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REPORT OF THE OIE *AD HOC* GROUP ON SUSCEPTIBILITY OF MOLLUSC SPECIES TO INFECTION WITH OIE LISTED DISEASES[1]

May–June 2021

This report covers the work of the OIE *ad hoc* Group on Susceptibility of mollusc species to infection with OIE listed diseases (the *ad hoc* Group) who met electronically between May and June 2021.

The list of participants and the Terms of Reference are presented in Annex I and Annex II, respectively.

Methodology

The *ad hoc* Group applied criteria, as outlined in Chapter 1.5. Criteria for listing species as susceptible to infection with a specific pathogen of the OIE *Aquatic Animal Health Code* (the *Aquatic Code*), to potential host species in order to determine susceptibility to infection with abalone herpesvirus[2]. The assessments were done using a three-stage approach, as outlined in Article 1.5.3. of Chapter 1.5., and further considerations are described below:

1) Stage 1: Criteria to determine whether the route of transmission is consistent with natural pathways for the infection (as described in Article 1.5.4.):

Consideration was given to whether experimental procedures mimic natural pathways for disease transmission. Consideration was also given to environmental factors given that these may affect host response, virulence and transmission of infection with abalone herpesvirus.

The table below describes the sources of infection accepted by the *ad hoc* Group for the assessments as well as some considerations when applying Stage 1 to support susceptibility to infection with abalone herpesvirus.

Source of infection	Considerations
1. Natural exposure included situations where infection had occurred without experimental intervention (e.g. infection in wild or farmed populations) OR	Invasive studies by intramuscular injection were not considered a natural route for transmission (Corbeil *et al.*, 2017 and Bai *et al.*, 2019).

1 Note: This report should be read in conjunction with the September 2021 report of the Aquatic Animal Commission, where the Commission's considerations and comments are noted. The Commission's report can be found at https://www.oie.int/en/what-we-do/standards/standards-setting-process/aquatic-animals-commission/#ui-id-2, and the proposed draft chapter can be found in its annex 14.

2 The nomination and classification status of abalone herpesvirus has been accepted by the International Committee on Taxonomy of Viruses (ICTV) as Haliotid herpesvirus 1 (HaHV-1) and represented as the unique member of the genus *Aurivirus* (family *Malacoherpesviridae*, order *Herpesvirales*).

2. Non-invasive experimental procedures: cohabitation with infected hosts; infection by immersion.	

2) Stage 2: Criteria to determine whether the pathogenic agent has been adequately identified (as described in Article 1.5.5.):

The *ad hoc* Group noted that unambiguous pathogenic agent identification might not have been carried out in older publications because molecular techniques were not available at the time. In these circumstances a weight of evidence approach, whereby the combined information from subsequent studies and additional information provided by the authors, was considered and used to conclude sufficiency of pathogen identification.

The table below describes the pathogen identification methods accepted by the *ad hoc* Group for the assessments as well as some considerations when applying Stage 2 to support susceptibility to infection with abalone herpesvirus.

Pathogen Identification	Considerations
1. Species-specific real-time PCR (for example ORF 49, 66 or 77) OR 2. Conventional PCR based on DNA polymerase and terminase region with subsequent sequence analysis (Chen *et al.*, 2012) OR 3. *In situ* hybridisation (ORF 66).	Although several genotypic variants have been reported based on genome sequence analyses, studies considering only a single variant were taken into consideration. Molecular data associated with microscopical examination (ISH) and transmission electron microscopy (TEM) was preferable but not compulsory to confirm the presence of the pathogen.

3) Stage 3: Criteria to determine whether the evidence indicates that presence of the pathogenic agent constitutes an infection (as described in Article 1.5.6.):

Criteria A to D, as described in Article 1.5.6. and presented below, were used to determine if there was sufficient evidence for infection with abalone herpesvirus in the suspected host species:

A. The pathogenic agent is multiplying in the host, or developing stages of the pathogenic agent are present in or on the host[3];

B. Viable pathogenic agent is isolated from the proposed susceptible species, or infectivity is demonstrated by way of transmission to naïve individuals;

C. Clinical or pathological changes are associated with the infection;

D. The specific location of the pathogen corresponds with the expected target tissues.

3 For the purposes of the assessments for susceptibility to infection with abalone herpesvirus, replication 'on the host' was not considered to apply.

Evidence to support criterion A alone was sufficient to determine infection. In the absence of evidence to meet criterion A, satisfying at least two of criteria B, C or D were required to determine infection.

The table below describes the criteria for assessment of Stage 3 to support susceptibility to infection with abalone herpesvirus.

Evidence for infection			
A: Replication	B: Viability / Infectivity	C*: Pathology / Clinical signs	D: Location
1) TEM observation of different developmental stages of the virus (nucleo capsids, empty capsids) **OR** 2) Demonstration of increasing copy number over time with real-time PCR (targeting DNA)[4].	1) Experimental cohabitation (Bai *et al.*, 2019) **OR** 2) Experimental immersion challenge system (Corbeil *et al.*, 2012a).	1) Clinical signs, such as: a) Weak or loss of righting reflex b) Reduced or loss of pedal adhesion c) Mortality **OR** 2) Macroscopic lesions, such as: a) Swollen mouth and prolapsed odontophore (Hooper *et al.*, 2007) b) Mantle recession (Chang *et al.*, 2005) c) Foot contraction **OR** 3) Microscopic lesions, such as: d) Necrosis of cerebral ganglion and nerve bundles in the muscle of the foot e) Increased cellularity (hemocytes and glial cells) accompanying lesions.	1) Cerebral and/or pleuropedal ganglion or peripheral nerve bundles (tissue may include surrounding muscle tissue) **OR** 2) Haemocytes (infiltrated in other tissues such as the hepatopancreas and mantle).

* Pathology/Clinical signs may be non-specific, variable and include some or all of the characteristics listed.

4 Demonstration of increasing copy number over time with real-time PCR (targeting DNA) was identified as a criterion for replication. However, the *ad hoc* Group did not find any references to this criterion in the papers reviewed.

Results

The *ad hoc* Group agreed that the four species currently included in Article 11.1.2. as susceptible to infection with abalone herpesvirus, i.e. Small abalone (*Haliotis diversicolor*), Greenlip abalone (*Haliotis laevigata*), Blacklip abalone (*Haliotis rubra*) and hybrids of Greenlip x Blacklip abalone (*Haliotis laevigata* x *Haliotis rubra*), meet the criteria for listing as susceptible to infection with abalone herpesvirus in accordance with Chapter 1.5. of the *Aquatic Code* and were proposed to remain in Article 11.1.2.

No new species were found to meet the criteria for listing as susceptible species to infection with abalone herpesvirus.

Two species, Japanese abalone (*Haliotis discus*) and Rainbow abalone (*Haliotis iris*) were assessed as having incomplete evidence of susceptibility and were proposed to be included in the second paragraph of Section 2.2.2. of Chapter 2.4.1., Infection with abalone herpesvirus, of the *Aquatic Manual*.

Assessments

The table below describes the different scores and outcomes of the assessments undertaken by the *ad hoc* Group.

Score	Outcome
1.	Species assessed as susceptible (as described in Article 1.5.7.). These species were proposed for inclusion in Article 11.1.2. of Chapter 11.1., Infection with abalone herpesvirus, of the *Aquatic Code* and Section 2.2.1. of Chapter 2.4.1., Infection with abalone herpesvirus, of the *Manual of Diagnostic Tests for Aquatic Animals* (the *Aquatic Manual*).
2.	Species assessed as having incomplete evidence for susceptibility (as described in Article 1.5.8.).
3.	Species assessed as not meeting the criteria or for which there was unresolved or conflicting information. These species were not proposed for inclusion in either the *Aquatic Code* or the *Aquatic Manual*. The exceptions were species where pathogen-specific positive PCR results had been reported, but an active infection had not been demonstrated. These species were proposed for inclusion in the second paragraph in Section 2.2.2., Species with incomplete evidence for susceptibility, of Chapter 2.4.1., Infection with abalone herpesvirus, of the *Aquatic Manual.*
4.	Species assessed as non-susceptible.
NS	Species not scored due to insufficient or irrelevant information.

The assessments for host susceptibility to infection with abalone herpesvirus together with the outcomes and relevant references are shown in the table below.

Family	Scientific name	Common name	Subspecies (if applicable)	Stages 1: Route of infection	Stage 2: Pathogen identification	Stage 3: Evidence for infection				Outcome	References
						A	B	C	D		
Score 1											
Haliotidae	*Haliotis diversicolor*	Small abalone	*Haliotis diversicolor supertexta*	N	Conventional PCR & sequencing	ND	ND	YES	YES	1	Chen *et al*., 2012
			Haliotis diversicolor supertexta	N and E and EI	Real-time PCR ORF 66	YES	YES	YES	YES	1	Bai *et al*., 2019
Haliotidae	*Haliotis laevigata*	Greenlip abalone		N	NO[5]	ND	NO	YES	YES	1	Hooper *et al*., 2007
				E	Real-time PCR ORF49 and ORF 66	ND	ND	YES	YES	1	Corbeil *et al*., 2016
Haliotidae	*Haliotis rubra*	Blacklip abalone	*Haliotis rubra*	E	Real-time PCR ORF49	ND	YES	YES	YES	1	Crane *et al*., 2013
			Haliotis rubra & Haliotis rubra conicopora[6]	E	Real-time PCR ORF49 and ORF 66	ND	ND	YES	YES	1	Corbeil *et al*., 2016
Haliotidae	*Haliotis laevigata x H. rubra*	Hybrid (greenlip x blacklip)		E	Real-time PCR ORF49 and ISH ORF66	YES	ND	YES	YES	1	Corbeil *et al.*, 2012a
				E	Real-time PCR ORF49	ND	YES	YES	YES	1	Dang *et al*., 2013

5 The Hooper *et al*., 2007 paper is scored as a 1 based on the molecular information provided from the same source population from Corbeil *et al*., 2016.

6 Corbeil *et al*., 2016 tested both *H. rubra* and *H. conicopora. H. conicopora* is considered as a junior synonym of *Haliotis rubra conicopora*.

Score 3											
Haliotidae	*Haliotis discus*	Japanese abalone	*Haliotis discus hannai*	N	Real-time PCR ORF49	ND	ND	ND	ND	3	Gu *et al*., 2019
			Haliotis discus hannai	N and E and EI	NO	ND	ND	NO	NO	4	Bai *et al*., 2019
Haliotidae	*Haliotis iris*	Rainbow abalone		E	Real-time PCR ORF66 and ISH ORF66	ND	ND	YES	YES	1	Corbeil *et al*., 2017
				E	Real-time PCR ORF66	NO	ND	NO	NO	4	Neave *et al*., 2019

Assessment Table Key

N: Natural infection
E: Experimental (non-invasive)
EI: Experimental (invasive)
YES: Demonstrates criterion is met.
NO: Criterion is not met.
ND: Not determined.

Note:

The scientific names of the species are in accordance with the World Register of Marine Species (WoRMS) https://www.marinespecies.org/index.php.

The common names of mollusc species are in accordance with FAOTERM (http://www.fao.org/faoterm/collection/faoterm/en/). Where the common mollusc name was not found in FAOTERM, the naming was done in accordance with https://www.sealifebase.ca.

Comments on the *ad hoc* Group's rationale and decision-making:

General comments

The *ad hoc* Group agreed to focus on studies published from 2000 onwards, when molecular testing was available. Papers published in earlier years were referred to when necessary to increase confidence of an assessment or when no recent paper was available for the assessment of a specific host species. When necessary to corroborate pathogen identification, the *ad hoc* Group:

(1) contacted authors of the studies to further describe pathogen identification methods, or

(2) utilized molecular information from parallel or subsequent studies on the same source population.

Although several genotypic variants have been reported based on genome sequence analyses (Cowley *et al*., 2011 and Corbeil *et al*., 2016), the *ad hoc* Group did not assess susceptibility of host species to the virus at the variant level and considered that it was sufficient to regard a species susceptible when it meets the criteria even for a single variant.

The *ad hoc* Group agreed that either two papers with a score of '1', or a single study with corroborative evidence, were enough to conclude susceptibility of a species. However, additional studies were still reviewed to check for any conflicting evidence. When additional papers were identified but the species had already been determined as susceptible by at least two other papers, these papers were still included in the list of references.

Species-specific comments

- The *ad hoc* Group considered publications on subspecies of *Haliotis discus* and *Haliotis diversicolor* (An *et al.*, 2013 and Wang *et al.*, 2004a) and these publications suggested that the subspecies of haliotids in other literature may not be an accurate reflection of subspecies taxonomic relationships. Based on this finding, the *ad hoc* Group did not assess subspecies of *Haliotis discus* or *Haliotis diversicolor*. Information about these subspecies has been included in the table of assessments for clarity, but the assessments were completed at the species level.

- *Haliotis conicopora* is indicated in WoRMS (https://www.marinespecies.org/index.php) as a junior synonym of *Haliotis rubra conicopora* and as a result has been assessed as *Haliotis rubra*.

- *Haliotis discus* was assigned an outcome of '3' due to unresolved conflicting information. One paper reported only PCR positives and the other paper supported non-susceptibility. Additional papers were assessed but did not provide further information for the final outcome as they were prior to 2000 and did not include pathogen identification. The *ad hoc* Group proposed to include *Haliotis discus* in Section 2.2.2., Species with incomplete

evidence for susceptibility, of Chapter 2.4.1., Infection with abalone herpesvirus, of the *Aquatic Manual* based on the PCR positive (Gu *et al.*, 2019).

- *Haliotis iris* was assigned an outcome of '3' due to unresolved conflicting information. One paper reported natural low infection in only a few of the animals exposed by immersion and the other paper supported non-susceptibility. Only two papers were available for review for *Haliotis iris* and based on the Corbeil *et al*., 2017 paper indicating the PCR positive as well as weak signal by ISH in the target tissue of naturally infected (by immersion) animals, the *ad hoc* Group proposed to include this species in Section 2.2.2., Species with incomplete evidence for susceptibility of Chapter 2.4.1., Infection with abalone herpesvirus, of the *Aquatic Manual*.

Listing of susceptible species at a taxonomic ranking of Genus or higher

- The *ad hoc* Group considered Article 1.5.9., Listing of susceptible species at a taxonomic ranking of Genus or higher, in Chapter 1.5., Criteria for listing species as susceptible to infection with a specific pathogen of the *Aquatic Code*, but considered it was not applicable for the hosts of abalone herpesvirus identified at this time as the susceptible species were limited to one Family, Haliotidae.

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Annex I

OIE *AD HOC* GROUP ON SUSCEPTIBILITY OF MOLLUSC SPECIES TO INFECTION WITH OIE LISTED DISEASES

May–June 2021

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Annex II

OIE *AD HOC* GROUP ON SUSCEPTIBILITY OF MOLLUSC SPECIES TO INFECTION WITH OIE LISTED DISEASES

May–June 2021

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Terms of reference

Background

Chapter 1.5, Criteria for listing species as susceptible to infection with a specific pathogen, was introduced in the 2014 edition of the *Aquatic Code*. The purpose of this chapter is to provide criteria for determining which host species are listed as susceptible in Article X.X.2 of each disease-specific chapter in the *Aquatic Code.* The criteria are to be applied progressively to each disease-specific chapter in the *Aquatic Code*.

These assessments will be undertaken by *ad hoc* Groups and the assessments will be provided to Members for comment prior to any change in the list of susceptible species in Article X.X.2 of the disease-specific chapters in the *Aquatic Code.*

For species where there is some evidence of susceptibility but insufficient evidence to demonstrate susceptibility through the approach described in Article 1.5.3, information will be included in the relevant disease-specific chapter in the *Aquatic Manual*.

Purpose

The *ad hoc* Group on Susceptibility of mollusc species to infection with OIE listed diseases will undertake assessments for the seven OIE listed mollusc diseases.

Terms of Reference

1) Consider evidence required to satisfy the criteria in Chapter 1.5.

2) Review relevant literature documenting susceptibility of species for OIE listed mollusc diseases.

3) Propose susceptible species for OIE listed diseases for molluscs based on Article 1.5.7.

4) Propose susceptible species for OIE listed diseases for molluscs based on Article 1.5.8.

Expected outputs of the *ad hoc* Group

1) Develop a list of susceptible species for inclusion in the relevant Article X.X.2. of mollusc disease-specific chapters in the *Aquatic Code.*

2) Develop a list of species with incomplete evidence for susceptibility for inclusion in Section 2.2.2. of the *Aquatic Manual.*

3) Draft a report for consideration by the Aquatic Animals Commission at its September 2021 meeting.