EU legislation*

The criteria for conducting surveillance, eradication programmes and obtaining and maintaining disease freedom from the listed diseases are included in Commission Delegated Regulation (EU) 2020/689 and subsequent amendments and integrations. Under this Regulation the samples collected for examining the presence of VHSV, IHNV and ISAV must be tested using one or more of the diagnostic methods in accordance with the detailed diagnostic methods and procedures laid down in Commission Delegated Regulation (EU) 2020/689 and the relevant details and guidance available on the website of the European Union Reference Laboratory (EURL) for Fish Diseases.[9]

Granting disease-free status for VHS and IHN

Annex VI Part II Chapter 1 of Delegated Regulation (EU) 2020/689 covers the disease-specific requirements for disease-free status for VHS and IHN.

[9] https://www.eurl-fish-crustacean.eu/da/fish/diagnostic-manuals.

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Unknown health status

A Member State, zone or compartment with unknown health status can be granted VHS-free or IHN-free status if all aquaculture establishments have undergone a risk-based surveillance using one of the following schemes – model A as per Table 1A and model B as per Table 1B.

Model A (2-year scheme):

- Health visits and sample collection over two consecutive years.
- All tests must be negative for VHS and IHN.

TABLE 1A Scheme for free status from VHS or IHN in a 2-year control period.

Type of establishment	Health visits per year	Samples per year	Number of growing fish	Number of broodstock fish
With broodstock	2	2	50 (first visit)/75 (second visit)	30 (first or second visit)
Broodstock only	2	1	0	75 (first or second visit)
Without broodstock	2	2	75 (first AND second visit)	0

Note: Maximum number of fish per pool: 10.

Model B (4-year scheme with reduced sample size) :

- Health visits and sample collection over four consecutive years.
- All tests must be negative for VHS and IHN.

TABLE 1B Scheme for free status from VHS or IHN in a 4-year control period (reduced sample size).

	Health visits per year		Samples per year		Number of growing fish		Number of broodstock fish	
Type of establishment	Years 1–2	Years 3–4	Years 1–2	Years 3–4	Years 1–2	Years 3–4	Years 1–2	Years 3–4
With broodstock	2	2	1	2	30 (second visit)	30 (first visit)	0	30 (second visit)
Broodstock only	2	2	1	2	0		30 (first or second visit)	30 (first AND second visit)
Without broodstock	2	2	1	2	30 (first or second visit)	30 (first AND second visit)	0	

Note: Maximum number of fish per pool: 10.

Granting the status free from VHS or IHN in compartments, zone or Member states known to be infected

A Member State, zone or compartment known to be infected with VHS or IHN can gain a free status, provided that all establishments keeping listed species within that Member State, zone or compartment have been subject to an eradication programme which meet the conditions outlined in Annex VI Part II Chapter 1 Section 3 of Delegated Regulation (EU) 2020/689, including investigation of non-infected establishments within the protection or restricted zone.

Maintenance of status free from VHS and IHN

To maintain the status free from VHS and IHN for a Member State, zone or compartment the following testing scheme must produce negative results: a sample size of **30** fish, with visit frequency determined by the risk level assigned to the establishment by the competent authority:

- **High risk**: one visit per year
- **Medium risk**: one visit every 2 years
- **Low risk:** one visit every 3 years

***Granting disease-free status for* HPR-deleted ISAV**

With regard to HPR-deleted ISAV, Annex VI Part II Chapter 2 of Delegated Regulation (EU) 2020/689 covers the disease-specific requirements for disease-free status.

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Unknown health status

A Member State, zone or compartment with unknown health status can be granted the status free from infection with HPR-deleted ISAV, if all aquaculture establishments have undergone risk-based surveillance using the following scheme (Table 2A:

- Health visits and sample collection over a minimum period of two consecutive years
- All tests must be negative for HPR-deleted ISAV

TABLE 2A Scheme for free status from infection with HPR-deleted ISAV in a -2year period.

Year of surveillance	Health visits per year	Laboratory examination per year	Number of fish
Year 1	6	2	75
Year 2	6	2	75

Note: Maximum number of fish per pool: 5.

Granting the status free from infection with HPR-deleted ISAV in Member states, zones and compartments known to be infected with HPR-deleted ISAV A Member State, zone or compartment known to be infected with HPR-deleted ISAV can gain a free status, provided that all establishments keeping listed species within that Member State, zone or compartment have been subject to an eradication programme which meet the conditions outlined in Annex VI Part II Chapter 2 Section 3, including investigation of non-infected establishments within the protection or restricted zone for HPR-deleted ISAV.

Maintenance of status free from HPR-deleted ISAV

To maintain the status free from infection with HPR-deleted ISAV of a Member State, zone or compartment the following testing scheme must produce negative results (Table 2B):

TABLE 2B Scheme for maintainance of free status from infection with HPR-deleted ISAV.

Risk level	Health visits per year	Laboratory examination per year	Number of fish in the sample
High	2	2	30
Medium	1	1	30
Low	1 every 2 years	1 every 2 years	30

Note: Maximum number of fish per pool: 5.

1.4.2 | WOAH code

Draft WOAH Chapter: https://www.woah.org/app/uploads/2024/10/a-aac-sept-2024-1.pdf

The new draft Chapter 4.Z of the WOAH Aquatic Animal Health Code titled **'Control of pathogenic agents in traded gametes and fertilised eggs of fish'** proposes a series of sequential RMMs for the safe trade of gametes and fertilised eggs of fish from an area other than a free country, free zone or free compartment. The scope is to improve upon the existing provisions in the Aquatic Code by offering a higher level of protection against diseases like VHS, IHN and infection with HPR-deleted ISAV. This draft chapter, developed in collaboration with industry, received general support from members during consultations in September 2024. Feedback from countries such as Canada and Norway, and the EU led to clarifications and additions, including guidance on biosecurity at collection and incubation centres and clearer definitions for testing timelines.

The purpose of the chapter, outlined in **Article 4.Z.1**, is to provide recommendations for trade of gametes and fertilised eggs of fish intended for aquaculture purposes and to define risk management for trade to a free country, free zone or free compartment when:

1. the intention is to grow out and harvest the fish, hatched from the traded eggs; or
2. the intention is to establish a new stock for aquaculture.

The scope of the chapter, explained in **Article 4.Z.2**, is to outline general recommendations for safe trade in gametes and fertilised eggs of fish from an area other than a free country, free zone or free compartment.

Article 4.Z.3 presents 'Specific measures required for trade of gametes and fertilised eggs of fish'. It specifies RMMs, requiring an **assessment of the health status of broodstock at the aquaculture establishment of origin**. Only broodstock that test negative for relevant pathogens may be transferred to collection and incubation centres, which must themselves be approved by the competent authority. If there is a positive detection, the importing competent authority should assess import risks, considering factors like the biosecurity plan to prevent contamination of gametes and eggs from parents which have individually tested negative. Fertilised eggs should be surface disinfected using proven methods, such as

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those detailed in Chapter 4.5. International consignments must be accompanied by an aquatic animal health certificate issued by the competent authority of the exporting country, confirming compliance with these measures.

Article 4.Z.4. 'Health status of broodstock at the aquaculture establishment of origin'. This outlines the requirements for broodstock health. Broodstock should be sourced from approved establishments under the competent authority's supervision, tested within 30 days before transfer, clinically healthy at the time of movement, and must not originate from a population experiencing recent or ongoing mortality or exposure to animals or other sources of disease.

Article 4.Z.5. 'Collection and incubation centres'. Collection and incubation centres should be approved by the competent authority and be supervised by an Aquatic Animal Health Professional or veterinarian. These centres must:

- comply with biosecurity plans;
- be structured to contain epidemiologically separate individual broodstock or groups of broodstock;
- ensure traceability of gametes or fertilised eggs to an epidemiologically separate individual or group;
- separate key activities like egg disinfection, incubation and waste disposal;
- use water from a source without contact with wild or farmed susceptible species known or suspected of being infected with the pathogenic agent.

Article 4.Z.6. 'Testing of broodstock at the collection and incubation centre'. Stripping and sampling must be overseen by the Aquatic Animal Health Professional or veterinarian responsible for the centre. Broodstock should be individually tested for the listed diseases as per the *Aquatic Manual* in an approved laboratory. In accordance with the biosecurity plan, gametes, fertilised eggs and fish from positive epidemiological groups should be disposed of in a biosecure manner and any affected facilities should be disinfected to prevent cross-contamination. If incubation is not carried out individually and a positive individual is detected, all eggs that were incubated together must be removed.

Article 4.Z.7. 'Conditions applicable to the collection and storage of milt and preparation of milt samples'. It covers additional provisions to address the handling, storage, and testing of milt, ensuring sterility and traceability throughout the process.

The Commission undertook a detailed evaluation of the new draft Chapter 4.Z. The primary goal of this assessment was to determine whether these general provisions, and the more disease-specific recommendations in Article 10.X.15, were suitable for inclusion in disease-specific chapters. Key considerations included the mode of pathogen transmission (with Chapter 4.Z providing higher risk mitigation for horizontally transmitted pathogens), the significance of trade in gametes and fertilised eggs, and the availability of validated egg disinfection protocols.

As a result of this assessment, the Commission agreed to only apply model Article 10.X.15 to certain fish disease chapters where it was deemed relevant and effective: Chapter 10.6. 'Infection with IHNV' and Chapter 10.10 'Infection with VHSV', and Article 10.4.20 to Chapter 10.4. 'Infection with ISAV' (with Article 10.4.20 applied).

For ISAV, the provisions of Article 10.X.15 were incorporated into **Article 10.4.20**. The chapter emphasises disinfection of fertilised eggs in accordance with the recommendations in Chapter 4.5, secure treatment to ensure inactivation of pathogen or disposal of all water (including ice), equipment and waste, and additional biosecurity measures such as disinfection (e.g. of the fertilised eggs) upon arrival in the importing country. The competent authority is tasked with ensuring compliance, supported by the mandatory health certificate accompanying each consignment.

1.4.2.1 | *Granting disease-free status in accordance with WOAH*

Under the Aquatic Code, as described in Chapter 1.4, **targeted surveillance** is one pathway to achieve a disease-free status, to regain a disease-free status following detection of the pathogenic agent and to maintain disease freedom.

Over the period of targeted surveillance, the combined number of aquaculture establishments and aquatic animals sampled should be sufficient to generate at least **95% confidence** that the pathogenic agent would be detected if present at or above the design prevalence in the country, zone or compartment. Design prevalence at the animal and higher levels of aggregation (i.e. pond, net pen, aquaculture establishment, etc.) should be set to a maximum of **2%**. Surveys should be designed in accordance with the recommendations provided in Article 1.4.16. At a design prevalence of 2%, a sensitivity and a specificity of 100%, **150** units should be sampled.

Targeted surveillance should be conducted for a defined period, as described in the relevant disease-specific chapter. For VHSV, IHNV and ISAV, a member country may make a self-declaration of freedom from infection for its entire territory if it can demonstrate that targeted surveillance has been in place for at least the last **2 years** without detection of the virus and basic biosecurity conditions have been continuously met and have been in place for at least 1 year before the commencement of targeted surveillance.

1.5 | Introduction to fish system production

1.5.1 | Fresh-water-sea-fresh-water

Marine aquaculture of Atlantic salmon and rainbow trout in the North Atlantic region of Europe is a technologically advanced and highly regulated industry. Atlantic salmon dominates the industry, accounting for approximately 95% of total

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production, but the method of production of rainbow trout when reared in this system follows the same methods as for Atlantic salmon (Figure 1). Broodstock are selected based on genetically desirable traits and are used for a single reproductive cycle. Broodfish (both female and male) are euthanised before gametes/eggs collection. fertilised eggs are disinfected and incubated in fresh-water hatcheries. The resulting fry are reared in fresh-water and typically vaccinated against six to seven common pathogens via intraperitoneal injection. Upon reaching the smolt stage, when the fish get physiologically adapted to seawater at approximately 1 year of age, the fish are transferred to marine grow-out facilities. These facilities most often consist of open-sea cages, each holding up to 200,000 individuals, with typical farms comprising 6–10 such units. Strict biosecurity protocols are implemented to reduce disease risk, and monitoring of fish health, growth, welfare and environmental parameters is comprehensive. After about 12–18 months at sea, new broodfish are selected and transferred to land-based quarantine facilities where they are monitored intensively for pathogens and disease before they are released for stripping of eggs and milt. Continuous innovation is observed in production infrastructure, including an increasing use of recirculating aquaculture systems (RAS) in fresh-water phases, and closed or semi-closed containment systems in seawater. The management of broodstock fish varies among breeding companies, with a notable trend toward minimising the time these fish spend in open-sea cages. Some operators maintain broodfish entirely in land-based indoor tanks (i.e. closed containment systems) throughout the life cycle of the fish.

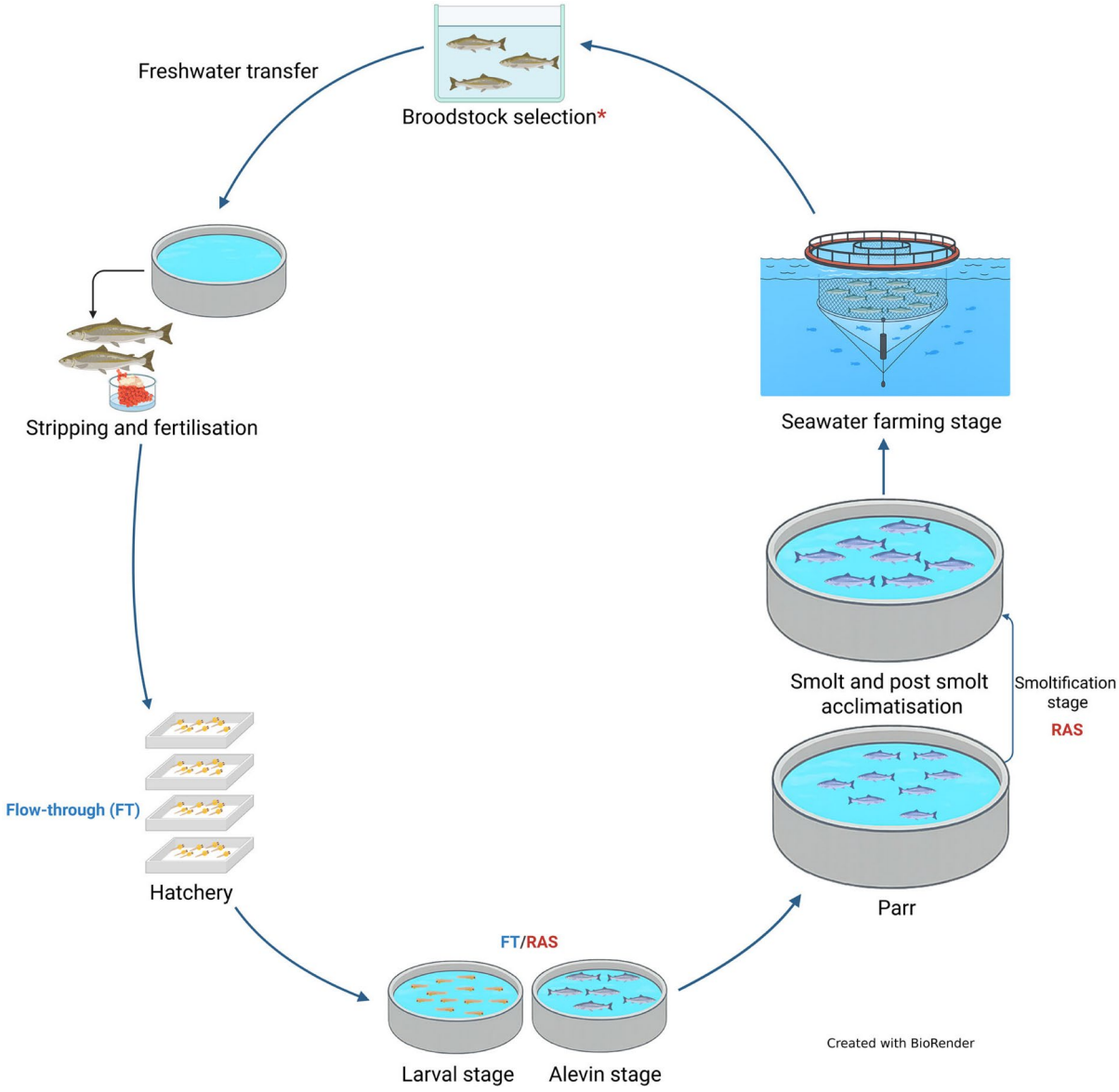

FIGURE 1 The breeding of Atlantic salmon brood fish from eggs stripping and fertilisation, fresh-water production of fry and smolt to seawater grow-out and selection of new brood fish generations.

1.5.2 | Fresh-water

In the European aquaculture setting rainbow trout broodstock are maintained in fresh-water under controlled environmental conditions to ensure optimal reproductive performance. Broodfish are typically held in ponds or raceways with

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flow-through water supply or RAS with water temperatures maintained between 8 and 12°C and photoperiod manipulation (e.g. 12L:12D or 16L:8D) to synchronise spawning.

Rainbow trout broodstock are usually kept at the same establishment during their whole life cycle (3–5 years). The water source for the establishment is often borehole or well water and in some cases surface water with no anadromous fish upstream. At sexual maturity, females are anaesthetised and manually stripped by applying gentle abdominal pressure to expel eggs into sanitised containers. Males are similarly stripped for milt, which is collected dry to avoid activation. Fecundation is achieved via the dry fertilisation method, where milt is added to the eggs followed by activation with clean, oxygenated water. The mixture is gently stirred to ensure uniform fertilisation. Post-fertilisation, eggs are in some cases rinsed and subjected to disinfection using an iodophor solution (green egg disinfection). Broodfish are typically reused from year to year and are not euthanised at stripping.

2 | DATA AND METHODOLOGY

The methodological approach adopted to address the terms of reference is described in the protocol given in Appendix A. The protocol was developed upfront of the initiation of the risk assessment. In this section, a more detailed description of the specific methodology used for each item is provided.

2.1 | ToR1 – Risk of introduction of VHSV, IHNV and HPR-deleted ISAV from non-disease-free areas into officially disease-free areas through fertilised eggs and gametes

To answer ToR1, two systematic literature reviews (SLR1 and SLR2) were outsourced by EFSA (see Sections 2.1.1 and 2.1.2) under the contract ref. EOI/EFSA/2022/01 – CT 22 BIOHAW.

The evidence collected through the SLRs was reviewed by experts and, where relevant, complemented with their collective expertise and experience, before being summarised into main conclusions. To assess the effectiveness of the RMMs identified in SLR2, a two-round elicitation process was conducted involving the elicitation of individual judgements, followed by collective discussion (see Section 2.1.3).

2.1.1 | SLR 1 – **Risk of vertical transmission**

The specific objective of SLR1 was to answer the following review question:

What is the existing evidence on the risk of vertical transmission of VHSV, IHNV and HPR-deleted ISAV *in the listed species, including the potential differences in transmission by unfertilised eggs, milt (sperm) and fertilised eggs?*

Following the definitions laid out in the Introduction (Section 1.2), both true vertical transmission and egg surface-associated transfer of infection from parents to progeny were considered in SLR1. Methodological details can be found in the SLRs protocol (Annex A). A total of 2435 unique references were identified after searching PubMed and Scopus on 25 October 2024. The initial search string included terms to identify any articles related to the three virus species in question, as well as any keyword referring to gametes, fertilised eggs or reproduction in general. Specific selection based on the objectives of this SLR1 was only applied during screening based on abstracts and subsequently full-texts.

Based on their relevance to the research question, a total of 31 references were selected for the extraction of the following data: Study period, Study type (experimental/field), Pathogen (IHNV, VHSV, ISAV), Fish species, Matrix (eggs/sperm/gametes), Sample size, Outcome description, Outcome value, Short conclusion.

2.1.2 | SLR 2 – **RMMs**

The specific objective of SLR2 was to answer the following review question:

What is the existing evidence on the effectiveness of RMMs to prevent VHSV, IHNV or HPR-deleted ISAV spread/introduction via movement of fertilised eggs, sperm or unfertilised eggs from a non-free to a free area?

The same general search (as in SLR1) aimed to retrieve any article referring to the viruses in question and transmission associated with reproduction was used, and 2454 unique records were screened. The methodological details can be found in the SLRs protocol (Annex A). After abstract and full-text screening, 13 references were selected for data extraction. The extracted data included: Study period, Study type (experimental/field), Pathogen (IHNV, VHSV, ISAV), Fish species, Matrix (fertilised egg/sperm/unfertilised egg), Intervention type (risk mitigation measure/critical control point), Intervention description, Sample size, Outcome description, Outcome value, Short conclusion.

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2.1.3 | Assessment method

The outcome of SLR2, along with an overview of the measures provided in WOAH's draft Chapter 4.Z were used to identify key RMMs for the safe trade of gametes and fertilised eggs. An expert knowledge elicitation to assess their effectiveness was conducted in two steps: elicitation of individual judgements, followed by collective discussion.

In the first round, each RMM/step was translated into questions that were assessed individually by all WG experts. The full list of questions is reported in Section 3.3, Tables 4 and 5. Each expert assigned a probability range to the likelihood of a measure failing to prevent vertical transmission (i.e. to the likelihood of vertical transmission occurring despite the application of a measure) for each virus.

The probability ranges in Table 3 were provided as guidelines, but experts were instructed to freely use any interval of their choice to reflect their judgement as well as their perceived uncertainty (reflected in the width of the interval chosen). Each expert was also asked to provide a short reasoning to support their answer.

TABLE 3 Probability ranges provided as references during expert elicitation.

Subject probability range (%)	Probability term
0–1	Almost impossible
1–5	Extremely unlikely
5–10	Very unlikely
10–33	Unlikely
33–66	About as likely as not
66–90	Likely
90–95	Very likely
95–99	Extremely likely
99–100	Almost certain

In the second round, the individual judgements were discussed and experts were asked to explain and justify their responses from Round 1. They were also given the opportunity to reconsider their estimates based on the discussion, with additional material/information provided as needed.

All values provided by experts were used to create a custom distribution of probabilities aggregating all their given ranges. This ensured the full range of values was accounted for, while also considering the weight of agreement (values overlapping are stacked). The result is a probability distribution for the efficacy of each RMM individually.

The results of the expert elicitation were used to estimate a combined probability of virus introduction. The final probability distribution was calculated assuming that virus introduction can only happen if all measures, applied in sequence, fail. For example, where two RMMs are applied in sequence, each with probability P_1 and P_2 of failure, the probability of introduction is calculated as $P_{introduction} = (P_1 \times P_2)$. Where an additional mitigation measure is applied, with probability P_3 of failure, $P_{introduction} = (P_1 \times P_2 \times P_3)$, and so on. Each probability in this case is a custom probability distribution constructed based on the expert's opinions, and the final result is also given as a probability distribution.

The final probability distribution is described in terms of its statistical characteristics – minimum and maximum values, median value and 95% percentile after 1 million random iterations.

Experts deemed that the probabilities should be assessed in conjunction for VHSV and IHNV, as the results would not be different for the two viruses for any of the steps considered. A separate independent assessment was performed for HPR-deleted ISAV. As described in Section 1.3.3, HPR0 ISAV is the ancestor of HPR-deleted ISAV and thus would pose a risk of ISA if introduced into an HPR0 ISAV-free area. EFSA experts thus chose to perform the elicitation considering HPR0 ISAV as well.

2.2 | ToR2 – Critical points to be controlled in the establishment of origin

To ensure the early detection of VHSV, IHNV and HPR-deleted ISAV or, if already present, to prevent their further spread within establishments where gametes and fertilised eggs of aquaculture animals are collected, the experts identified a series of critical points that could be effectively controlled. These critical points encompass various aspects of biosecurity and management practices. A consensus-based approach was adopted to determine the most relevant factors influencing control at each critical point.

2.3 | ToR3 – Implementation of effective RMMs

To gather relevant practical information on the implementation of RMMs identified, EFSA's experts developed a structured survey reported (see Annex C). The survey was designed to collect the experience and expertise of fish health practitioners working directly with establishments that are egg producers or receivers in Europe (inside and outside the EU).

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The objective was to capture real-world applications of RMM s. It comprised a mix of closed and open-ended questions. Questions were formulated to assess how these specific mitigation measures are implemented, their perceived effectiveness and any obstacles encountered in practice.

EFSA's working group experts identified 18 fish health practitioners whose countries of practice cover 20 European countries (not all EU Member States) and actively sought their engagement. This targeted sample was considered the most appropriate way to ensure that participants took the time to respond to the survey thoroughly. The survey was kept anonymous to ensure that opinions and experiences would be shared candidly, but before sharing the link of the survey the working group met with all the practitioners enlisted to carefully review the questions and ensure that everyone had the same understanding of the questions and of the objective of the exercise.

2.4 | ToR4 – Uncertainty

For ToR 1, as explained above in Section 2.1.3, a quantitative assessment was performed based on expert opinion. Uncertainty in this case was first captured individually – for each expert their uncertainty is captured in the range of probabilities they give for the efficacy of any individual RMM. These individual ranges were then combined into a probability distribution, and the total uncertainty for each RMM efficacy estimation was captured by this distribution. For the final probability of failure, after all RMMs are applied, results are presented after 1 million random iterations of this final probability distribution.

Uncertainty in ToRs 2 and 3 was evaluated qualitatively through the expert's description of critical control points (ToR2) complemented with the reported experience of several practitioners of the practical implementation of these critical controls (ToR3). For these ToRs, major sources of uncertainty were identified and the uncertainty on the conclusions were discussed among the expert group following the methodology recommended in EFSA's Guidance on Uncertainty Analysis in Scientific Assessments (EFSA Scientific Committee, 2018). A final certainty scored reflecting consensus judgements was assigned based on the probabilities in Table 3.

3 | ASSESSMENT (RESULTS)

3.1 | ToR1 – Risk of vertical transmission – Results from the SLR1 complemented with expert expertise and experience

The full results of SLR1 are given in Annex A. This section reports the main findings of the SLR.

3.1.1 | Infection with VHSV

True vertical transmission of VHSV has never been demonstrated. Chaves-Pozo et al. (2010) showed that VHSV is able to replicate in the ovarian tissue, which triggers a strong immune response, thereby preventing true vertical transmission of the pathogen. It has also been suggested that antiviral factors within yolk components inactivate the virus or interrupt its replication (Al-Hussinee et al., 2010). Several reviews (Bovo et al., 2005; Rimstad et al., 2019) mention a study conducted by Vestergård Jørgensen (1974) in which viable VHSV could only be detected up to 3.5 h after fertilisation of naturally infected non-disinfected eggs. In the literature review conducted in the context of the EU FP6 project Fish Egg Trade report 'Hazard identification for vertical transfer of fish disease agents' published in 2005 (Bovo et al., 2005), it was concluded that so far there has been no evidence of true vertical transmission of VHSV.

Egg surface contamination with the virus cannot be excluded. Some phylogenetic analyses showed that outbreaks occurring in regions with no prior history of VHS were most likely originated from embryonated eggs imported from endemic regions, e.g. the introduction of VHSV into Iran was probably due to such an import (Ahmadivand et al., 2021). However, referring to unpublished field observations, Jørgensen (1974) concluded that the virus may be present as a contaminant on the egg's surface, but that it apparently does not survive the egg incubation period. The author concluded that vertical transmission of VHSV seems highly unlikely, because, as of 1974, European trout eggs had been exported to all parts of the world without VHS being introduced into any country outside Europe.

These observations agree with research that indeed indicates that VHSV might contaminate the egg's surface but cannot survive the egg incubation period (Bovo et al., 2005).

When fertilised eggs undergo recommended disinfection procedures (WOAH, 2024), the virus has not been demonstrated to be transmitted vertically. In line with this, there have been cases where the introduction of untreated green eggs from infected farms during the incubation phase of a VHSV outbreak has led to subsequent VHS outbreaks (H. Korsholm, Danish Veterinary Services, personnel communication) (EFSA 2007).

The virus has been found in sperm (Eaton et al., 1991) from wild coho salmon, but Mulcahy and Pascho (1984) were unable to demonstrate the adsorption of the virus to Chinook salmon sperm.

In conclusion, no studies were found that demonstrate true vertical transmission via fertilised eggs or gametes. The available data indicate that while contamination of fertilised egg surfaces by VHSV may be observed due to the presence

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of the virus in reproductive fluids or a contaminated environment, the virus is not transmitted to progeny if appropriate disinfection of the egg surface is implemented.

3.1.2 | Infection with IHNV

Although several studies pointed out the presence of IHNV in progeny originating from eggs (Meyers, 1998; Meyers et al., 1990; Mulcahy & Bauersfeld, 1983; Mulcahy & Pascho, 1985) most studies indicate that IHNV-infected parents do not produce IHNV-infected offspring when the eggs have been raised in virus-free water or undergone disinfection with an iodophor solution (Wingfield & Chan, 1970; Amend 1972; Ratliff et al., 1982; Traxler et al., 1997; Bovo et al., 2005; Oidtmann et al., 2014).

Experimental studies failed to demonstrate vertical transmission via gametes (Traxler et al., 1997; Yoshimizu et al., 1989). It has been suggested that components of the egg yolk may inhibit viral replication. The capacity of IHNV to replicate following injection into the egg appears to be linked to the progression of embryonic development, as it correlates with a reduction in yolk components (Bovo et al., 2005; Yoshimizu et al., 1989).

It has been demonstrated that IHNV can be quickly adsorbed by the surface membrane of steelhead trout and Chinook salmon sperm (Mulcahy & Pascho, 1984), suggesting thereby that IHNV attached to the sperm originating either from the male fish or from infected ovarian fluid could deliver the virus directly into the egg during fertilisation. However, the contamination of salmon sperm with IHNV did not result in the infection of eggs in the study from Yoshimizu et al. (1989), and there is no direct evidence that sperm can transmit IHNV to eggs (Rimstad et al., 2019).

The conclusion of the EU FP6 project Fish Egg Trade report 'Hazard identification for vertical transfer of fish disease agents' (Bovo et al., 2005) was that, based on experiences in the laboratory and in the field, the risk of true vertical transmission of IHNV is negligible.

Introduction of IHNV into Europe (Italy, France and Spain) has been linked to the introduction of eyed rainbow trout eggs from the USA (Baudin-Laurencin, 1987; Bovo et al., 1987), as has the introduction of IHNV into Iran from Europe (Ahmadivand et al., 2021). Whether these were due to failure in disinfection procedures or true vertical transmission has not been resolved, but based on previous experiences in laboratory settings, failure in procedures or post-disinfection contaminations are the most plausible reasons for these introductions.

To conclude, there is clear evidence of presence of the virus in reproductive fluids, but no evidence of true vertical transmission of IHNV. There is also no clear evidence of the possibility of vertical transmission posed by non-fertilised eggs or sperm.

3.1.3 | Infection with HPR-deleted ISAV

Several phylogenetic analyses showed that the most likely explanation for the emergence of ISAV in Chile was vertical transmission because at that time the country imported a lot of Atlantic salmon embryos from Norway (Vike et al., 2009). However, this has not been demonstrated to be true vertical transmission and could have been due to egg surface-associated transfer. Until 1998, Chile imported millions of Atlantic salmon eggs from Norway without requirements that the eggs should be derived from ISAV-free broodstock. Despite this, ISAV was not reported in the progeny of Atlantic salmon in Chile until 2007 (Bovo et al., 2005; Godoy et al., 2008).

The possibility that the first ISAV outbreak in Chile, which occurred during first feeding of Atlantic salmon fry, might have been a case of vertical transmission was discussed without a definite conclusion in Nylund et al. (1999). Potential contamination in the experimental facilities makes the conclusions of this study unreliable, according to EFSA experts' opinion. According to Marshall et al. (2014), HPR-deleted ISAV isolates were recovered from the interior of eggs originating from naturally infected Atlantic salmon from Chile, suggesting the possibility of vertical transmission of the variant to fry and juvenile salmon. However, several experimental (Thorud, 1988; Melville & Griffiths, 1999; Polinski et al., 2024), field (Bovo et al., 2005) and epidemiological (Lyngstad et al., 2008) studies failed to demonstrate true vertical transmission via eggs.

The findings of the EU FP6 project Fish Egg Trade report 'Hazard identification for vertical transfer of fish disease agents' (Bovo et al., 2005) indicated that there was no solid evidence for true vertical transmission inside the gamete or the egg, and that true vertical transmission is insignificant in the epidemiology of the infection.

Further empirical evidence supports these findings. Around 20–30 million fertilised eggs are imported annually from Iceland and Norway to the Faroe Islands. The Faroese HPR0 ISAV variants are circulating as house strains in all Faroese smolt farms. Since 2006, the Faroe Islands have had three ISAV outbreaks. All three HPR-deleted variants were derived from the Faroese HPR0 ISAV circulating in the Faroes. Icelandic or Norwegian ISAV variants have never been detected on the Faroe Islands. In the context of Norwegian salmon farming, the consensus is also that HPR-deleted ISAV is rarely, if ever, transmitted vertically. This is based on accumulated empirical evidence demonstrating a lack of embryonic mortality (which would be expected if HPR-deleted ISAV were capable of infecting embryos of fertilised eggs) and a lack of observed infection or disease in fingerlings during early fresh-water stages. It has been shown that these fish stages are susceptible to ISAV (Nylund et al., 1999). However, no disease cases have been reported in farmed salmon fingerlings over the past 25 years. Approximately 500 million fertilised eggs are produced annually in Norway, amounting to more than 10 billion eggs over

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recent decades. Despite this large-scale production, there has been no evidence of disease resulting from vertical transmission of HPR-deleted ISAV under farming conditions.

In conclusion, although true vertical transmission of HPR-deleted ISAV has been demonstrated in one infected broodfish, several other studies and mounting empirical evidence has found no evidence for true vertical transmission of HPR-deleted ISAV.

3.2 | ToR1 – RMMs – Results from the SLR2 complemented with expert expertise and experience

The success of programmes, such as the Sockeye Salmon Culture Policy (SSCP) implemented in 1981 in Alaska to control IHNV (Meyers et al., 1990; Meyers et al., 2003) or the ISAV control measures used in the Norwegian salmon egg production industry (Farstad et al., 2007), demonstrate that multiple interventions could be applied at each step of the production process to reduce the risk of VHSV, IHNV or HPR-deleted ISAV transmission via fertilised eggs or sperm. These measures should be able to detect infections early on to prevent pathogens from spreading down the production line.

3.2.1 | Broodstock

Prevention of virus presence in gametes and fertilised eggs starts with the broodstock. Utilising pathogen-free broodstock is a widely acknowledged method for preventing vertically transmitted diseases (Brock & Bullis, 2001; Dixon et al., 2016; Winton, 1991). However, maintaining broodfish free from pathogens is not always feasible in areas where the pathogens are endemic. Pathogen-free stock, screening and removal of infected broodstock are key strategies applied to broodstock and are also recommended by WOAH (Brock & Bullis, 2001; WOAH, 2024). Most experts from the Norwegian Scientific Committee for Food Safety concurred that screening broodfish for ISAV infection using real-time RT-PCR is one of the most effective strategies for identifying infections in healthy fish (Farstad et al., 2007). Similarly, in Japan, systematic health checks of broodstock are carried out to ensure that the fish are free from certain key pathogens (IHNV included in the study). Specialised diagnostic methods and regular inspections are necessary to create broodstock that is free of specific pathogens (Yoshimizu, 2009).

3.2.2 | Separation of fish in different production phases in dedicated areas

The definition of specific, segregated production areas is an essential aspect of disease management in aquaculture.[10] Kasai and Nishikawa (2018) mentioned that individual broodstock should ideally be separated during fertilisation and reared in separate containers until the broodstock is certified as pathogen-free. By doing this, if a positive sample is detected, only the infected lot is contained and eliminated. In the Alaskan SSCP, each egg lot is separately water-hardened and eggs are then placed in incubators that allow separation into small lot sizes.

3.2.3 | Fertilised eggs

Another essential mitigation measure is the disinfection of egg surfaces. The SLR findings indicate that most of the existing research on mitigation measures is focused on iodophor disinfection. This method has been widely used since the 1970s to minimise the spread of viral pathogens linked to eggs or sperm. It is used after egg fertilisation, before transportation from brood fish facilities to hatcheries, and often also after arrival at hatcheries (Winton, 1991; Farstad et al., 2007). Both field and experimental studies have demonstrated that iodine compounds, recommended for the disinfection of salmonid fertilised eggs, can efficiently reduce the risk of transmission of the viruses of interest (Amend & Pietsch, 1972; Goldes & Mead, 1995; Meyers et al., 2003; Farstad et al., 2007; Tuttle-Lau et al., 2009; Groocock et al., 2013). Moreover, it has been shown that iodine treatment did not compromise the survival or development of eggs (Bowzer et al., 2011).

There have also been studies reporting evidence of infection in the progeny of infected broodstock despite disinfection of the egg's surfaces (Dixon et al., 2016; Goldes & Mead, 1995; Mulcahy & Bauersfeld, 1983; Ratliff et al., 1982; Winton, 1991; Yoshimizu et al., 1989). Several factors can contribute to the inadequate disinfection of eggs, such as inadequate contact time, concentration, water temperature or pH; or the presence of organic debris and mucus, which can reduce the efficacy of iodophors. The eggs can also be recontaminated if they are placed back into contaminated water or handled with non-sterile equipment. Other studies have suggested that viral titres may have been so high in ovarian fluid that disinfection

[10]This is not to be confused with 'compartmentalisation'. In line with the WOAH Aquatic Health Code, in this scientific opinion we reserve the term compartment to mean 'one or more aquaculture establishments under a common biosecurity management system containing an aquatic animal population with a distinct health status with respect to a specific disease or diseases for which required surveillance and control measures are applied and basic biosecurity conditions are met for the purpose of international trade'. The risk mitigation measure described here refers to the segregation of fish within an establishment into dedicated areas for specific production phases, in line with the proposed WOAH RMMs as described later in this document.

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protocols were insufficient, and also that a small proportion of virions might have been concealed in the irregular surface of the egg membrane and thus inaccessible to the iodophor (Dixon et al., 2016; Goldes & Mead, 1995).

Timing of disinfection in relation to egg activation (hardening) can influence the outcome of disinfection, because during the activation of the egg the surface changes considerably (Winton, 1991; Bovo et al., 2005; Farstad et al., 2007).

While fertilised egg disinfection with iodophors might not be completely effective, it significantly lowers the risk of transmission of the pathogens of interest and is so far the best available disinfectant for fertilised egg surfaces. Fertilised egg disinfection with iodophors is commonly implemented in numerous production facilities (Brock & Bullis, 2001; Farstad et al., 2007; Dixon et al., 2016). The current WOAH recommendation for surface disinfection of salmonid eggs at hatcheries is indeed to use iodophors, such as povidone–iodine solutions (100 ppm for a minimum of 10 min) and subsequent incubation in virus-free water (WOAH, 2024). To enhance the effectiveness of this protocol, it is recommended to apply it only to hardened, newly fertilised or eyed eggs, starting by rinsing eggs in pathogen-free saline to remove organic matter and maintaining the pH of the iodophor solution between 6 and 8. Other disinfectants such as malachite green and formalin have also been used in the past for disinfecting fertilised eggs, but have been shown to be less effective than iodophors (Amend & Pietsch, 1972; Bovo et al., 2005; Farstad et al., 2007). Moreover, malachite green is prohibited for use in food-producing animals in the EU. Iodophores were originally designed to be used in fresh-water and recommended doses are not necessarily effective in seawater. Earlier studies with plaice (*Pleuronectes platessa*), cod (*Gadus morhua*) and Atlantic halibut (*Hippoglossus hippoglossus*) have indicated that glutaraldehyde has a potential as a surface disinfectant for marine fish eggs (Harboe et al., 1994; Salvesen & Vadstein, 1995; Vadstein et al., 1993). Glutaraldehyde, is an attractive candidate for disinfection of egg in saline conditions (Salvesen et al., 1997).

3.2.4 | Gametes (milts and unfertilised eggs)

No specific RMMs were found in the literature review which could be applied to gametes. This is in line with the experience of the experts.

A recent qualitative risk assessment on the risk of transmission of infectious disease through trade of cryopreserved milt (Rimstad et al., 2019) reported that there are no effective disinfection procedures for fish seminal fluids described in the literature. Thus, the pathogens, which could originate from the brood fish or from contamination following stripping, may be present in frozen milt. However, the report also mentions that the primary practical application of salmonid milt is for the fertilisation of salmonid eggs, which are typically subject to a disinfection procedure after fertilisation.

3.2.5 | Water

Since VHSV, IHNV and ISAV are all able to survive in water (Bovo et al., 2005; Farstad et al., 2007), maintaining pathogen-free water for incubation and rearing is a key factor for controlling the diseases (Dixon et al., 2016; Meyers et al., 1990). One main point to consider is the source of the water. Spring water (or from a borehole) is considered to be a safe source with regards to biosecurity, but special care is needed when using water from reservoirs populated with susceptible species. Common disinfectants used to treat water in aquaculture applications include ultraviolet (UV) light, chlorine or ozone, which have all been shown to be effective in producing virus-free water in laboratory settings (Brock & Bullis, 2001; Liltved et al., 2006; Øye & Rimstad, 2000; Wedemeyer et al., 1978). UV-C irradiation has been demonstrated to inactivate 99.9% of VHSV (Øye & Rimstad, 2000) and ISAV (Liltved et al., 2006; Øye & Rimstad, 2000). IHNV inactivation has been demonstrated by protocols using iodine (Batts et al., 1991), chlorine and ozone exposure (Wedemeyer et al., 1978), also with inactivation rates of 99.9%.

In conclusion, the outcomes derived from the current SLR emphasise the need to implement a comprehensive set of mitigation measures and procedures at the various stages of the fertilised egg and sperm production processes. There is evidence that these combinations of measures can effectively mitigate the risk of transmitting VHSV, IHNV and HPR-deleted ISAV via the movement of fertilised eggs or sperm from a non-free to a free area.

3.3 | ToR1 – Effectiveness of RMMs to prevent introduction of VHSV, IHNV and ISAV from non-disease-free areas into officially disease-free areas through fertilised eggs or gametes

The evidence gathered from SLR1 showed that there is no evidence of true vertical transmission of VHSV, IHNV and ISAV, but that these viruses can contaminate the surface of eggs or sperm (milt), and thus that RMMs are necessary to reduce the risk of virus introduction via movement of fertilised eggs or gametes originating from non-free areas. Therefore, the assessment focused on the RMMs. Without any RMMs the contamination of gametes or fertilised eggs with infectious viruses (external contamination) was assumed.

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3.3.1 | Proposed list of RMMs to be included in the risk assessment

3.3.1.1 | *Fertilised eggs*

Considering the results of SLR2 (Section 3.2), and also the specific conditions listed in the new draft Chapter 4.Z of the WOAH Aquatic Animal Health Code, all the RMMs listed in Table 4 were considered for assessing the risk of virus introduction to areas declared free through the introduction of fertilised eggs. Table 4 details which RMM were subject to assessment and the specific assumptions made about their adoption. Specifically, the RMMs indicated in Table 4 as RMM1, RMM2 and RMM3 were quantitatively estimated via elicitation process.

TABLE 4 **Risk mitigation measures** from the new draft Chapter 4.Z of the WOAH Aquatic Animal Health Code 'Control of pathogenic agents in traded gametes and fertilised eggs of fish', **and details of how they were incorporated into the risk assessment on fertilised eggs**.

Risk mitigation measures	Conditions	Assumptions and assessment applied
Risk mitigation at the collection centre (exporter)		
The health status of the broodstock at the aquaculture establishment of origin must be determined	As specified in 4.Z.3 item 1	While assumed to be a necessary condition for the trading of fertilised eggs, for the purposes of the RA, this measure was always considered to fail. That is, the *RA assumes the worst-case scenario* and assesses how effective all other RMM would be in preventing vertical transmission if parents were infected (or could not be considered free with 100% certainty).
Gametes and fertilised eggs should originate from a collection and incubation centre which has been approved for that purpose by the competent authority of the place of origin	As specified in 4.Z.3 item 2	The establishment of origin is assumed to be compliant in terms of biosecurity plan, separation of different production phases into dedicated areas, and controlled water sources.
RMM1: testing of broodstock at the collection and incubation centre	As specified in 4.Z.6	Individual testing of broodstock using internal organs after stripping was the primary scenario considered and assessed (Scenario 1). Alternatively, a scenario of testing through sampling fish in the same closed establishment was also assessed (Scenario 2, see notes in the text following this table).
Provisions in the event of a positive result during the previous step (RMM1)[a]	As specified in 4.Z.6, items 3, 4 and 5	Establishment is assumed to have a system in place to ensure that gametes or fertilised eggs can be traced back to an epidemiologically separate individual or group, and *in the event of any positive result, gametes or fertilised eggs from that epidemiological group should be disposed of a biosecure manner. In this RA no gametes or fertilised eggs are traded from the establishment where RMM1 resulted in a positive detection*.
RMM2: disinfection of fertilised eggs at the collection and incubation centre before to trading	As specified in 4.Z.3 item 4 and 4.5. 4.5.2.2[b]	Iodine disinfection following the specific protocol outlined is assessed for its effectiveness. Two disinfection points are considered: green eggs (RMM2a), and eyed eggs (RMM2b). Their effectiveness was explicitly assessed by the experts.
Risk mitigation at the receiving establishment (importer)		
RMM3: disinfection of fertilised eggs at the destination (importing establishment)	As specified in 10.X.15	A specific step of additional disinfection at arrival on the importing establishment is not prescribed in the draft WOAH chapter, but its effectiveness in reducing the overall risk was included in the assessment

Abbreviations: RA, risk assessment; RMM, risk mitigation measure; '4.Z.X' refers to the specific article referenced.

[a]Positive results according to procedures described in WOAH specific chapters or EU diagnostic manuals.

[b]Or another method demonstrated to be equally effective. The disinfection procedure applied should be justified by providing evidence that the chosen method is sufficiently active against the particular virus.

In relation to RMM1, note that in the draft chapter Article 4.Z.5, a specific provision is made for the option of having animals treated as epidemiologically separated groups, rather than individuals. Specifically, in item 4:

*'[*the collection and incubation centre*]* Has a system in place to ensure that gametes or fertilised eggs can be traced back to an *epidemiologically separate individual or group*, and which includes documentation of test results. Where the system only allows tracking to the group and not to the individual level, *the measures referred to in 4.Z.6 number 5 should apply to the group'*.

While Article 4.Z.6., item 2 mentions that 'At stripping the broodstock should be *individually* sampled', item 4 of Article 4.Z.5 quoted above specifically states that measures referred to in 4.Z.6. could be applicable to the epidemiological group, rather than the individual.

The expert group felt strongly that a further scenario, where animals are not individually tested at stripping, is needed to reflect the current reality of fresh-water production systems in Europe (currently mainly used for rainbow trout production).

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The possibility of group testing was specifically addressed in the RA through consideration of a dedicated scenario for RMM1 (Scenario 2).

All assessments for fertilised eggs were therefore performed for two scenarios:

Scenario 1: Animals are raised in sea water at an establishment defined as the 'establishment of origin' and later transferred as broodstock to a collection and incubation centre (different units in the same establishment or a different establishment). **All conditions in Article 4.Z.3 apply** and at the time of egg collection, animals will be stripped, killed and tested individually. This scenario was only considered applicable to the current reality of large-scale production of Atlantic salmon or rainbow trout in fresh-water-sea-fresh-water production systems.

Scenario 2: Animals spend their entire life in the same physical establishment, compliant with the operation and biosecurity conditions listed in Chapter 4.Z. Broodstock are not individually tested and not necessarily killed for testing after stripping. Testing is based on a sample of animals in the same closed epidemiological unit. The health status of the establishment is regularly monitored. This scenario was considered to better reflect practices applicable to the fresh-water-only production system described in 1.5.2, which is used for some species of salmonids, in particular fresh-water farmed trout, and in which fish are kept in broodstock establishments for several cycles of egg production, and not killed or individually tested after every egg collection. The same RMMs as per scenario 1 will be applied, except for individual testing. This scenario can only be considered compliant with Chapter 4.Z if the broodstock are considered to be a single epidemiological unit, and the conditions in the chapter are acceptable to be applied to the epidemiological unit, not the individual animal.

In **Scenario 2, RMM1**, testing of broodstock is assumed to occur via **population testing**, defined as targeted sampling and testing of animals from the population of fish in the same physical establishment at least once a year. The broodstock group is not specifically tested, but a sample of animals in the same establishment is killed and subjected to testing of internal organs using a testing protocol approved by the EURL. Specifically, for the assessment, experts considered testing 75 individuals (as per surveillance testing scheme in the event of unknown health status, Table 1A).

Scenario 2 is in principle applicable to other fresh-water species. As stated in Section 1.2, this risk assessment only concerns salmonid fish species. However, a number of non-salmonid farmed species are listed as susceptible to infection with VHSV, IHNV and ISAV. For all susceptible fresh-water species (e.g. pike, yellow perch, pike perch etc) the same RMMs as for salmonid species can be followed. Iodophor treatment is, however, not possible in sea water, and thus not suitable for VHSV susceptible species such as turbot, Senegalese sole, Japanese flounder, cod, lumpfish or Atlantic halibut, where glutaraldehyde might be used as an alternative.

3.3.1.2 | *Gametes*

No specific disinfection protocols are currently available for gametes. Unfertilised eggs, in particular, are less resistant to the adverse effects of disinfectants. They are also fragile and therefore not a commodity that can easily be transported.

Assuming the establishments are fully compliant with the conditions listed in the new draft Chapter 4.Z of the WOAH Aquatic Animal Health Code (including Article 4.Z.7, detailing conditions applicable to the collection and storage of milt and preparation of milt samples), the only risk mitigation steps available relate to ensuring the health of the population of origin, and specifically the health of the broodstock.

Trading **unfertilised eggs** was not considered viable.

For trade of **milts**, as no further RMMs can be applied at the origin of the trade, EFSA's experts further discussed measures that could be applied at the establishment receiving the imported milt. The complete list of RMMs evaluated is given in Table 5.

TABLE 5 **Risk mitigation measures** from the new draft Chapter 4.Z of the WOAH Aquatic Animal Health Code 'Control of pathogenic agents in traded gametes and fertilised eggs of fish', **and how they were incorporated into the risk assessment on milt**.

Risk mitigation measures	Conditions	Assumptions and assessment applied
Risk mitigation at the collection centre (exporter)		
The health status of the broodstock at the aquaculture establishment of origin must be determined	As specified in 4.Z.3 item 1	As specific disinfection procedures are not currently available, this should be considered a major risk mitigation step.
Gametes and fertilised eggs should originate from a collection and incubation centre which has been approved for that purpose by the competent authority of the place of origin	As specified in 4.Z.3 item 2	The establishment of origin is assumed to be compliant in terms of biosecurity plan, separation of different production phases into dedicated areas and controlled water sources.
RMM1: testing of broodstock	As specified in 4.Z.6	Individual testing of broodstock was the primary scenario considered and assessed (Scenario 1). Risk mitigation in a scenario where only population testing is applied (Scenario 2) was also assessed.
Provisions in the event of a positive result in the previous step (RMM1)	As specified in 4.Z.6, items 3, 4 and 5	Establishment is assumed to have a system in place to ensure that gametes or fertilised eggs can be traced back to an epidemiologically separate individual or group, and *in the event of any positive result, no gametes or fertilised eggs from the establishment are traded*.

(Continues)

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TABLE 5 (Continued)

Risk mitigation measures	Conditions	Assumptions and assessment applied
Conditions applicable to the collection and storage of milt and preparation of milt samples	As specified in Article 4.Z.7	The establishment of origin is assumed to be compliant.
Risk mitigation at the receiving establishment (importer)		
RMM2: disinfection of fertilised eggs at the destination (importing establishment)	As specified in 4.5.2[a]	After milts are used for egg fertilisation, fertilised eggs can be subject to disinfection protocols as previously discussed for fertilised eggs. The previous assessment of its effectiveness was considered to also apply here.
Disinfection of effluents		As shown in the results of SLR2, disinfection protocols are available which can be applied directly to the water. Their effectiveness is documented in literature (see Section 3.2.5). RMM2 would prevent vertical transmission, and the disinfection of effluents would prevent virus release into the environment in officially free areas.

Abbreviations: RA, risk assessment; RMM, risk mitigation measures; '4.Z.X' refers to the specific article referenced.

[a]Or another method demonstrated to be equally effective. The disinfection procedure applied should be justified by providing evidence that the chosen method is sufficiently active against the particular virus.

3.3.2 | Assessment of the effectiveness of the RMMs individually

3.3.2.1 | *Fertilised eggs*

Considering the RMMs listed in Tables 4 and 5, the assessment has been estimated, explicitly, for each of the viruses:

- **RMM1:** The probability of failing to **detect infected animals** in a collection and incubation centre, if an infected animal has been introduced into the establishment.
- **RMM2:** The probability that any virus associated with the surface of eggs would remain viable after the **disinfection of fertilised eggs**, applied once at the stage of green eggs (RMM2a) or also applied to eyed eggs (RMM2b), if the parents were infected and somehow not detected;
- **RMM3:** The probability of virus survival if the **disinfection of eggs is also applied on arrival** at the importing facility.

Results of the expert assessment of the probability of failure of each of the RMMs in **Scenario 1** are given in Figures 2 and 3. The detailed individual probabilities and their reasoning are provided in Annex B.

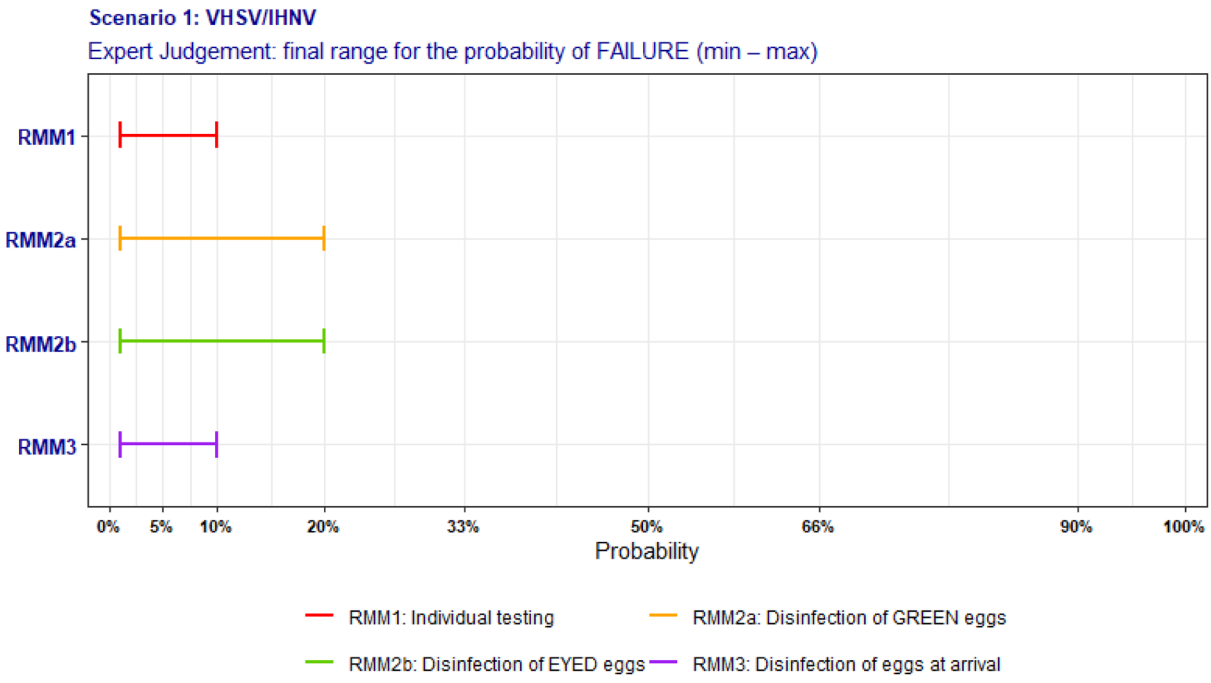

FIGURE 2 Final range for the probability of FAILURE (min. – max.) for the RMMs considered in Scenario 1 for VHSV and IHNV (elicited by the five EFSA experts).

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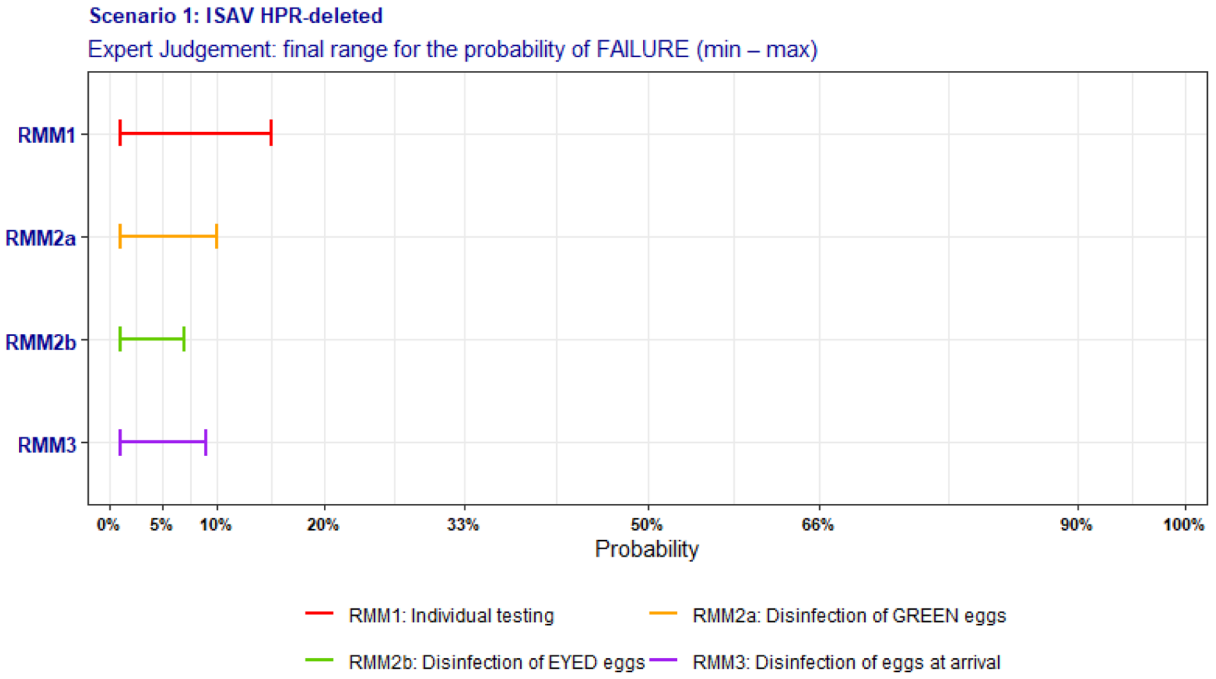

FIGURE 3 Final range for the probability of FAILURE (min – max) for the RMMs considered in Scenario 1 for HPR-deleted ISAV (elicited by the five EFSA experts).

Experts also chose to perform the exercise for HPR0 ISAV. In this case, the experts judged the probability of egg disinfection to have exactly the same effect as for HPR-deleted ISAV (same ranges for RMM2a, RMM2b and RMM3). However, experts stressed that testing internal organs, as is done for the specific goal of detecting HPR-deleted ISAV, will fail to detect HPR0 ISAV in 50%–99% of cases. To specifically detect HPR0 ISAV, gills instead of internal organs should be tested, and then the probability of failure to detect the virus is 10%–1%.

The results for **Scenario 2** are shown in Figure 4 for VHSV and IHNV, and Figure 5 for HPR-deleted ISAV. In this scenario, testing to detect HPR0 ISAV was considered to have a probability of failure from 50% to 100% if using internal organs only.

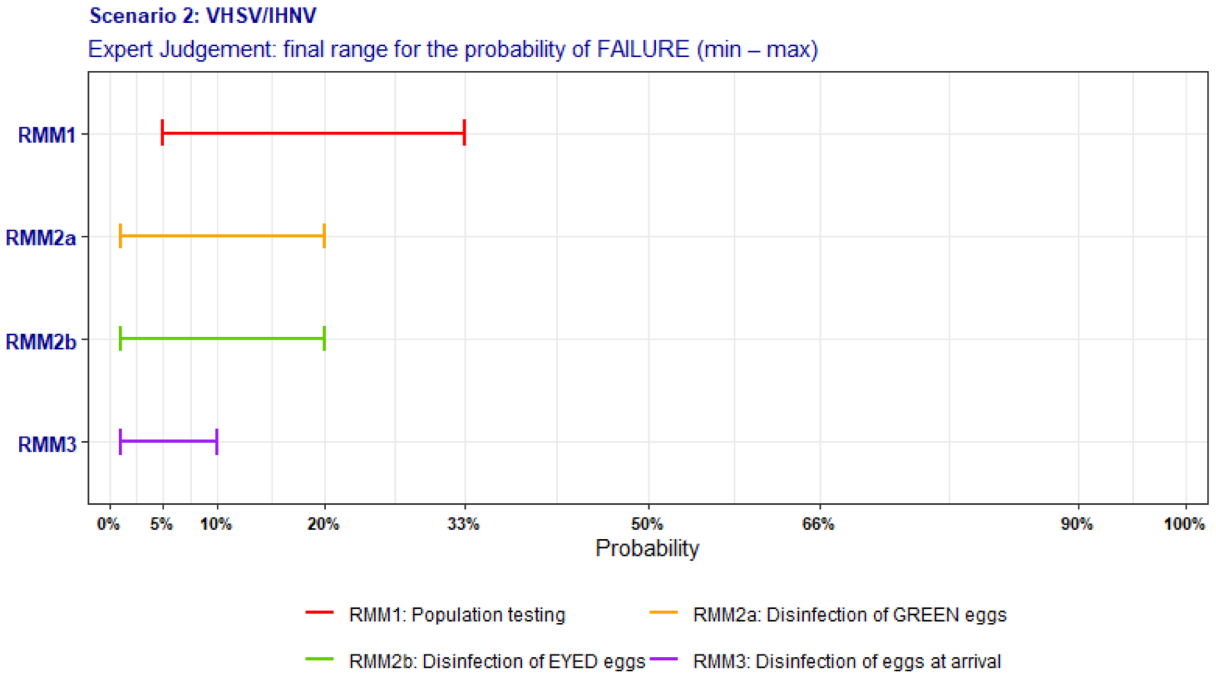

FIGURE 4 Final range for the probability of FAILURE (min – max) of the RMMs considered in Scenario 2 for VHSV and IHNV (elicited by the five EFSA experts).

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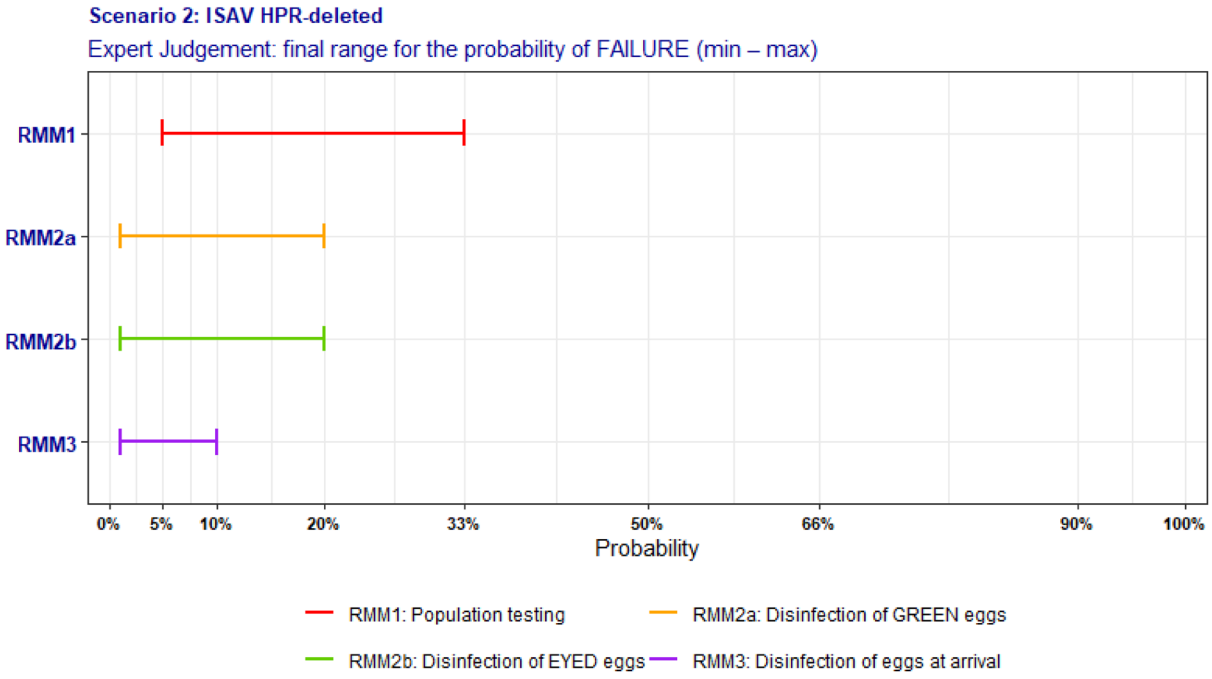

FIGURE 5 Final range for the probability of FAILURE (min – max) for the RMMs foreseen in Scenario 2 for HPR-deleted ISAV (elicited by the five EFSA experts).

3.3.2.2 | *Milt*

No specific additional assessments were performed for milt – the same probabilities presented in Figures 2–5 were considered for the RMMs applicable for RMM1, individual testing of parents; and RMM2, disinfection of fertilised eggs.

For the additional RMM to avoid virus release into the environment, as per SLR2, water treatment protocols are available, such as UV-radiation, which would be 99.9% effective in inactivating the viruses (see Section 3.2.5).

3.3.3 | Overall risk of introduction of VHSV, IHNV and HPR-deleted ISAV from non-disease-free areas into officially disease-free areas through fertilised eggs or gametes

The assessments of the individual RMMs were used to estimate a **combined probability of virus introduction** in areas declared free of disease, when importing:

- fertilised eggs to which RMM1 and RMM2 above have been applied;
- fertilised eggs to which the additional step RMM3 is implemented by the receiving establishment;
- milt to which the measure on monitoring the health status, RMM1, RMM2 (at the receiving establishment) and treatment of water effluent have been applied.

Results for fertilised eggs are presented in Table 6 for Scenario 1, and Table 8 for Scenario 2. Milt is discussed in the sequence.

3.3.3.1 | *Fertilised eggs*

Tables 6 and 8 present the final probability ranges for each scenario. The probability distributions after simulating a million random scenarios are not uniformly distributed within these ranges, and in general, distributions are skewed to the left. The probability distributions are shown in Tables 7 and 9.

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TABLE 6 Combined results of the assessment of RMMs to provide a final estimation of the probability of virus introduction in areas declared free of disease, when importing fertilised eggs under Scenario 1.

Risk mitigation measures applied	Virus	Final range for the probability of virus introduction (min. – max.)
RMM1. Individual testing of broodstock when collecting eggs + RMM2. Surface disinfection of eggs before shipment	VHSV or IHNV	**0.01%–2%** if eggs are disinfected only after hardening **0%–0.40%** if eggs are disinfected *before and after* hardening
	HPR-deleted ISAV	**0.01%–1.50%** if eggs are disinfected after hardening **0%–0.11%** if eggs are disinfected *before and after* hardening
RMM1. Individual testing of broodstock when collecting eggs + RMM2. Surface disinfection of eggs before shipment + RMM3. Surface disinfection of eggs upon arrival at the receiving establishment	VHSV or IHNV	**0%–0.20%** (0%–0.20% if at origin disinfected only after hardening and 0%–0.04% if disinfected twice at origin)
	HPR-deleted ISAV	**0%–0.13%** (0%–0.09% if at origin disinfected only after hardening and 0%–0.01% if disinfected twice at origin)

Abbreviations: HPR-deleted ISAV, highly polymorphic region-deleted infectious salmon anaemia virus, IHNV, infectious haematopoietic necrosis virus, VHSV, viral haemorrhagic septicaemia virus; RMM, risk-mitigation measure.

TABLE 7 Full probability distributions (after 1 million simulations) for the final estimation of the probability of virus introduction in areas declared free of disease, when importing fertilised eggs under Scenario 1.

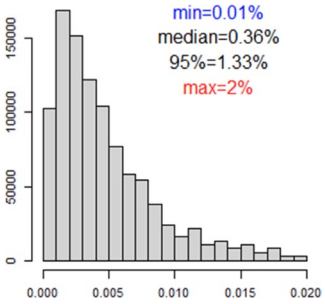
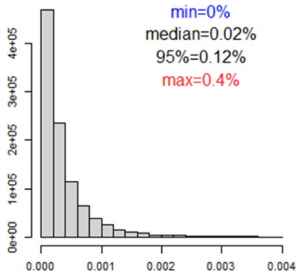
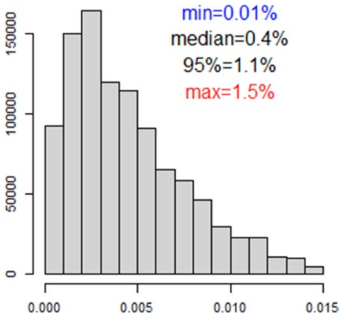
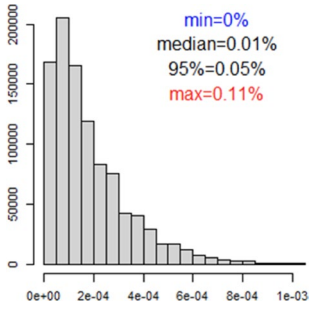
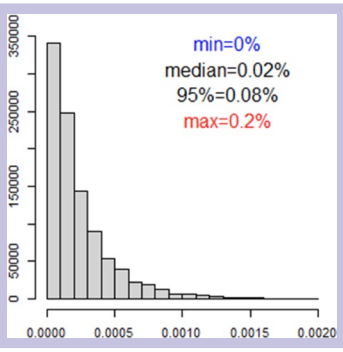
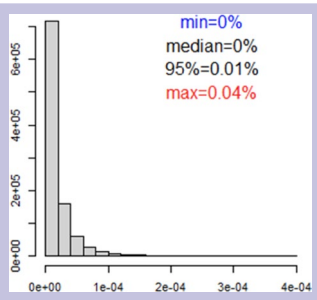
Risk mitigation measures applied	Virus	Final range for the probability of virus introduction	
		if eggs are disinfected only after hardening	**if eggs are disinfected *before and after* hardening**
RMM1 + RMM2	VHSV or IHNV		
	HPR-deleted ISAV		
RMM1 + RMM2 + RMM3	VHSV or IHNV		

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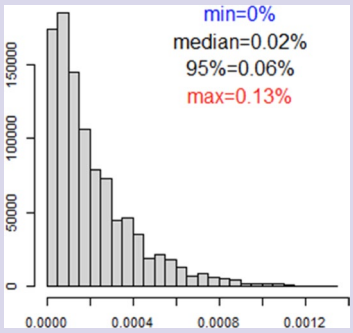
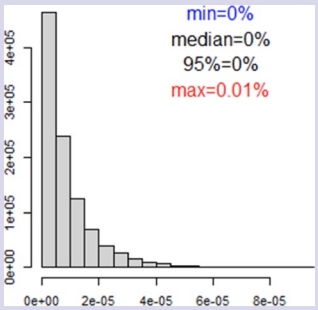
TABLE 7 (Continued)

Risk mitigation measures applied	Virus	Final range for the probability of virus introduction	
		if eggs are disinfected only after hardening	**if eggs are disinfected *before and after* hardening**
	HPR-deleted ISAV		

Abbreviations: HPR-deleted ISAV, highly polymorphic region-deleted infectious salmon anaemia virus, IHNV, infectious haematopoietic necrosis virus; VHSV, viral haemorrhagic septicaemia virus; RMM, risk-mitigation measure.

TABLE 8 Combined results of the assessment of risk mitigation measures to provide a final estimation of the probability of virus introduction in areas declared free of disease, when importing fertilised eggs under Scenario 2.

Risk mitigation measures applied	Virus	Final range for the probability of virus introduction (min – max)
RMM1-. Population testing + RMM2. Surface disinfection of eggs before shipment	VHSV or IHNV	**0.05%–6.6%** if eggs are disinfected only after hardening **0%–1.3%** if eggs are disinfected *before and after* hardening
	HPR-deleted ISAV	**0.05%–6.6%** if eggs are disinfected once, either before hardening or after hardening **0%–1.3%** if eggs are disinfected *before and after* hardening
RMM1-. Population testing + RMM2. Surface disinfection of eggs before shipment + RMM3. Surface disinfection of eggs upon arrival at the receiving establishment	VHSV or IHNV	**0%–0.66%** (0%–0.66% if at origin disinfected only after hardening, and 0%–0.13% if disinfected twice at origin)
	HPR-deleted ISAV	**0%–0.66%** (0%–0.66% if at origin disinfected only after hardening and 0%–0.13% if disinfected twice at origin)

Abbreviations: HPR-deleted ISAV, highly polymorphic region-deleted infectious salmon anaemia virus; IHNV, infectious haematopoietic necrosis virus; RMM, risk-mitigation measure; VHSV, viral haemorrhagic septicaemia virus.

TABLE 9 Full probability distributions (after 1 million simulations) for the final estimation of the probability of virus introduction in areas declared free of disease, when importing fertilised eggs under Scenario 2.

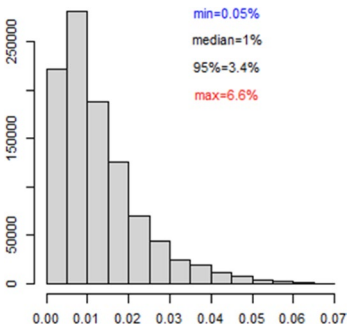
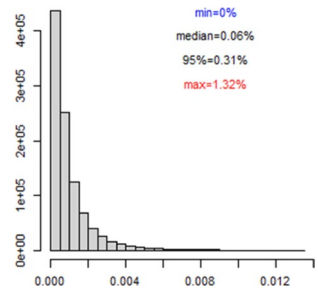
Risk mitigation measures applied	Virus	Final range for the probability of virus introduction	
		if eggs are disinfected only after hardening	**if eggs are disinfected *before and after* hardening**
RMM1 + RMM2	VHSV or IHNV		

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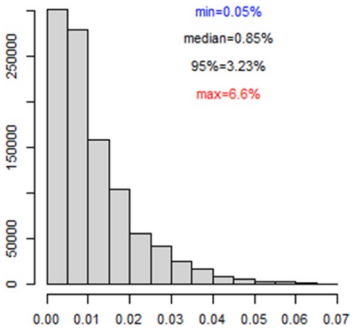
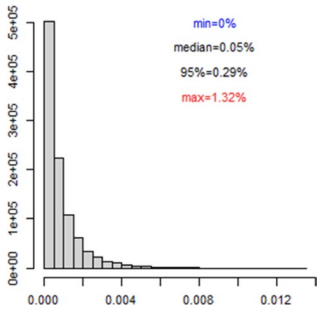
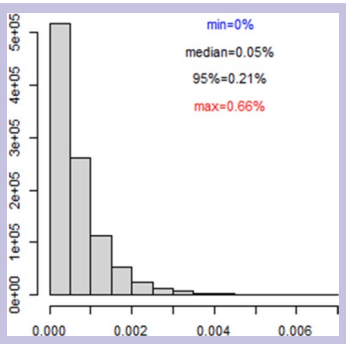
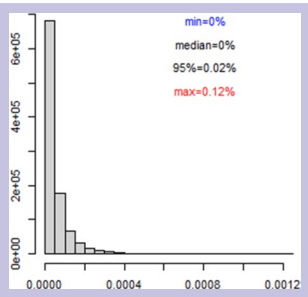
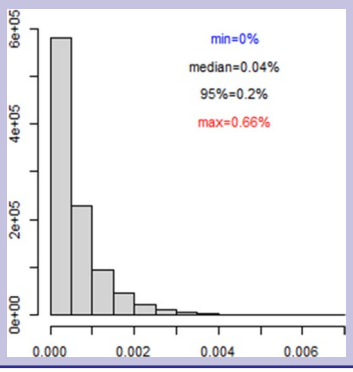
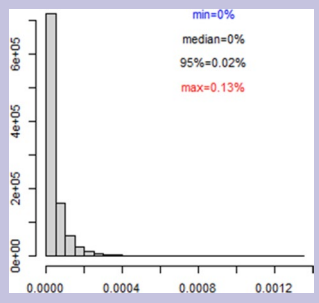
TABLE 9 (Continued)

Risk mitigation measures applied	Virus	Final range for the probability of virus introduction	
		if eggs are disinfected only after hardening	**if eggs are disinfected *before and after* hardening**
	HPR-deleted ISAV		
RMM1 + RMM2 + RMM3	VHSV or IHNV		
	HPR-deleted ISAV		

Abbreviations: HPR-deleted ISAV, highly polymorphic region-deleted infectious salmon anaemia virus; IHNV, infectious haematopoietic necrosis virus; VHSV, viral haemorrhagic septicaemia virus; RMM, risk-mitigation measure.

3.3.3.2 | *Milt*

For milt, as disinfection protocols which can be applied directly to the products are not currently available, testing of parents is the only RMM available at the origin.

For **Scenario 1**, the probability of a false-negative in tests applied individually (individually testing of broodstock), was presented in Figure 2 for VHSV/IHNV (1%–10%), and Figure 3 for HPR-deleted ISAV (1%–15%). These probabilities were assessed, however, assuming the worst-case scenario, in which animals are infected but do not show clinical signs and can go unnoticed. More realistic scenarios should consider that the broodstock will be selected from populations with monitored health status. Following the provisions in the new draft Chapter 4.Z.3 item 1 and 4.Z.4 item 3 of the WOAH Aquatic Animal Health Code, for example, if the animals are tested at origin to ensure 95% confidence of freedom of viruses (2% design prevalence), and then individually tested (with probabilities of failure of 1%–10% for VHSV/IHNV and 1%–15% for HPR-deleted ISAV), then the **probability of an infected parent not being detected is 0.1%–0.5% for VHSV or IHNV** (with median 0.3% and 95% upper percentile of 0.5%) **and 0.1%–0.8% for HPR-deleted ISAV** (with median 0.45% and 95% upper percentile of 0.8%).

For importation, RMMs can also be applied at the destination to ensure that, even if milts could have been contaminated with virus, the virus will not be passed to the progeny or be released into the officially disease-free importing area. After these milts are used to fertilise eggs, two further RMMs can be applied: (i) disinfect the eggs as discussed in the dedicated section above; and (ii) treat the water to ensure disinfection before elimination as effluents.

For Scenario 1, the final **probability of vertical transmission** associated with the importation of milt from areas not officially recognised as disease-free, if the health status of broodstock is determined at the establishment of origin, then individually tested after spawning, and disinfection is applied to the eggs fertilised with the milt at destination varies from

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0% to 0.3%. The probability of virus release into the environment if the water that was in contact with the imported milts is treated before release from the establishment is lower than 0.01.

For **Scenario 2**, where animals are kept at the same establishment their entire life, and the confidence of disease freedom is given only by population testing, the probabilities of failure for RMM1 given in Figure 4 for **VHSV/IHNV (5%–33%)**, and Figure 5 for **HPR-deleted ISAV (5%–33%)**, apply. That is, there is up to 33% probability of milt being produced from infected parents, which would result in potential contamination of milt with the viruses (surface-associated contamination). When disinfection is applied to the fertilised eggs at destination, the **probability of vertical transmission** is reduced to **0.05%–6.6%**, and introduction of the virus into disease-free areas can be further ensured by water treatment of the effluents as described before.

3.3.3.3 | *Unfertilised eggs*

On unfertilised eggs the same results reported for milt applies, however it should be considered that currently there is no movement of unfertilised eggs.

3.4 | ToR2 – Critical points to be controlled in the establishment of origin

Table 10 lists critical points that could be implemented at establishments that produce fertilised eggs and gametes in order to ensure the effectiveness of RMMs for detecting and preventing the spread of VHSV, IHNV or HPR-deleted ISAV identified under ToR 1. The table also includes a description of the biosecurity and management practices associated with these control points.

TABLE 10 Critical points which could be controlled at the establishments where the fertilised eggs and gametes of aquaculture animals are produced, to avoid the spread of ISAV, IHNV and VHSV and ensure early detection if they occur.

Critical points	Description of biosecurity and management practices which could positively affect the critical point
Health and surveillance	
Systematic control of the health status of the population in the establishment producing eggs/gametes	Dead and moribund fish collected daily and tested for ISAV, IHNV and VHSV upon suspicion. Targeted sampling regularly conducted following predetermined plans for laboratory examinations. Regular visits by fish health personnel (Competent Authority or accredited by them) during the growth of brood fish. The health visits must be planned and carried out based on an assessment of the risk of introduction of infection into the facility, development of disease and spread of infection from the facility. Factors that should be considered when scheduling relevant health visits and sampling include: • increased unexplained mortality • clinical signs and necroscopy findings • abnormal behaviour • reduced feed intake • movement of fish into or out of the facility • knowledge of recent disease history at the establishment and in neighbouring farms (e.g. 5 or 10 km radius zones) or at all farms located upstream in the watercourse. The presence of any of the aforementioned factors should initiate suspicion or rather trigger further investigation
Clinical surveillance	In the event of unexplained increased mortality or other signs of disease (in any of the fish populations in the establishment, including the larvae/fish from imported eggs/gametes), the competent authority or health personnel accredited by them should be informed and carry out health visits followed up with testing for the presence of VHSV, IHNV and HPR-deleted ISAV.
Testing of broodstock	Testing of broodstock for IHNV, VHSV and/or HPR-deleted ISAV is conducted by targeted sampling in connection with regular clinical inspections of the fish. Inspections and samplings are done by the competent authorities or personnel approved for this. Testing only to be conducted by laboratories approved by the competent authorities. In addition, broodstock or part of these are tested individually for HPR-deleted ISAV, IHNV and/or VHSV, following stripping.
Compliance with reporting obligations	Any suspicions/positive tests for VHSV, IHNV and/or HPR-deleted ISAV must be reported to the competent authorities
Establishment (egg-producing)	
Water source	**In production systems with an open seawater phase:** The distance between the establishment where the fish that are intended to become broodstock are kept and other establishments with salmonids should be known. No specific minimum distance is specified, but the information on the proximity to other establishments with salmonids should be included in the risk assessment in the event of importation. **In production systems with fresh-water only**: Inlet water should be from sources free from the pathogens in question (i.e. borehole water or surface water that does not have anadromous wild fish populations or, for fish farms located upstream in the watercourse, their health status should be free from those infections). Alternatively, removal of particles by filtration and disinfection by ultraviolet treatment and/or ozone is necessary, with the functional requirements of inactivating the agents in question.

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TABLE 10 (Continued)

Critical points	Description of biosecurity and management practices which could positively affect the critical point
Time since last outbreak of the diseases in question at the establishment and in the neighbouring area	Eradication procedure, duration of quarantine period following the last outbreak must be documented. The time since the last outbreak should be documented and will be used as part of the risk assessment for importing.
Water flow within the establishment	In facilities where both eggs and grow-out fish are kept, water flow should be unidirectional and only from eggs to grow-out fish, but can be recirculated within the specific fish production stage and within the egg sector.
Biosecurity procedures	
Separation of fish and controlled movement within the establishment producing eggs/gametes	**In production systems with an open seawater cycle**, different fish groups/age classes are kept in separate epidemiological units/sub-compartments with hygiene barriers between them to restrain and localise any infection that could occur in a tank. Before fish are moved from one unit to the next, it is recommended to check their health status. Tanks are washed and disinfected between movement of fish. ***In fresh-water only systems***, the only separation applied is between the sectors with eggs and those for grow-out of fish. In RAS, escaped fish living in the pipeline system, but outside the tanks, must be removed.
Origin of the broodfish	Either from disinfected eyed eggs hatched at the establishment or establishments that comply with the same critical control points (at least with documented level of assurance of the health status of the population). Fish can also be introduced when originating from establishments in a zone or compartment that are officially free from the pathogens in question.
Quarantine of any introduced fish	**In production systems with an open seawater cycle**, new fish should be kept under observation for 4–8 weeks to detect clinical sign of disease, before being introduced to the main facility. Not relevant for ***fresh-water-only systems*** (only officially disease-free are introduced).
Egg disinfection procedures	Protocols used for green egg and eyed egg disinfection and their implementation should be well documented.
Compliance with biosecurity plan	A quality assurance system with standard operating procedures and where critical operations are logged and documented needs to be implemented, i.e. RMMs.
Traceability	Ensure there is a system in place to trace all movement of fish/eggs/gametes within and between establishments.
Fomites	Separated for the different sectors within the establishment (including collection, storage and disposal of dead fish). Fomites are washed and disinfected regularly.
Personnel	There should be requirements for personnel education/training. All sectors within the establishment are entered by personnel through hygiene barriers with designated clothes and shoes. Ideally, personnel movement within a work day is unidirectional from eggs to fish though this may not always be possible. However, efficient hygiene barriers can mitigate the risk of virus transmission associated with movement of personnel.
Visitors	Keep records of any non-employees entering the farm, including service people and check whether they have visited other farms. Visitors enter through hygiene barriers with designated clothes and shoes.
Disinfection of services	Documentation of protocols and implementation of disinfection of vessels/trucks for transport, feed, etc. Measures to avoid spillage of water during loading and unloading should be taken.
Protecting establishment from access of birds and mammals	Additional biosecurity measures, such as protecting the establishment from access by birds and mammals (e.g. by wires and nets) would avoid these animals spreading the diseases
Diagnostic laboratory	
Quality assurance and certification	Diagnostic laboratories should follow ISO 17025 standards

Abbreviations: HPR-deleted ISAV, highly polymorphic region-deleted infectious salmon anaemia virus; IHNV, infectious haematopoietic necrosis virus; RAS, Recirculatory Aquaculture System; VHSV, viral haemorrhagic septicaemia virus.

In addition to the critical points identified in Table 10, experts also highlighted the following biosecurity issues:

- Fish can leave the tanks and enter the RAS pipes. These 'pipe fish' can be a reservoir for pathogens and establish contact between different fish groups within an RAS. However, the relatively small number of Atlantic salmon in a broodstock RAS facility makes 'pipe fish' unlikely/uncommon. Nevertheless, clear group separation should be maintained with periodical inspection and emptying the pipe system.
- Interaction with wild fauna. Physical barriers include nets or wires over the farming unit to avoid the entrance of predatory birds (e.g. cormorants and herons), as well as screens or water flow drop at the outlet of the farm to avoid wild fish swimming upstream and entering the farm.

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3.5 | ToR3 – Implementation of effective RMMs

All 18 fish health practitioners answered the survey and a summary of their answers is presented here. The full results are given in Annex D.

3.5.1 | Characterisation of the establishments represented in the survey

The 18 respondents work across 20 countries in Europe (including non-EU countries). Six respondents work in more than one country, and 12 in one country only. Thirteen respondents indicated that they work in the private sector. Overall, four respondents work only with Atlantic salmon establishments, eight only with rainbow trout establishments and six respondents work with both species. Three respondents reported working only with establishments that are providers of eggs; three only with egg receivers; and 11 work both with providers and receivers (one respondent indicated not working with either).

3.5.2 | Adoption of the RMMs (testing and disinfection)

In agreement with the expectation of the working group, **individual testing** at stripping was not a practice conducted among establishments where broodstock stay their entire life in fresh-water (scenario applicable to rainbow trout). **Population testing** based on the current legal EU framework (Commission Delegated Regulation (EU) 2020/689 and subsequent amendments and integrations, see Introduction, Section 1.4) was reported to be widely adopted. However, respondents did report challenges such as high costs, scheduling and coordination issues and incomplete sampling of all year classes or production units. The low frequency of inspections and targeted sampling were also mentioned as challenges. In these types of production, broodstock are commonly reused after stripping and individual testing based on testing of internal organs is not applicable. Testing of ovarian fluid could be feasible but it is not currently common practice.

Among establishments where broodstock spend part of their life in seawater all eight respondents reported **individual testing** for HPR-deleted ISAV (half reported pooling of samples before testing). Only four of the eight respondents reported individual testing generally of pooled samples for VHSV and IHNV. Key challenges reported for individual testing at stripping included the need for sufficient incubator capacity to keep eggs separated until test results arrive and the associated cost.

Disinfection of eggs with iodine was reported as a key biosecurity measure, applied at least once after eggs are fertilised. Practices vary due to differing national regulations and product authorisations within the EU. Small farms can often skip disinfection for locally used eggs, especially when operating in disease-free zones. However, for trade, disinfection is usually applied.

Disinfection at the establishment of destination is not always applied, but at the establishment of origin it is common to apply disinfection to both green and eyed eggs (12 out of 16 respondents). Green egg disinfection is, however, not common in smaller broodstock facilities providing rainbow trout eggs. The protocol recommended by WOAH is generally the protocol of choice.

3.5.3 | Practical experience with the critical control points

The 18 respondents were presented with the list of critical points compiled by the EFSA experts (Table 10) and asked to provide comments based on expertise and practical experience of the major bottlenecks for implementation, difficulties in adoption, common failures or best practices. Their answers were reviewed by the EFSA experts and compiled considering the type of production system with which the respondent worked to work, i.e. production systems where broodstock stay part of their life in seawater (which is the production system under which Atlantic salmons are raised, and which can also be used for production of rainbow trout) versus production systems in which broodfish spend their whole life cycle in fresh-water (used only in rainbow trout production). Note that the production systems with a seawater phase are considered by the EFSA experts to more closely align with the Scenario 1 in this evaluation, in which broodfish can be subjected to individual testing during stripping. Scenario 2, in which population testing is applied, is considered to more closely relate to the current reality of fresh-water-only systems. This has been confirmed by the survey, as described above in the results for the adoption of RMMs.

3.5.3.1 | *Health and surveillance*

Regarding the **systematic control of the health status of the population in an establishment producing eggs/gametes** as detailed in Table 10, respondents working with production systems with a seawater phase emphasised the necessity of regular health checks at salmon farms, recommending at least 12 veterinary visits per year, including diagnostic testing. Responsible farm staff must promptly notify the authorities of any change in fish health status, such as abnormal behaviour or increased mortality. These practices of regular health checks and mandatory notification are well established in legislation and strictly regulated in some countries (respondents reported specifically Norway), forming a key part of

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official inspections. In addition, one respondent suggested e-DNA testing as a tool to strengthen surveillance. Practitioners working in fresh-water only systems agreed on all the factors that should be considered when assessing what constitutes relevant health visits and sampling. In addition to the factors given, biosecurity measures, such as preventing access by predatory birds and mammals and knowledge of the movement of fish in and out of the establishment, were also highlighted. Regular health inspections are conducted by the competent authorities in certain Member States in accordance with the EU regulations and the risk profile of the fish farms. According to the respondents' answers, if not specifically regulated at national level, health visits should be more frequent – e.g. every month or every 2 months – and carried out by experienced personnel, especially after spawning when fish are more vulnerable. In addition, EFSA experts suggested that the farm size should also be taken into account when planning the number of visits. The respondents were of the opinion that there needs to be clearer roles and collaboration between private veterinarians and control authorities, especially for small-scale farms across Europe. According to their responses, more training, awareness and financial support would improve disease monitoring and response. Respondents working in both production systems highlighted a shortage of trained fish veterinarians, which may slow down health interventions. Economic constraints make it difficult to perform all the desired health visits and tests, especially for small-scale farms. Norwegian regulations were considered a strong model for systematic health control both in salmon and trout production.

In relation to **clinical surveillance**, fish health practitioners working with production systems with a seawater phase emphasised that salmon farms primarily test for HPR-deleted ISAV, but the EFSA experts noted that the respondents in this group were from Norway and the Faroe Islands, which have disease-free status for both VHS and IHN, and could explain why farms do not regularly test for these. Clinical surveillance and health visits are regulated by legislation, and in Norway and the Faroe Islands these were reported to be a key element of the competent authority inspections. However, a major challenge is the shortage of trained veterinarians, which can delay timely response to disease signs or increased mortality. Practitioners working in fresh-water only systems reported that trout farmers show mixed awareness of 'unexplained increased mortality or other signs of disease' and it is difficult to define when the farmer should inform the authorities. Farmers normally contact their own veterinarian, who often responds quickly, but many farmers do not fully recognise the need for immediate action. Health visits should ideally occur on the same day that issues arise, but in some regions, private vets should contact the competent authority upon suspicion of VHS, IHN or ISA, who will then conduct sampling for virological examination. The lack of direct reporting to the authorities might reduce the number of suspect cases and delay early detection. In the opinion of the respondents, clearer procedures and better coordination between public and private vets could improve disease prevention and control. For both production systems, the respondents highlighted a shortage of trained veterinarians. Clearer protocols for suspicion-based testing and mortality thresholds are needed to ensure timely action. Commenting on **compliance with reporting obligations**, fish health practitioners working with production systems that include a seawater phase agreed that compliance with reporting obligations is a key focus during inspections by the competent authority. Both private and official laboratories are expected to notify the authorities of any findings of VHSV, IHNV and HPR-deleted ISAV. Practitioners working in fresh-water-only systems highlighted the fact that reporting of VHSV, IHNV and HPR-deleted ISAV is mandatory in the EU, but many farmers are unaware of their reporting duties, though private veterinarians do comply. Standardised formats could improve communication and consistency, and respondents suggested that stronger enforcement of standardised formats is required. For both production systems, the respondents reported that both European and Norwegian regulations are viewed as strong compliance models, but clearer protocols and broader enforcement are required.

3.5.3.2 | *Establishment (egg-producing)*

In relation to the listed requirements for **water sources** in production systems with a seawater phase, fish health practitioners stated that the goal is to have land-based, closed broodstock production. Pumped seawater should be disinfected with UV light and ozone to reduce pathogen risk, and the fresh-water source (well water or surface water) should meet a similar biosecurity standard to minimise the risk of the pathogen being present. EFSA's experts note that even in systems where the broodfish spend part of their life in open pens in the sea, closed fresh-water production systems are always used when the broodstock reach sexual maturity. There are not sexually mature broodstock kept in seawater. In production systems with fresh-water-only, respondents agreed that broodstock fish should only be kept in facilities supplied with borehole or well water, as surface water poses risks regardless of the health status of wild fish. The distance to other facilities and upstream farm health must be factors in risk assessments. Birds, wild animals and feral trout in water sources are considered infection risks. Respondents working in both production systems believed that open-sea broodstock systems pose a high risk of infection. Disease-free status is unlikely without individual testing and strict controls. Closed, land-based systems with reliable pathogen-free water (borehole or well water) or treated water were considered the ideal approach.

As shown in Table 10, EFSA's experts pointed out that the **time since the last outbreak of the diseases in question at the establishment and in the neighbouring area** is an important aspect when considering the safety of an establishment producing eggs/gametes. Asked to comment on general adoption of measures for control of outbreaks and any bottlenecks, survey respondents working in systems with a seawater phase reported that HPR-deleted ISAV outbreaks trigger restriction zones, set by authorities, which affect future production. They reinforced the need for broodstock compartments to be isolated and protected by disinfection of the water source. Those working in fresh-water-only systems also stressed the critical need for the disinfection of farms, their surroundings and equipment if disease is detected. Use of sentinel fish was suggested by one respondent to confirm virus absence post-eradication. Record-keeping was reported

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to often be poor at small farms, affecting traceability. There is broad agreement among respondents working in both production systems on the importance of documenting outbreak history and quarantine measures adopted. Broodstock from restriction zones cannot be used post-ISA outbreaks until restrictions are lifted by the authorities. There was general agreement on the fact that strict compliance, traceability and documentation are necessary, especially for import risk assessments.

Regarding the **waterflow within an establishment**, respondents working with production systems with a seawater phase agreed that water used for the incubation of eggs must be separated from water used for fish production. Recirculation in broodstock facilities is acceptable only with full water treatment, including ozone post-biofilter. Water treatment standards must be equivalent to those for new inlet water. Those working in fresh-water-only systems consider that pathogen presence leads to rapid spread, making internal flow less critical – if the pathogen enters a farm, all sectors must be culled. Parallel tank systems are preferred to create epidemiological subunits. Recirculation with water treatment may help to reduce the risk of disease if properly managed. Respondents working in both production systems agreed that the control of water flow is a critical biosecurity measure. Strict separation and treatment of water for broodstock and eggs are widely supported. Shared water or facilities across sites increase the risk of virus transmission. Recirculation is commonly used in the later broodstock stages, with UV-treated water reused in egg incubation areas.

3.5.3.3 | *Biosecurity procedures*

Separation of fish and controlled movement within an establishment. According to the experience of the respondents, in systems with a seawater phase, 'all-in, all-out' systems are recommended to avoid mixing age groups (and are mandatory in Norway and the Faroe Islands). Open-sea cages for broodstock are discouraged due to the high infection risk, and the challenges of maintaining separate epidemiological units. In fresh-water-only systems, separation was reported to be ideal but difficult without dedicated spaces and equipment. The 'all-in, all-out' system is also considered ideal, but not always possible and less commonly applied. Parallel tank management is preferred to create subunits and reduce disease spread. Broodstock should be isolated with hygiene barriers. In both production systems, respondents highlighted the need for strict water treatment and biosecurity protocols within RAS. Lack of compliance leads to pathogen buildup, especially in land-based and RAS systems. Respondents suggested that third-party biosecurity audits and equipment screening are recommended, but didn't specify how often they should be applied. 'Pipe fish' and biofilter contamination are noted risks in RAS.

Origin of the broodfish. Responses were similar across production systems. Fish health practitioners consider the risk of introduction of different pathogens (not only those listed) is always present, but it can be minimised by using only broodstock obtained from disinfected eggs and farmed only in the same broodstock fish farm. The highest risk of pathogen introduction comes from fish transferred between sites, especially from open pen systems. Knowledge of the history of diseases at the farm of origin was also considered important. Concerns were expressed about the use of wild fish as broodfish.

Quarantine of any introduced fish. According to the experience of the respondents, quarantine of broodfish of a farm's own production in land-based closed-confinement systems is considered essential for systems with a seawater phase and should be coupled with sampling and laboratory testing on moribund or dead fish and on other matrices (e.g. water). Quarantine was not considered critical in fresh-water-only systems. Testing of the fish before introduction in the broodstock facility seems more reliable. It was noted that open-sea pens are unsuitable for quarantine due to water flow.

Compliance with a biosecurity plan. Respondents working in both systems considered standard operating procedures (SOPs) and quality assurance systems important but difficult to implement consistently (practical application remains the main challenge), making the impact of written procedures debatable. They suggested that accreditation bodies should enforce compliance more strictly. They also commented that companies must show a stronger commitment to procedures and documentation, as compliance depends on individual effort and awareness. Biosecurity plans and SOPs should be reviewed and updated annually. Staff training is essential for effective implementation.

Traceability. Respondents working in both systems agreed that traceability systems are applied and widely supported, and these were seen as essential for biosecurity implementation and disease control. Clear documentation of movements should be expected. There was general consensus on the importance and use of traceability practices across sectors.

Fomites. Survey respondents working in both systems considered separation of different sector fomites and disinfection as essential and reported it to be regularly performed by farms. One respondent suggested that farm design and functionality should be a legislative requirement. Some specific comments provided were that dead fish should be handled as hazardous material and that shared equipment like boats in open-sea farms poses a biosecurity risk.

Personnel. There was general agreement among respondents working in both systems on the importance of training and hygiene. As they highlighted, training should be renewed annually for all staff. Personnel must follow hygiene protocols and wear designated gear. Education on movement and behaviour within the farm is essential. Small units may struggle with unidirectional workflows, but hygiene remains a key concern. Hygiene barriers must be placed strategically to avoid disrupting routines. Visitor tracking and movement reporting were suggested.

Visitors. Visitor records and hygiene protocols were reported to be standard practice. Proper handling of contractors and service personnel was emphasised. Ensuring awareness of biosecurity measures was considered essential. Personal protective equipment should be provided to all visitors. Visiting other farms was not always seen as a major threat if proper

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disinfection and correct procedures are followed. Consensus supports the maintenance of clear records and hygiene standards.

Disinfection of services. Respondents working in both systems considered the disinfection of transport services to be of utmost importance, highlighting that it should be standard procedure. Respondents suggested strict control from the authorities of any inter-farm fish movements. Once again respondents highlighted that audits and hygiene inspections should be mandatory. All service equipment should follow disinfection protocols. Among those working in systems with a seawater phase it was explicitly mentioned that authority approval is required for all inter-farm movements and equipment, and that transport units must be certified and designed to prevent effluent spills.

Protecting establishments from the access of birds and mammals. Pest control was considered important in general, even if there was no agreement on the real role of pests in spreading diseases (especially for mammals; birds and wild fish are considered to pose a higher risk). Respondents working in both systems reported that pest control is generally applied and is more efficient in closed buildings, although complete avoidance of the entry of pests at a facility is rarely achievable.

3.5.3.4 | *Diagnostic laboratory*

Across production systems, ISO 17025 certification was reported to be universally applied and considered essential. Further, national reference laboratories and accredited laboratories need to participate regularly in interlaboratory proficiency tests to monitor and validate the quality of their results. Certification builds trust in diagnostic results from egg producers. Although labs must always be certified, respondents highlighted that cut-off values for PCRs may vary between labs, as well as the assays used. They recommended that national authorities should define assay requirements to ensure consistency and supervise laboratories, especially those in the private sector. One respondent suggested that other methods (i.e. PCR and serological methods) should be allowed under self-monitoring regimes.

3.6 | ToR4 – Uncertainty

3.6.1 | ToR1 – Risk of vertical transmission

There is a very high level of certainty in the conclusions on vertical transmission. The results of the SLR, complemented by the expertise of EFSA's experts, lead to the conclusion that almost certainly *(99%–100% certainty)*: (i) true vertical transmission does not occur; (ii) all three viruses can contaminate the surface of eggs and milt, and therefore in the absence of adequate risk mitigation there remains a risk of viral introduction into officially disease-free areas via the importation of gametes or fertilised eggs from non-disease-free areas.

3.6.2 | ToR1 – Effectiveness of the RMMs

The effectiveness of the RMMs was estimated by five EFSA experts, considering each of the three viruses, under two scenarios of testing (Scenario 1 – individual testing at stripping, Scenario 2 – population testing). Detailed estimations from the experts are presented in Annex B and summarised in Figures 2 and 3 for Scenario 1, and Figures 4 and 5 for Scenario 2.

The assessment of the experts for the individual effectiveness of the measures suggested a high level of certainty as demonstrated by the narrow ranges of the individual judgements and their high degree of overlap.

The final probability distributions presented combine the uncertainty from all experts on the effectiveness of each of the individual RMMs considered. As the results are already presented as probability ranges, no further uncertainty assessment was applied.

The estimated probabilities were applied, when relevant, to the assessment of risk associated with the importation of milt. Less literature and field evidence is available for the application of risk mitigations to milt, than for to fertilised eggs so, the conclusions provided for milt are based on expert opinion. Uncertainty was captured in the same way, by providing estimates as probability ranges.

3.7 | ToR2 and ToR3 – Critical points to be controlled at the establishment of origin and implementation of effective RMMs

The list of critical control points proposed was drawn up with full consensus among EFSA's experts. They are almost certain (99%–100% certainty) of the completeness of the list, and of its importance in ensuring the health status of broodstock. The results of the survey, which gathered the opinion and experience of 18 additional experts in Europe, corroborated these results. There was generally little to no disagreement across the opinions expressed in the survey, and no conflicting results on the recommendations made by EFSA's experts about the critical control points.

The results of the survey have been summarised in Section 3.5 (full description in Annex D), and best practice and practical difficulties are provided in Sections 3.5.2 and 3.5.3. The 18 responses across Europe are considered by EFSA's experts

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to be representative of the field experience, and to sufficiently capture the variability in practices adopted across establishments and countries represented in the survey.

In the opinion of EFSA's experts, and as demonstrated through the survey, the main uncertainty remains as to whether the iodine disinfection protocol is implemented properly (respecting time and concentration), and the degree of compliance with the critical control points. The iodine protocol was shown to already be routinely used in the industry. Compliance with the critical control points was highly variable according to size of establishments, the type of production, the involvement of establishments in trading, and the national legislation (prescribed legislation and enforcement). Strong national legislation and enforcement are important to reduce uncertainty around compliance with the critical control points, and adoption of the RMMs using proper protocols. All participating experts elicited are active in Europe. Compliance and enforcement outside Europe were not assessed.

4 | CONCLUSIONS

4.1 | ToR1 – Risk of introduction of VHSV, IHNV and ISAV from non-disease-free areas into officially disease-free areas through fertilised eggs and gametes

4.1.1 | Risk of vertical transmission

4.1.1.1 | *Infection with VHSV*

The available evidence does not substantiate the occurrence of true vertical transmission of infectious VHS virus. The virus can contaminate the surface of eggs and has been detected in reproductive fluids and the surrounding aquatic environment. However, studies indicate that it does not survive the egg incubation period and is not transmitted from parents to progeny if appropriate disinfection measures of the eggs surface are applied. In the absence of adequate risk mitigation, there remains a risk of VHSV introduction into officially VHS-free areas via the importation of gametes or fertilised eggs from non-disease-free areas.

4.1.1.2 | *Infection with IHNV*

The available evidence does not substantiate the occurrence of true vertical transmission of infectious IHNV. The virus can contaminate the surface of eggs and has been detected in reproductive fluids and the surrounding aquatic environment, and the evidence for that is even stronger for IHNV than for VHSV as more studies have reported it. In the absence of adequate risk mitigation, there remains a risk of IHNV introduction into officially IHN-free areas via the importation of gametes or fertilised eggs from non-disease-free areas.

4.1.1.3 | *Infection with HPR-deleted ISAV*

The available evidence does not substantiate the occurrence of true vertical transmission of infectious ISAV (both HPR0 and HPR-deleted). The virus can contaminate the surface of eggs and has been detected in reproductive fluids and the surrounding aquatic environment. In the absence of adequate risk mitigation, there remains a risk of ISAV introduction into officially ISA-free areas via the importation of gametes or fertilised eggs from non-disease-free areas.

4.1.2 | Effectiveness of RMMs

The following conclusions are based on the assumption of disinfection of fertilised eggs being compliant with the disinfection protocol set out in WOAH Aquatic Animals Code Article 4.5.2 or other protocol demonstrated to be similarly effective at the establishment of origin, and compliance of establishments with biosecurity measures that enable their effectiveness and prevent contamination of eggs after disinfection.

This assessment focused on the risk of VHSV, IHNV and HPR-deleted ISAV introduction without considering the consequences following possible introduction of those pathogens. Putting in place the eradication programme described in Regulation (EU) 2020/689 after the introduction of a listed pathogen in a disease-free area will require extensive logistic and financial effort to regain the official freedom status.

4.1.2.1 | *Fertilised eggs*

The probabilities reported in the conclusions below refer to the introduction associated with importation of a batch of eggs under the worst-case scenario (at least one fish was infected with the respective virus in the group of broodstock from which eggs were harvested in a batch, and that infection introduction in the establishment has gone unnoticed).

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These results should be contextualised considering the different host-specificities for each virus. VHSV and IHNV are most frequently reported in rainbow trout in fresh-water-only production systems (Scenario 2), whereas HPR-deleted ISAV has mainly been observed in farmed Atlantic salmon at marine sites (Scenario 1).

Results of the SLRs and expert elicitation allow leading the following conclusions:

1. There is no evidence of true vertical transmission (i.e. vertical transmission which cannot be prevented by disinfections of fertilised eggs) of VHSV, IHNV or ISAV. However, these viruses can contaminate the surface of eggs, therefore RMMs are necessary to reduce the risk of virus introduction via movement of fertilised eggs originating from non-disease-free areas.
2. Distributions for the final probability of transmission due to external contamination of fertilised eggs (egg surface-associated transmission) of VHSV, IHNV and HPR-deleted ISAV were estimated, assuming disinfection of eggs being properly applied compliance. The final probability distributions were:

    a. *For VHSV and IHNV, the median probability was 0.36% (with a 95% upper percentile of 1.33% and maximum of 2%) and for HPR-deleted ISAV the median probability was 0.4% (with a 95% upper percentile of 1.1% and maximum of 1.5%)*. The median probabilities are reduced to 0.02% and 0.01%, respectively, if fertilised eggs are disinfected twice (as green eggs and then as eyed eggs), with maximum probabilities of 0.4% for VHSV and IHNV introduction, and 0.11% for HPR-deleted ISAV.
    b. If the provisions of the WOAH draft chapter are applied at the origin **AND the imported fertilised eggs are disinfected upon arrival** in the importing establishment, the median probability of introduction is reduced to 0.02% for all viruses with one disinfection at the origin (maximum 0.2% for VHSV and IHNV, and 0.13% for HPR-deleted ISAV), and to virtually zero when two disinfections have been applied at the origin (maximum 0.04% for VHSV and IHNV, and 0.01% for HPR-deleted ISAV).

3. In the specific scenario of fresh-water trout production where broodstock are not necessarily killed and individually tested after spawning, and RMMs considered were **population testing** with negative results, coupled with disinfection of fertilised eggs, the **median probabilities of introduction were 1% for VHSV and IHNV** (95% upper percentile of 3.4%, maximum 6.6%) **and 0.85% for HPR-deleted ISAV** (95% upper percentile of 3.23%, maximum 6.6%) if disinfection of eggs is applied once at the origin. **Disinfection upon arrival** would further reduce the median probability of introduction of VHSV and IHNV to 0.05% (maximum 0.66%) and of HPR-deleted ISAV to 0.05% (maximum 0.66%), in the scenario of one disinfection at the origin. Two disinfections at the origin plus disinfection at the destination result in median probabilities of introduction of virtually zero (maximum 0.12% for VHSV and IHNV, and 0.13% for HPR-deleted ISAV).

4.1.2.2 | *Milt*

1. There is no evidence of true vertical transmission of VHSV, IHNV and HPR-deleted ISAV. However, it cannot be excluded that milt from infected parents could be contaminated with the viruses (surface contamination). Therefore, RMMs are necessary to reduce the risk of virus introduction via movement of milt originating from non-disease-free areas.
2. Disinfection procedures, which can be applied directly to the milt before trading, are not currently available; therefore, the absence of infection in the broodstock is the main RMM.
3. If the broodstock come from an establishment which has been tested to provide a 95% confidence of freedom from disease, and are **individually tested** after stripping, with negative results in accordance with procedures described in specific WOAH chapters or EU diagnostic manuals, the risk of introduction via the importation of milt ranges from 0.1% to 0.5% for VHSV or IHNV, and 0.1%–0.8% for HPR-deleted ISAV. In addition to documenting the health status of the broodstock as being free of virus, RMMs can be applied at the establishment receiving imported milts.
4. If eggs fertilised **using the imported milt are properly disinfected**, the final probability of vertical transmission (because of the contamination of the surface of the eggs) associated with the importation of milt from areas not officially recognised as disease-free is reduced to a minimum of 0% and a maximum of 0.3%.
5. If parents are not individually tested, there is a 5%–33% probability that milt would be produced from infected, undetected broodstock, which could cause viral contamination (surface-associated) of milt. When disinfection is applied at destination to the eggs fertilised using the imported milt, the probability of vertical transmission due to contamination of the surface of the eggs in this scenario is reduced to a range of **0.05%–6.6%**.
6. In addition to the measures aiming to prevent vertical transmission, RMMs should also be adopted to reduce the risk of potential release of virus into the environment in disease-free areas. Protocols to disinfect water effluent from the hatching unit, especially in the period between fertilisation and disinfection (e.g. by UV light, ozone, heat or chlorine or a combination, with a documented efficacy of 99.9%), can be adopted before releasing the effluent into the environment.

EFSA's experts note that importation of frozen milt is not, currently, a common practice. Less literature and field evidence is available for the application of risk mitigations to milt, than for fertilised eggs so the conclusions provided for milt are based on expert opinion.

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4.1.2.3 | *Unfertilised eggs*

All the conclusions presented for milt are in principle applicable to unfertilised eggs too. As for milt, there is no currently available method for disinfection because unfertilised eggs are less resistant to the adverse effects of disinfectants than fertilised eggs. However, unfertilised eggs are in general fragile and therefore not a commodity that can easily be transported.

4.2 | ToR2 and ToR3 – Critical points to be controlled at the establishment of origin and implementation of effective RMMs

Methods to enforce and monitor quality in compliance with the critical control points is a critical step to ensure the effectiveness of the RMMs recommended in ToR1. EFSA's experts agreed unanimously on the proposed list of critical control points, and these were corroborated by the survey respondents. These critical points are also given in the WOAH draft Chapter 4.Z 'Control of pathogenic agents in traded gametes and fertilised eggs of fish'.

Conclusions on the feasibility of their adoption and enforcement of compliance are provided below.

4.2.1 | Health and surveillance

Systematic control of the health status and regular clinical surveillance are pivotal to monitor disease presence at establishments. Systematic control of the **health status of the broodstock** population is fully implemented in countries producing large numbers of Atlantic salmon, with 12 compulsory visits per year and strict health monitoring (e.g. Norway and the Faroe Islands). In other European countries, which mainly produce rainbow trout, the number of official health visits in a year is generally lower. The EU Animal Health Law (Regulation (EU) 2016/429), for instance, specifies a number of visits based on the risk profile of the establishments, varying from one visit per year to one visit every 2 or 3 years (see Section 1.3.1). Complementing the official inspections with more regular health visits by trained fish health personnel, was mentioned by the fish experts as being a critical measure to strengthen early detection of any disease introduction and to ensure compliance with the critical control points.

Individual testing of broodstock at stripping (manual extraction of gametes) is only being carried out in countries producing a large number of salmonids (e.g. Norway and the Faroe Islands) which adopt the fresh-water-sea-fresh-water system.

Implementing individual testing is not realistic for traditional rainbow trout production in fresh-water only (Scenario 2) because the broodfish are reused for several years, and there are practical and financial constraints (e.g. shortage of trained personnel, cost of testing). In this scenario the broodfish spend their entire lifespan in water sources free of the pathogens in question, and individual testing at stripping can be replaced by **population testing** before sampling, if compliance with all critical control points and application of the proposed RMMs can be ensured.

4.2.2 | Establishments (egg-producing)

Establishments must document that their water source is free of the pathogens in question, and that all biosecurity measures are implemented and in place to prevent the introduction of VHSV, IHNV and HPR-deleted ISAV.

The basic rules and guidelines for biosecurity and management of establishments with broodstocks, as listed by EFSA's experts, were confirmed by the fish health practitioners to be commonly adopted or easy to implement. The ability of establishments to present a thorough documentation of all the RMMs identified is critical to reduce the risk of introduction of VHSV, IHNV or HPR-deleted ISAV to very low or negligible, particularly for trade.

4.2.3 | Biosecurity measures

A critical control point is the design and implementation of a proper biosecurity plan, customised to the establishment features, and subject to approval by the competent authorities. All critical points listed in Table 10 should be included in the biosecurity plan.

There was consensus among the surveyed fish health practitioners on the list of measures to be implemented in the biosecurity plans. However, a remaining bottleneck is the risk of disease introduction associated with the sea phase of broodstock in systems that include a seawater phase. In this type of system, the conclusion on the importance of individual testing of broodstock is ratified.

As a critical biosecurity measure, the EFSA experts and fish health practitioners highlighted that biosecurity SOPs and plans, including a traceability system, should be reviewed and updated at least once a year. They also considered that to be complete, the biosecurity plan should include provisions for third-party audits on its implementation, and there should be education and training of personnel with regular updates.

For broodstock farms intending to export fertilised eggs, adoption of the 'all-in–all-out' production approach was also considered a critical biosecurity point.

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The fish health practitioners surveyed considered that the risk of introduction of different pathogens, including VHSV, IHNV and HPR-deleted ISAV, is always present and can be minimised by using either broodfish from officially disease-free establishments or broodstock obtained from disinfected eggs and farmed at the same broodstock facility. Alternatively, quarantine could be implemented. However, quarantine can be difficult at establishments using fresh-water only systems due to space and infrastructural limitations.

Disinfection of eggs in accordance with the WOAH protocol is generally adopted at least once at facilities producing fertilised eggs and was reported as a key biosecurity measure. Disinfection of both green and eyed eggs (two disinfections before leaving the establishment of origin) was also commonly reported. Disinfection at the establishment of destination is not always applied, but it was considered feasible by health experts and to be in place for importation from non-disease-free areas.

4.2.4 | Diagnostic laboratory

Health certification is supported by laboratory testing for the specific pathogens considered in this scientific opinion (VHSV, IHNV and HPR-deleted ISAV). Regardless of the type of production and scenario considered, the quality and reproducibility of test results are essential.

The use of laboratories accredited under ISO 17025 to perform the testing was listed among the critical control points. All national reference laboratories in Europe operate under these guidelines.

Survey respondents raised concerns on the application of different thresholds/cut-off values across different laboratories, and advocated for the intervention of an independent controller.

4.3 | ToR4 – Uncertainty

There was high certainty (99%–100%, almost certain) about the effectiveness of RMMs (i.e. eggs and milt disinfection at establishment) in preventing introduction of the viruses into free areas in all scenarios evaluated, as assessed in ToR1.

The main source of uncertainty on ToR2 and 3 relates to the degree of compliance of establishments with identified critical controls. The health control of establishments where gametes originate is a specific requirement in the WOAH chapter being evaluated. For this scientific opinion, EFSA's experts produced a thorough list of control points based on their collective experience and expert opinion. There was consensus among them on the relevance of each point listed. There was a high degree of certainty (99%–100%, *almost certain*) that the main critical control points had been identified and of their importance, as demonstrated by broad agreement among EFSA's experts and the fish health practitioners surveyed. The degree of compliance however can vary with the size of the establishments, the type of production, the involvement of the establishment in trade, and the national legislation (prescribed legislation and enforcement).

While uncertainties around the adoption of the critical control points can impact the confidence in the health status of the broodstock, the risk can be mitigated with proper application of the RMMs (RMMs). Certainty in the effectiveness of iodine disinfection relies on proper adoption of the active compound concentration and time of application.

5 | RECOMMENDATIONS

5.1 | ToR1 – Risk of introduction of VHSV, IHNV and HPR-deleted ISAV from non-disease-free areas into officially disease-free areas through fertilised eggs and gametes

Proper implementation of the measures included in the new draft Chapter 4.Z of the WOAH Aquatic Animal Health Code is recommended to prevent the introduction of VHSV, IHNV and HPR-deleted ISAV when moving gametes and fertilised eggs from non-disease-free to disease-free areas. Those measures include: (i) determining the health status of the broodstock at the aquaculture establishment of origin with protocols that provide at least 95% confidence of freedom; (ii) ensuring that gametes and fertilised eggs originate from a collection and incubation centre which has been approved for that purpose by the competent authority of the place of origin; (iii) individual testing of broodstock at the collection and incubation centre (Scenario 1); (iv) specific provisions in the event of a positive result to ensure destruction of all biological materials and disinfection of the establishment; and (v) disinfection of fertilised eggs at the collection and incubation centre before to trading.

As measures (iii), i.e. individual testing at stripping of broodstock, (Scenario 1) was not reported to be commonly applied and not considered to be applicable in fresh-water only systems, replacement of individual testing with population testing (Scenario 2) to certify the health status of broodstock in these systems is recommended to provide an extremely low risk of introduction (0.05% in contrast to 0.02% for individual testing), along with the other measures in Chapter 4.Z.

According to (v) above, it is recommended that fertilised eggs are disinfected twice (once as green eggs and again after hardening, eyed eggs) before being traded.

Disinfection of fertilised eggs at the establishment receiving imported materials is recommended to further reduce the risk.

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For the trade of gametes, the measures included in the new draft Chapter 4.Z of the WOAH Aquatic Animal Health Code are also recommended to prevent the introduction of VHSV, IHNV and HPR-deleted ISAV into free areas. Disinfection of eggs after fertilisation at destination using the imported milt is recommended. For gametes, it is further recommended to disinfect the water effluents from the hatching unit (e.g. by UV light, ozone, heat or chlorine or a combination of those) before releasing them into the environment to avoid virus spread.

5.2 | ToR2 and ToR3 – Critical points to be controlled at the establishment of origin and implementation of effective RMMs

Methods to enforce and monitor quality in compliance with the critical control points at the establishments of origin (producing fertilised eggs and gametes for exportation) should be in place as those are critical steps to ensure the effectiveness of the RMMs and ensure low probabilities of virus introduction.

The number of health visits (official by CA and complementary by trained fish health personnel) per year should be planned, carried out and documented to ensure systematic control of the health status of the broodstock population.

For establishments where broodstock spend part of their life in the sea, **individual testing** of broodstock at stripping is a critical RMM (Scenario 1 in this opinion). In fresh-water-only systems individual testing is not considered feasible and it is recommended to be replaced by **population testing** before sampling (Scenario 2 in this opinion), if compliance with all critical control points and application of the proposed RMMs can be ensured.

It is recommended to strictly comply with the WOAH **disinfection protocol for fertilised eggs** or with any other protocol which has been proven to effectively eliminate VHSV, IHNV and HPR-deleted ISAV from egg surfaces. For importation of fertilised eggs from non-disease-free areas, it is recommended to disinfect eggs more than once at the producer establishment, which is a practice commonly reported. Disinfection at the establishment of destination, even if not systematically applied, is also recommended when feasible.

Biosecurity SOPs and plans, including a traceability system, should be reviewed and updated at least once a year. Third-party audits of the implementation of the biosecurity plan should be embedded in the plan itself. It is also recommended to maintain and update the education and training of personnel.

Basic rules and guidelines for biosecurity and management of establishments with broodstock are, in general, implementable. For trade, it is recommended for the establishments to present thorough documentation of all the RMMs identified as critical to reduce risk of introduction of VHSV, IHNV or HPR-deleted ISAV to 'very low' or 'negligible'.

Health certification is supported by **laboratory testing** for specific pathogens (VHSV, IHNV, ISAV HPR-deleted). Regardless of the type of production and the scenario considered, the quality and reproducibility of test results is essential. Only a laboratory accredited under ISO 17025 should be used. Participation in interlaboratory proficiency tests, as provided by the EURL, are recommended to harmonise procedures and cut-off values.

ABBREVIATIONS

EURL	European Union Reference Laboratory
HPR-deleted ISAV	highly polymorphic region-deleted infectious salmon anaemia virus
ISAV HPR0	non-pathogenic HPR0 (non-deleted HPR) infectious salmon anaemia virus
IHNV	infectious haematopoietic necrosis virus
PCR	polymerase chain reaction
RA	risk assessment
RAS	recirculating aquaculture systems
RMM	risk mitigation measure
RT-qPCR	reverse transcription quantitative polymerase chain reaction
SLR	systematic literature review
SOP	standard operating procedure
SSCP	Sockeye Salmon Culture Policy
ToR	term of references
VHSV	viral haemorrhagic septicaemia virus
WOAH	World Organisation for Animal Health

GLOSSARY

Broodstock	Reproductively mature, sexually active animals, often fish or other aquatic species, that are intentionally kept to breed and produce offspring.
Collection and incubation centre	According to the new glossary presented as Annex 10 in the Report of the Meeting of WOAH Aquatic Animal Health Standards Commission (18–25 September 2024) it means a facility approved by the competent authority in conformity with the provisions of Chapter 4.Z. for holding broodstock, the collection of eggs, fertilisation and incubation, and the collection, processing and storage of milt.

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Compartment	According to the glossary of the Aquatic Animal Health Code it means one or more aquaculture establishments under a common biosecurity management system containing an aquatic animal population with a distinct health status with respect to a specific disease or diseases for which required surveillance and control measures are applied and basic biosecurity conditions are met for the purpose of international trade. Such must be clearly documented by the competent authorities.
Eyed eggs	Fertilised eggs of fish where the eyes of the embryo are visible. These fertilised eggs may be transported (According to the new glossary presented as Annex 10 in the Report of the Meeting of WOAH Aquatic Animal Health Standards Commission (18–25 September 2024)).
Fertilised eggs	According to the new glossary presented as Annex 10 in the Report of the Meeting of WOAH Aquatic Animal Health Standards Commission (18–25 September 2024) it means a viable fertilised ovum of an aquatic animal.
Gametes	Haploid reproductive cells, the term is used to refer to milt (sperm) and unfertilised eggs (ova).
Green eggs (eggs under hardening)	After fertilisation, during hardening until they become eyed.
Newly fertilised ova of fish	(According to the new glossary presented as Annex 10 in the Report of the Meeting of WOAH Aquatic Animal Health Standards Commission (18–25 September 2024)).
Risk mitigation measures	Measures which can be applied to mitigate or reduce the risk of the virus being present in gametes and fertilised eggs. These include measures to ensure the health status of the broodstock, measures to ensure biosecurity in the aquaculture establishment of origin, as well as measures applied during collection and storage of gametes and fertilised eggs, including disinfection procedures.
Sector	Separated epidemiological unit within the same establishment.
Vertical transmission	Transfer of virus particles from parents to progeny. True vertical transmission cannot be prevented by disinfection of the fertilised eggs. All other vertical transmission is considered 'egg surface-associated transfer'.

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APPENDIX A

Protocol: translation of Terms of Reference into assessment questions and sub-questions

A.1 | STEP 1: FORMULATE THE PROBLEM

A.1.1 | Translation of the mandate into assessment questions

Following the Technical report on Problem Formulation (Risk Sciences International, 2022), the A(gent), P(athway), R(eceptor), I(ntervention) and O(utput) – APRIO – approach was used to draft the assessment questions and subsequent sub-questions to be answered in this scientific opinion. The process for questions formulation can be found in Table A.1, where each terms of reference was formulated in a single assessment question.

A.1.2 | Define the sub-questions of each assessment question and their relationship (conceptual model)

Following the assessment items mentioned in the terms of references, the assessment questions were broken down into four higher order questions (Q) and five lower order sub-questions (SQ).

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TABLE A.1 APRIO elements for formulating the assessment question and sub-questions.

Number as appeared in the mandate	Mandate element	Agent	Pathway	Receptor	Intervention	Output	Lower or higher order sub-questions
ToR1	Risk assessment of the introduction of VHSV, IHNV and HPR-deleted ISAV from a non-disease-free to a disease-free area	VHSV, IHNV and HPR-deleted ISAV	True- and egg surface-associated vertical transmission	Listed fish species	Risk mitigation measures	Qualitative assessment of the risk of transmission via movement of eggs, sperm and gametes	Q1: What are the potential risks of introducing VHSV, IHNV and HPR-deleted ISAV into areas declared free of these pathogens through the introduction of fertilised eggs and gametes originating from regions not free of them?
1.1	Assessment of the risk of vertical transmission of VHSV, IHNV and HPR-deleted ISAV	VHSV, IHNV and HPR-deleted ISAV	Vertical transmission	Listed fish species	Not relevant	Description of the existing evidence on the risk of vertical transmission of VHSV, IHNV and HPR-deleted ISAV	SQ1.1: What is the existing evidence on the probability of vertical transmission of VHSV, IHNV and HPR-deleted ISAV in the listed species? SQ1.2: What are the factors associated with the potential differences in vertical transmission via gametes and fertilised eggs?
1.2	Assessment of all available risk mitigation measures and their ranking in accordance with their effectiveness at preventing the spread or introduction of VHSV, IHNV or HPR-deleted ISAV	VHSV, IHNV and HPR-deleted ISAV	Vertical transmission	Listed fish species	RMMs	Identification of all available risk mitigating measures, along with a description and an assessment of their effectiveness	SQ1.3: What measures can mitigate the risk of spread or introduction of VHSV, IHNV or HPR-deleted ISAV via the movement of gametes or fertilised eggs from a non-disease-free to a disease-free area? SQ1.4: What is the existing evidence supporting the effectiveness of each of these RMMs? SQ1.5: What is the effectiveness of such RMMs?
ToR 2	Identification of the critical points to be controlled at establishments where the fertilised eggs and gametes of aquaculture animals are produced	VHSV, IHNV and HPR-deleted ISAV	Vertical transmission	Listed fish species	Detection of the virus	Identification and description of critical control points at the establishment level	Q2: What are the critical points of those mitigation measures that can be implemented at the establishments where the fertilised eggs and gametes are produced to ensure detection and prevent further spread of VHSV, IHNV or HPR-deleted ISAV?

(Continues)

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TABLE A.1 (Continued)

Number as appeared in the mandate	Mandate element	Agent	Pathway	Receptor	Intervention	Output	Lower or higher order sub-questions
ToR3	Describe the implementation of these effective risk mitigation measures	VHSV, IHNV and HPR-deleted ISAV	Vertical transmission	Listed fish species	RMMs	Description of how effective risk mitigation measures are currently implemented, including practical examples	Q3: How are these effective risk mitigation measures currently implemented, what are potential difficulties for their implementation, and what can be recommended as best practices?
ToR 4	Uncertainty	VHSV, IHNV and HPR-deleted ISAV	Vertical transmission	Listed fish species	Related to the terms of reference	Analysis of the uncertainties that can arise	Q4: What are the uncertainties surrounding the terms of reference?

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A.2 | Step 2. Plan the methods for conducting the assessment

Mandate elements	Sub-question	Evidence needs	Data collection	Assessment methods to be used
(1.1) Risk assessment of the introduction of VHSV, IHNV and HPR-deleted ISAV from a non-disease-free to a disease-free area	SQ1.1: What is the existing evidence on the probability of vertical transmission of VHSV, IHNV and HPR-deleted ISAV in the listed species?	Scientific evidence on occurrence of vertical transmission of VHSV, IHNV or HPR-deleted ISAV.	Systematic literature review (SLR) integrated, as appropriate, with expert knowledge and critical appraisal.	Descriptive analysis of the information collected (summary table and descriptive text)
	SQ1.2: What are the factors associated with the potential differences in vertical transmission via gametes and fertilised eggs?	Scientific evidence on the potential differences in transmission by gametes and fertilised eggs.	SLR integrated, as appropriate, with expert knowledge and critical appraisal.	Descriptive analysis of the information collected (summary table and descriptive text)
(1.2) Assessment of all available risk mitigation measures and their ranking in accordance with their effectiveness at preventing the spread or introduction of VHSV, IHNV or HPR-deleted ISAV	SQ1.3: What measures can mitigate the risk of spread or introduction of VHSV, IHNV or HPR-deleted ISAV via the movement of gametes or fertilised eggs from a non-disease-free to a disease-free area?	Information on all available RMMs, including those proposed in Chapter 4.Z. of the WOAH Aquatic Code.	SLR integrated, as appropriate, with expert knowledge and critical appraisal.	Description of the various available RMMs, including those proposed in the WOAH Aquatic Code
	SQ1.4: What is the existing evidence supporting the effectiveness of each of these RMMs?	Scientific evidence on the effectiveness of the identified measures.	SLR integrated, as appropriate, with expert knowledge and critical appraisal.	Descriptive analysis of the information collected (summary table and descriptive text)
	SQ1.5: What is the effectiveness of such RMMs?	Scientific evidence on the effectiveness of the identified measures.	Working group internal discussion, potentially complemented by an expert knowledge elicitation by a larger expert group using criteria for the definition of effectiveness.	Summary table of the various risk mitigation measures ranked according to their effectiveness
(2) Identification of the critical points to be controlled at establishments where the fertilised eggs and gametes of aquaculture animals are produced	Q2: What are the critical points of those mitigation measures that can be implemented at the establishments where the fertilised eggs and gametes are produced to ensure detection and prevent further spread of VHSV, IHNV or HPR-deleted ISAV?	Information on those critical control points at the establishment level.	SLR integrated, as appropriate, with expert knowledge and critical appraisal.	Description of the various critical control points identified
(3) Describe real case implementation of these effective risk mitigation measures	Q3: How are these effective risk mitigation measures currently implemented, what are potential difficulties for their implementation, and what can be recommended as best practices?	Information on effective risk mitigations measures currently applied by Member States.	Survey sent to relevant experts across different Member States.	Summary table and description of relevant examples of effective risk mitigations measures currently applied in Member States
(4) Uncertainty	Q4: What are the uncertainties surrounding the terms of reference?	Information on the uncertainties that arise.	Working group internal discussion.	Descriptive analysis of the information collected (summary table and descriptive text) and quantification of the uncertainty when possible/needed

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