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REPORT OF THE MEETING OF THE OIE *AD HOC* GROUP ON OIE ELECTRONIC *AD HOC* GROUP ON THE OIE LIST OF AQUATIC ANIMALS DISEASES (FINFISH TEAM)

December 2010–January 2011

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The OIE *ad hoc* Group on the OIE List of Aquatic Animal Diseases (Finfish) (the *ad hoc* Group) met electronically during December 2011 and January 2011.

The members of the *ad hoc* Group are listed at Annex 1. The agenda adopted is given at Annex 2.

The *ad hoc* Group was convened to undertake an assessment of pancreas disease against the *Criteria for Listing Aquatic Animal Diseases* provided in Chapter 1.2. of the OIE *Aquatic Animal Health Code* (*Aquatic Code*) taking into consideration the assessment provided by Chile.

Overall, the *ad hoc* Group concluded that there is insufficient data to support listing at this point, but this should be reviewed should such evidence be provided.

Summarised discussions and key recommendations made by the *ad hoc* Group are provided under the following headings:

1. Review of the Chilean submission.
2. Conclusions and recommendations (1)
3. *Ad hoc* Group's *de novo* evaluation of the case for listing of PD
4. Conclusions and recommendations (2)

1. Review of the Chilean submission

The Chilean submission requesting listing of Pancreas Disease (PD) in the Aquatic Animal Diseases list of the OIE is included as Annex 3. The ad hoc Group reviewed the submission against the criteria, parameters and explanatory notes laid down in the *Aquatic Code* Article 1.2.1. and made the following comments:

General comments on the submission

Case definition, etc.

The submission requests listing of pancreas disease (PD), a term that is generally used to refer to describe the clinical outcome of infection in Atlantic salmon (*Salmo salar* L.). We recommend that this be reconsidered and would suggest that "infection with salmon pancreas disease virus (SPDV)" be used instead. While the name salmonid alphavirus (SAV) is widely used, and accepted, by those working in this field, the International Committee on Viral Taxonomy (ICTV) lists SPDV as the official virus species name, with sleeping disease virus (SDV) being listed as a strain or subtype (http://www.ictvdb.org/Ictv/fs_togav.htm; accessed 14/01/11). Article 1.2.1 of the *Aquatic Code* stipulates that the submission should be accompanied by a case definition. This is provided, but refers to PD i.e. "Detection of the aetiological agent of PD in susceptible species with or without manifest clinical symptoms" The group suggests that this should be amended to "Infection of susceptible species with SPDV, with or without manifest clinical signs".

References cited

There are a number of items that needed revision:

i. Page 1, last para- Brown and Deegan 2006 is cited; the listing of this in the references should be amended to the standard format and relocated in the list.

ii. The reference list also contains a Brown and Deegan 2008 which is not cited.

iii. Page 1, last para- McLoughlin et al. 1998 is cited but not included in the list of references

iv. Page 2, McLaoughlin *et al.* 2007 is cited but not included in the list of references

v. Page 3, McLoughlin and Graham 2007 is cited but not included in the list of references

vi. Page 4, Graham *et al.* 2010 is cited but not included in the list of references

vii. Page 5, Jewhurst *et al.* 2004 is cited but not included in the list of references

viii. Page 5, Graham *et al.* 2008 is cited but not included in the list of references

ix. Hodneland *et al.* 2006 is cited on a number of occasions, but not listed, while Hodneland 2006 (thesis) is listed but not cited

x. Page 6, Graham *et al.* 2006 is listed but not cited

xi. Page 7, Kristoffersen 2009 is listed but not cited

A. Consequences (parameter 1- the agent has been shown to cause significant production losses at a national or multinational (zonal or regional) level).

Overall, the group believe there is a clear case to support "significant production losses...". The explanatory notes in 1.2.1 indicate that these losses should be "..related primarily to the agent and not management or environmental factors." The submission does not perhaps provide evidence in support of this, although the group does consider this to be the case.

A. Consequences (parameters 2 and 3)

Parameter 2 (the disease has been shown to...negatively affect wild aquatic animal populations that are an asset worth protecting for economic or ecological reasons)- the only evidence presented is from the recent paper by Snow et al (2010) which found real time RTPCR signals in flat fish, although no virus was isolated. The group do not believe that this in itself is sufficient evidence in light of the explanatory notes in 1.2.1.

Parameter 3 (the agent is of public health concern)- no data presented.

B. Spread Parameter 4 (Infectious aetiology of the disease is proven).

Overall, the group consider that the infectious aetiology is proven, although we have a few comments re the submission.

a) Para 1- the subtypes are defined according to partial sequence data of E2, rather than "...based on differences in geographical location, susceptible species and their presence in sea or fresh water.."

b) Para 1- SAV subtype 4 has also been detected in outbreaks of PD in Ireland (Graham et al. 2010).

c) Para 2- it is inaccurate to refer to "The aetiological agent of SAV1...". rather, the aetiological agent of PD identified by Nelson et al. 1995 is now considered to belong to SAV subtype 1.

d) Para 3 refers to the "..identity of both (SPD and SD)..." and the "genomes of PD and SD..."- these should refer to the viruses ie SPDV and SDV, and in the latter case the genome sizes quoted strictly speaking only refer to the two strains sequenced.

e) Para 4 refers to "clinical signs…..weight loss and increased mortality": The increased mortality usually occurs before the weight loss and the development of runts.

f) Para 5- while the inability to secrete digestive enzymes may indeed contribute to decreased body condition it is not the only cause- e.g. fish are also inappetant. Also & as above, the decrease in body condition usually follows after the onset of the disease (or the infection).

B. Spread Parameter 5 (...aetiology is not yet known)

No data presented/not applicable

Potential for international spread, including via live animals, their products or fomites (parameter 6)

The data provided gives support for direct transmission of the agent rather than via vectors or fomites. In relation to the actual text.

a) Para 1. Should read "...average half life of at least 5.7." rather than "...average life..."

b) Para 1. Graham 2007 a, b, c are all cited to support spread of virus through water between sites- not all are relevant in this context.

c) Para 1. Graham et al. 2010 is cited as having "...showed the persistence of the virus in the environment." Rather, this provided supporting evidence for persistence of virus in recovered fish which could then be transferred to naive populations; an alternative hypothesis could be that both populations were exposed from a common environmental source.

d) Para 2. Karlsen et al. Found the SAV subtype 3 strains which they examined to be very homogenous- thus caution is required when using these sequences for molecular epidemiological purposes, as is done here.

e) Para 3. The sentence "To support this hypothesis, we..." needs clarification. The precise meaning is unclear, and the conclusion open to question: "...this leads to establish that the healthy population is infected from fish that had been moved to the sea after recovery from the disease.." especially as this implies that the original source of of the infection was in freshwater. Rewording of this sentence would make the intended meaning clearer.

f) Para 5.this paragraph would read better if it began with e.g. "All non-salmonid alphaviruses share a common...".

   i) Despite the above points, the group agree with the evidence provided that virus can be directly transmitted between fish.

   ii) The group agree that there is a potential risk of movement of virus with movement of live fish, particularly if these are currently undergoing an active infection.

g) In relation to vertical transmission via eggs, the group consider the potential for this to be unproven, particularly when the explanatory notes of 1.2.1 are taken into consideration i.e. that "...under international trading practices, entry and establishment of the disease is a likely risk". See the group's review in the second section of the report for further information.

h) Overall, the group felt that there was insufficient data presented to confirm that infection with SPDV met this criterion. It would be helpful to have further supporting data in relation to international trading practices, and the likelihood of both introduction and establishment of disease by live fish, their products or fomites.

Potential for spread (parameter 7 − several countries or countries with zones may be declared free of the disease)

Evidence is presented for several countries currently being free:

a) Chile, based on cell culture and RT-PCR surveys (it is not clear if PCR was used to confirm cell cultures as negative from 2003 (para 2) or only in the survey conducted in para 1. This should be clarified.

b) Iceland, Denmark and Australia have been declared free, based on cell culture surveillance.

c) The group had a number of comments and concerns in relation to this section of the submission:

d) Concerning this parameter 7, referring to chapter 1.4. of the *Aquatic Code*, the document from Chile does not give information about the surveillance programmes, the sample sizes for virological analysis etc

   i) The precise scope of these declarations is not clear- do these relate to (Atlantic) salmon only, or other species also (including rainbow trout; *Oncorhynchus mykiss*).

ii) It is not clear on what basis these declarations have been made- are these by the countries themselves, or by Chile on receipt of suitable data? The group are not aware that any of this data in support of freedom has been published via peer-review. It would be helpful if the submission contained more detail, including the basis/bases for such declaration, i.e. consistent with various pathways laid down in Chapter 1.4 of the *Aquatic Code*, including the statistical validity of the approach taken. In relation to the requirements for listing it is recognised that it may be sufficient for Chile alone to be able to demonstrate freedom but parameter 7 of the listing criteria requires that 'several countries or countries with zones may be declared free…', not just one.

iii) In relation to the testing described, the group would have have reservations about declarations based solely on cell culture and cpe, without either immunostaining or RTPCR to confirm cultures as negative. This is based on the absence of cpe which can occur, particularly at low passage. (Graham et al. (2003); Karlsen et al. (2005)).

Diagnosis (parameter 8 − a repeatable and robust means of detection/diagnosis exists).

The group accept that a range of diagnostic methods are available to permit diagnosis of infection with SPDV.

2. Conclusions and recommendations (1)

Infection with SPDV meets several of the criteria for listing by the OIE. However, without additional information relating to points 6 and 7, as outlined above, the *ad hoc* Group is unable to endorse the assessment of Chile for these two criteria.

It is recommended that the submission be revised and resubmitted, providing additional supporting information in relation to these two criteria.

3. Ad hoc Group's *de novo* evaluation of the case for listing of PD

The *ad hoc* Group considered the case for the listing of infection of susceptible species with SPDV against each of the criteria, in the *Aquatic Code* Article 1.2.1., based on the peer-reviewed literature and their own knowledge and experience and in relation to each of the parameters conclude:

1. A. Consequences (parameter 1- the agent has been shown to cause significant production losses at a national or multinational (zonal or regional) level). The economic significance of infection with SPDV, in both Atlantic salmon and rainbow trout is well recognised, and accept that this criterion is met.

2. A. Consequences (parameter 2- the disease has been shown to...negatively affect wild aquatic animal populations that are an asset worth protecting for economic or ecological reasons). The group consider that there is currently insufficient evidence available to satisfy this criterion.

3. A. Consequences (parameters 3- the agent is of public health concern). The group is unaware of any evidence to support this claim.

4. B. Spread Parameter 4 (Infectious aetiology of the disease is proven). The group accepts that infection with SPDV causes the conditions known as pancreas disease and sleeping disease, for which strains of the virus are considered to be the aetiological agent.

5. B. Spread Parameter 5 (...aetiology is not yet known) Not relevant.

6. B. Potential for international spread, including via live animals, their products or fomites.

In order to satisfy this criterion, it is necessary to establish firstly that international trade exists or is likely to develop. The group recognises that this is the case, particularly in terms of eggs and products e.g. fillets. Secondly, that international trading practices are likely to facilitate the entry and establishment of disease.

In relation to this second point, a thorough import risk analysis would be required to satisfactorily answer this question. However, in relation to entry of the disease, this could potentially occur via live fish, eggs, or products. In relation to entry via live fish, it is conceivable that if fish were introduced when viraemic, the potential for direct horizontal transmission exists. In addition, the detection of RTPCR signals for prolonged periods following infection has been demonstrated in both experimental and field studies (Christie et al. 2007, Graham et al. 2010, Jansen et al. 2010b), and there is evidence to support the claim that this is indicative of a carrier state, although this remains to be definitively proven. The risk of establishment of infection associated with such movements would obviously be influenced by a range of factors, including frequency and size of shipments and husbandry factors post-import.

In relation to introduction and spread by eggs- the role of vertical transmission in the epidemiology of SPDV infections has caused much debate. Bratland et al. (2009) reported very low incidence of RT-PCR signal for SPDV in ova, eyed eggs and fry from early maturing broodstock, although all parr and pre-smolts tested were negative, as were all sample types derived from normally maturing broodstock. In a similar RT-PCR-based study of freshwater sites, Jansen et al. (2010a) failed to detect any positive signals that would provide evidence in support of vertical transmission. Castric et al. (2005) demonstrated that a high concentration of SDV injected intraperitoneally to rainbow trout two weeks before spawning resulted in the presence of the virus in the fertilised eggs, even after surface disinfection. However a recent detailed experimental study by Kongtorp et al. (2010) failed to find evidence of vertical transmission, with screening of freshwater sites in the same study also yielding consistently negative results. The Norwegian Scientific Committee for Food Safety has recently made a risk assessment on brood fish surveillance and vertical transmission of infection, concluding that the risk of vertical transmission of SAV is insignificant (Anon, 2011).

Disinfectants commonly used in aquaculture, including for the disinfection of eggs, have been shown to be effective against SPDV (Graham et al. 2007b). Moreover, there have been very large numbers of eggs moved from Europe to Chile, and from Norway to Scotland. If there were a likely risk of introduction by this means, it is expected that SPDV would have been introduced to Chile and that SAV subtype 3 strains (which thus far have only been detected in Norway) would emerge in Scotland. To date there is no evidence of either of these having occurred, suggesting that the risk of introduction via movement of eggs under international trading practices, is low to negligible.

So far as the group is aware, there are no peer-reviewed publications describing the introduction of SPDV via fish products. There is anecdotal evidence that SAV subtype 2 may have been introduced to the United Kingdom via imported trout fillets (Graham et al. 2003), although there is no confirmatory evidence to support this. However, it would seem likely that the flesh of fish that were viraemic at slaughter would contain SPDV and that importation of such products would represent a potential means of introduction of the virus.

Overall, the group considers that there is a likelihood that SPDV could be introduced to a free country or zone by imports of live fish and it is likely that more trade will develop as and when it becomes economically profitable, but there is need for evidence to confirm that movements of live fish of the SPDV-susceptible species are part of international trade to countries believed to be free of SPDV. In the absence of definitive evidence supporting the likelihood of entry and establishment by live fish, eggs or by products, the group consider that there is insufficient evidence that SPDV meets this criterion.

7. B Potential for spread (parameter 7- several countries or countries with zones may be declared free of the disease).

In the absence of peer-reviewed publications or provision of additional data the group is not able to confirm that SPDV meets this criterion. Any such data relating to declarations of freedom should satisfy OIE requirements and be carried out using appropriate diagnostics tests.

8. C Diagnosis (parameter 8- a repeatable and robust means of detection/diagnosis exists).

The group agree that there are a range of diagnostic methods which when used appropriately allow repeatable detection and diagnosis. These include histopathology, virus isolation and RT-PCR. In addition, the use of virus neutralization assays to detect antibodies to SPDV has been extensively used and is a valuable method for evaluating the sanitary status for fish. The group has concerns about the use of virus isolation by cell culture for diagnosis, even in diseased fish. The presence of virus does not always induce a cytopathic effect (Graham et al. 2003), and even when present may not be readily identified by inexperienced observers, especially in the first passages, although these problems can largely be overcome by the use of immunostaining when reading cultures (Jewhurst et al. 2004). Additionally, clinically affected fish are often culture negative by the stage of the disease process at which clinical signs develop. As already mentioned, RTPCR signals in tissues persist for longer periods than that for which virus can be isolated.

4. Conclusions and recommendations (2)

The two areas where the *ad hoc* Group was currently unable to confirm that the necessary evidence is available to support the criteria are for 6 and 7. It may be possible to provide additional data in support of these points in a revised submission by Chile.

Overall, the *ad hoc* Group assessed infection with SPDV against each of the criteria as follows and concluded that there is insufficient data to support listing at this point, but this should be reviewed should such evidence be provided:

Disease considered by the AHG	**Assessment Against the OIE Listing Criteria in the *Aquatic Code***								**Ad hoc Group conclusion**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	
Infection with SPDV	+	-	-	+	NA	?	?	+	There is insufficient data to support listing at this point, but this should be reviewed should such evidence be provided

Additional References

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Annex 1

MEETING OF THE OIE ELECTRONIC *AD HOC* GROUP ON THE OIE LIST OF AQUATIC ANIMALS DISEASES

(FINFISH TEAM)

December 2010–February 2011

List of participants

MEMBERS OF THE *AD HOC* GROUP

Dr David Graham (Chair)
Animal Health Ireland
c/o 42 Ballymaglave Road,
Ballynahinch, Co. Down,
NORTHERN IRELAND
BT24 8QB
Email: david@animalhealthireland.ie

Dr Jeanette Castric
Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (Anses)
Site de Brest,
Technopôle Brest Iroise,
FR - 29280 Plouzané,
FRANCE
E-mail: jeannette.castric@anses.fr

Dr Torunn Taksdal
National Veterinary Institute
Section of Fish Health
Po box 750 Sentrum
0105 Oslo
NORWAY
Email: torunn.taksdal@vetinst.no

Dr Barry Hill (President, AAHSC)
CEFAS -Weymouth Laboratory
Barrack Road, The Nothe
Weymouth, Dorset DT4 8UB
UNITED KINGDOM
Tel.: (44-1305) 20.66.00
Fax: (44-1305) 20.66.01
E-mail: b.j.hill@cefas.co.uk

OIE HEADQUARTERS

Dr Gillian Mylrea
Deputy Head
rnational Trade Department
OIE
E-mail: g.mylrea@oie.int

Annex 2

MEETING OF THE OIE ELECTRONIC *AD HOC* GROUP ON THE OIE LIST OF AQUATIC ANIMALS DISEASES

(FINFISH TEAM)

December 2010–February 2011

Adopted agenda

1. Undertake an assessment of pancreas disease against the *Criteria for Listing Aquatic Animal Diseases* provided in Chapter 1.2. of the *Aquatic Animal Health Code*, taking into consideration the assessment provided by Chile.

2. Submit a report to the OIE Aquatic Animal Health Standards Commission by 28th January 2011.

Annex 3

GOBIERNO DE CHILE
SERVICIO NACIONAL DE PESCA

Request for listing Pancreas Disease (PD) in the Aquatic Animal Diseases list of the OIE

The following document is submitted in addition to what was required by Chile regarding the comments on the report of the October 2009 of the Aquatic Animals Commission's meeting, in which a request was made to evaluate the incorporation of Pancreas Disease (PD) to the diseases listed.

Case definition of PD: Detection of the etiologic agent of PD in susceptible species with or without manifest clinical symptoms.

Below is the information regarding the criteria and parameters that the disease meets for its incorporation in the list.

A. CONSEQUENCES

Parameter No. 1: The disease has been shown to cause significant production losses at a national or multinational (zonal or regional) level.

There are several subtypes of salmon Alphavirus (SAV), which are divided into etiologic agents of PD in Atlantic salmon, Salmo salar (Nelson *et al.*, 1995), and sleeping disease (SD) in rainbow trout, Oncorhynchus mykiss (Castric *et al.*, 1997) which are further classified by their geographical distribution (Fringuelli *et al.*, 2008).

PD is a contagious viral disease that primarily affects salmonids in the marine production phase, causing among other signs, sudden loss of appetite, lethargy, increased number of faecal casts in the cages and mortality (McVicar, 1987; McLoughlin *et al.*, 2002). It was first described in Scotland by Munro *et al.* (1984). Since then, the disease has been reported also in Ireland, Norway and the U.S. (Kent and Elston 1987, McLoughlin *et al.*, 1996; Taksdal *et al.*, 2007; Fringuelli *et al.*, 2008), where the most severe economic impacts have been recorded in Ireland, Norway and Scotland (Rodger & Mitchell, 2007). The outbreak described in U.S. occurred in 1987 and there was no detection of the virus and is considered as the only outbreak of PD outside Europe (Kent and Elston, 1987), however, there has also been a record of a double infection with Infectious Salmon Anemia Virus and a Toga-like virus in New Brunswick, Canada, but there is no further information published regarding that agent (Kibenge *et al*., 2000).

Sleeping Disease, described in France in 1994, causes a condition similar to PD in rainbow trout, Oncorhynchus mykiss in the freshwater phase and it has been reported in addition to France, in Italy, Spain, Germany and the UK (Castric *et al.*, 1997; Graham *et al.*, 2007a; Franguelli *et al.*, 2008).

PD has been recognized as a disease of economical importance in salmon aquaculture, in part, due to the high mortality rates that may occur during outbreaks, which can vary from 0.1 to 63%. The mean duration of increased mortality following an outbreak of PD in a cohort study by Jansen *et al.* (2010), was 2.8 months (range 1-6). There were also detrimental effects on growth rate and problems caused by fish movement restriction due to illness (Crockford *et al.*, 1999; McLoughlin *et al.*, 2003; Ruane *et al.*, 2005, Brown and Deegan, 2006; Rodger and Mitchell, 2007). PD was a major problem for the salmon industry in Ireland during the decade of 1990, presenting mortality rates of up to 50% in the sites affected (Menzies *et al.*, 1996), there was a reduction in clinical severity of the disease during the years that followed, although it was possible to detect mild infections (McLoughlin *et al.*, 1998). PD has since re-emerged with a

Annex 3 (cont.)

considerable increase in incidence, with 59% of affected sites in 2002, while in 2003 and 2004, outbreaks occurred in 62% and 86% of sites, respectively, with a loss on the growth rate of 11.4% in the period of two years (Rodger & Mitchell, 2007) causing a significant decrease in the production of Atlantic salmon during the sea phase (Menzies *et al.*, 1996; McLoughlin *et al.*, 2003). It is estimated that PD in Ireland, has resulted in a monetary loss of 35 million Euros. In Norway, the economic loss is estimated at 100 million Euros per year. Finally in Scotland, PD has been responsible for losses that have been quantified by half a million Euros per site affected (Ruane *et al.*, 2008).

These results reveal the economic threat posed by the disease to salmon farming in these three countries. Considering the economic losses associated with outbreaks of PD, the disease was listed as a notifiable disease in the B list of diseases of the Norwegian Food Safety Authority (NFSA) in 2007 (Parsons, 2005; Ruane *et al.*, 2008; Skjelstad *et al.*, 2008).

An economic model to estimate the costs directly associated with PD outbreaks in Norway including biological losses, treatments, prevention and insurance payments, and other costs, reveals that in one site with 500,000 smolts, compared with a site in the same conditions without the disease, direct costs would be NOK (Norwegian crowns) 14.4 million, reducing the population for sale to 70% of the biomass and generating an increase in the cost of production of 6 NOK per kilogram (Aunsmo *et al.*, 2010).

Parameter No. 2: The disease has been shown to or scientific evidence indicates that it is likely to negatively affect wild aquatic animal populations that are an asset worth protecting for economic or ecological reasons.

A study by Snow *et al.* (2010), detected SAV in wild fish by real time RT-PCR. The species in which the virus was detected were Limanda limanda, Pleuronectes Hippoglossoides platessoides and platesa, all flatfish that were caught from sea water in the vicinity of areas with aquaculture activities.

B. SPREAD

Parameter No. 4: Infectious aetiology of the disease is proven.

To the date, 6 subtypes of Salmon Alphavirus (SAV) have been described; they have been defined based on differences in geographic location, susceptible species and their presence in sea or fresh water (Fringuelli *et al.*, 2008). SAV1 (Scotland, Ireland) and SAV3 (Norway) are the etiologic agent of PD in Atlantic salmon, Salmo salar (Nelson *et al.*, 1995) and SAV2 (France, England, Scotland, Italy, Spain, Germany) is the agent of SD in rainbow trout, Oncorhynchus mykiss (Castric *et al.*, 1997; Villoing *et al.*, 2000; Hodneland *et al.*, 2005), (Table 1. McLaughlin *et al.*, 2007) and was later isolated from Atlantic salmon in Scotland (Fringuelli *et al.*, 2008). Subtypes SAV4, SAV5 and SAV6 are also etiologic agents of PD and are responsible for outbreaks in Atlantic salmon in the United Kingdom (Fringuelli *et al.*, 2008, Snow *et al.*, 2010).

The etiologic agent of SAV1 was initially described as a Togavirus (Nelson *et al.*, 1995), later, in 1999, a study where a partial comparison of genomic sequence with a known Alphavirus arthropods was made, and based on the genomic organization of the region analyzed and the homologies of structural proteins, it was able to be classified as the first reported Alphavirus in fish (Weston *et al.*, 1999). The genomic organization between SAV1 and SAV3 is identical and the similarity with SAV2 is 92.9% and 91.6%, respectively (Hodneland *et al.*, 2005).

Annex 3 (cont.)

It has also been described that PD and SD share similar histopathological lesions in heart, muscle and pancreas and that fish can acquire cross-protection (Boucher and Laurencin, 1996, Weston *et al.*, 2002).

Table 1 Summary of salmonid alphavirus virus (SAV) infections, their geographical distribution and species susceptibility

Virus name	Virus subtype	Location	Species	Disease	Expt. infections
Salmon pancreas disease virus	SAV 1	Ireland, Scotland	Atlantic salmon	Pancreas disease	Atlantic salmon, rainbow and brown trout
Sleeping disease virus	SAV 2	France, England, Scotland, Spain, Italy, Germany	Rainbow trout	Sleeping disease	Atlantic salmon, rainbow and brown trout
Norwegian salmon alphavirus	SAV 3	Norway	Atlantic salmon, rainbow trout	Pancreas disease	Atlantic salmon and rainbow trout

Nelson *et al.* (1995) reported the first isolation of virus, demonstrating its ability to be reproduced experimentally. It was described as an RNA virus of spherical (65.5 +/- 4.3 nm in diameter), enveloped, sensitive to chloroform, rapidly inactivated at pH 3.0 and 50 º C and has a buoyant density in cesium chloride of 1, 20 g mL. Moreover, Castric *et al.* (1997) reported the first isolation of the causative agent of SD in CHSE-214. Partial studies of genome sequencing of SD, also suggest that it is a member of the genus Alphavirus (Villoing *et al.*, 2000a). The identity of both (SPD and SD) as a new aquatic Alphavirus was confirmed in a subsequent study, conducted by Weston *et al.* (2002), in which complete genomes were sequenced from F93-125 and S49P, reference strains of SPD and SD, respectively. SAV subtypes have a characteristic genomic organization of the Alphaviruses, with a single-stranded genome of approximately 11.8 kb in size. The 5' end encodes the four nonstructural proteins (nsP1-nsP4) which are essential for virus replication, whereas the 3' organizes the genes for the structural proteins E1 - E3. The genomes of PD and SD are composed of nucleotides 11919 and 11900, respectively (Villoing *et al.*, 2000a; Weston *et al.*, 2002; Hodneland *et al.*, 2005; Hodneland *et al.*, 2006).

Clinical signs associated with PD are in order of appearance, sudden loss of appetite, lethargy, increased number of faecal casts, weight loss and increased mortality (McVicar, 1987; McLoughlin *et al.*, 2002; Norris *et al.*, 2008). Affected fish may lose the ability to maintain its normal position in the water, due to muscle damage, which predisposes to ulceration of the skin and fins (Ferguson *et al.*, 1986).

The main findings in the necropsies, are the absence of food in the gut, sometimes petechiae are identified on the surface of the pyloric caeca and the surrounding fat. The fish decrease their body condition due to the inability of the pancreas to secrete digestive enzymes, and these fish are more susceptible to secondary bacterial and parasitic infections (McLoughlin and Graham, 2007; Taksdal *et al.*, 2007; Norris *et al.*, 2008).

In the exocrine pancreas tissue, there may be necrosis identified histologically in the affected area, combined with skeletal myopathy, including cardiomyopathy and esophageal lesions (Ferguson, 1986; Munro *et al.*, 1984; Murphy *et al.*, 1992; McLoughlin *et al.*, 2002; Taksdal *et al.*, 2007).

In SD, the clinical feature of the affected fish is the lateral presentation in the bottom of the cage, which is mainly due to extensive necrosis of skeletal muscle. The lesions in the pancreas and heart, are very similar to those described for PD (Boucher and Laurencin, 1996).

Annex 3 (cont.)

Parameter No. 6: Potential for international spread, including via live animals, their products or fomites.

Studies on horizontal transmission of PD, have shown that it can survive for long periods in sea water, with an average life of at least 5.7 days at 10 º C. It has been shown that virus survival is inversely proportional to the temperature and reduces its viral load in the presence of organic matter, where the half life of the virus may have a range of 61.0 to 1.5 days (4-under sterile conditions, 10, 15 and 20 º C in sea water and sweet, with and without organic matter), which means that the virus can remain in the water and be transferred to adjacent sites through water, without human or animal intervention, directly or indirectly (Graham *et al.*, 2007a, b, c; Viljugrein *et al.*, 2009). Graham *et al.* (2010) conducted a prospective longitudinal study of two outbreaks in Atlantic salmon in Ireland in the marine production phase, which showed the persistence of the virus in the environment. The partial genome sequence of the virus causing the outbreak was identical to the strain of SAV detected in earlier populations of Atlantic salmon in the affected farms that overlap in time and space to new populations.

Regarding the movement of fish from one marine site to another, a study by McLoughlin *et al.* (2003), found that farms that moved fish during the marine production cycle, in Ireland, were 6 times (OR 6.88, P = 0.064) more likely to have a PD outbreak than those farms that do not move fish in the sea. The method of transporting fish in the sea water indicated that the sites that used a towing vessel presented greater risk of a PD outbreak (OR=14, P= 0.09), compared to the use of well boats. Between 2003 and 2004, the first outbreak caused by SAV is described in northern Norway, 800 km from the endemic area west of the same country (Karlsen *et al.*, 2006) demonstrating the transmission of the disease from one area to another, a condition that could be related to the transportation of smolts by well boats from the infected area to the free one. In addition, Karlsen *et al.* (2006), reported that three isolated cases of SAV were found with identical genomic regions, on different sites within the same body of water, which is consistent with local transmission from one site to another, whether it is by water or indirectly via fomites.

On the other hand, the viral RNA can be detected in tissues such as heart and gills for up to 140 days post experimental infection and can be detected by RT-PCR in serum for 14 days or more after infection.

This suggests that fish can be carriers with persistent or latent infection, which poses a risk to healthy fish who enter the sea with fish that have recovered from PD (Christie *et al.*, 2007; Ruane *et al.*, 2008). To support this hypothesis, we conducted a study of molecular characterization of strains present in a population of fish where there was a case that joined a group of initially healthy fish with one that had recovered from PD, subsequently presenting an outbreak, the strains found were indistinguishable, this leads to establish that the healthy population is infected from fish that had been moved to the sea after recovery from the disease (Ruane *et al.*, 2008).

Kongtorp *et al.* (2010), conducted a study to determine the possibility of vertical transmission of SAV3 in Atlantic salmon gametes, in which the results of all samples were negative, concluding in their study that the disease is not transmitted vertically and in the eventuality of such occurrence, it would not be a path of great importance. On the other hand, it is important to mention that Castric *et al.* (2005) were able to re-isolate SAV2 from ova lots and two months old offspring coming from experimentally infected broodstock. For these reasons, vertical transmission cannot be entirely excluded as a possible via of transmission of different SAV subtypes.

All Alphavirus animals share a common epidemiology, where transmission of infection is through arthropods. To date, no invertebrates vectors have been identified for SAV and it has been shown that SAV infections can be transmitted without an insect vector in fish (McLoughlin *et al.*, 1996), as a result further studies are needed to determine the potential role of lice or other parasites in SAV infections (McLoughlin and Graham 2007).

Parameter No. 7: Several countries or countries with zones may be declared free of the disease based on the general surveillance principles outlined in Chapter 1.4. of the *Aquatic Code*

In Chile, in May 2008, Pancreas Disease (PD) was incorporated in the List of High Risk Diseases (List 1) of mandatory reporting, since it belongs to an exotic disease, produces high mortality and therefore, high economic impact besides being transmissible, conditions required by the enforced regulations to be considered High Risk.

Given the above, during the 2008-2009 period, Sernapesca completed an Official Investigation assigning the Universidad Austral de Chile, a study where samples were collected from blood and Atlantic salmon organs (Salmo salar) and rainbow trout (Oncorhynchus mykiss) from farms, lake centers, processing plants and marine sites in the IX, X, XI, XII and XIV Regions. For virus isolation CHSE-214 cells were used, incubated at 14±1ºC, and the detection of Alphavirus subtypes, qPCR were used. The results concluded that there is no presence of Alphaviruses caused by PD and SD in salmon farms in Chile. Furthermore, studies we made in wild fish samples from 15 lakes in the south of Chile, also applying the same technique of diagnosis finding no detection of the presence of these viruses. Given the incorporation of PD in the High Risk Diseases List above, an active surveillance in cultivated animals is required by Chilean law from 2009. This surveillance includes biannual surveys of all the sites with susceptible species in accordance with OIE guidelines. Currently, the official technique for this purpose in Chile is real time RT-PCR, with a Taqman probe according to the technology described by Hodneland and Endresen (2006). To date, this monitoring has not detected the presence of Alphaviruses causing the disease.

It should be equally noted that from 2003, the biannual monitoring formally indicates the use of cell strings CHSE-214 and BF-2 or EPC in fish farms, none of which have shown the presence of this virus. In addition to Chile, there are countries where the disease has not been detected, based on active and passive surveillance programs.

It should be noted, for example, that under health certification requirements required by Chile for salmon ova, Iceland, Denmark and Australia have been declared free of the disease under active surveillance conducted by analysis in cell strings sensitive to Alphaviruses such as the CHSE-214, BF-2 and EPC.strings.

C. DIAGNOSIS

Parameter No. 8: A repeatable and robust means of detection/diagnosis exists.

The diagnosis of PD and SD, is performed based on clinical signs in combination with histopathological findings (Jansen *et al.,* 2010). There have been tropism studies of the virus allowing us to know the chosen organ for diagnosis, determining that the gills and the heart are the most useful samples (Andersen *et al.,* 2007).

The isolation of the virus in fish cell culture, and the subsequent identification using specific antibodies, can be used to verify the etiology of the disease, performing routine isolations (Todd *et al.,* 2001; Graham *et al.,* 2003; Jewhurst *et al.,* 2004; Graham *et al.,* 2008). However, it is not possible to clearly distinguish SAV subtypes in cell culture (Holdneland *et al*., 2006).

Molecular methods and the techniques as real time RT-PCR have been developed for a number of viral diseases in fish and has successfully demonstrated that the detection rate increases. Villoing *et al.* (2000b) used RT-PCR in two steps to for the detection of the SD virus in naturally infected salmonids, which was also useful for the amplification of the PD virus from experimentally infected fish.

Since this technique is highly sensitive, specific and reproducible it can detect and differentiate RNA from different variants (as these are properly sequenced and their homology is known), with the potential to differentiate and quantify all SAV subtypes within the host (Hodneland *et al.*, 2006).

Annex 3 (cont.)

Monoclonal antibodies have been developed for the detection of SAV, which have been successfully proven in diagnosis by immunofluorescence and immunohistochemistry (Todd, 2001, McLoughlin and Graham, 2007).

Conclusion

With the previous scientific information presented, we conclude that the agent that causes Pancreas Disease in salmonids, meet the criteria set out in Chapter 1.2 of the Aquatic Code to be listed as an OIE disease and in view of the above, suggests the possible review of the Alphaviruses situation affecting the fish.

Chile requests, according to the background study presented, that OIE, through the Commission of Aquatic Animal Health Regulations can deliver an expert opinion accepting these diseases as listed by the OIE.

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