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

January–June 2020

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) between January and June 2020. During this period, the *ad hoc* Group met twice (a three-day physical meeting followed by a series of virtual meetings).

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

Methodology

The *ad hoc* Group applied the criteria to potential host species to determine susceptibility and non-susceptibility to infection with *Bonamia ostreae*. This was done by the three-stage approach, outlined in Article 1.5.3 of the *Aquatic Code*, to assess susceptibility of a species to infection with *B. ostreae*, as described below:

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

Stage 1: Criteria to determine whether the modality of exposure is consistent with natural pathways (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 *B. ostreae*.

The table below describes additional considerations made by the *ad hoc* Group when applying Stage 1 to support susceptibility to infection with *B. ostreae*.

Stage 1: Source of infection	Comment
Natural exposure includes situations where infection has occurred without experimental intervention (e.g. infection in wild or farmed populations) OR Non-invasive experimental procedures[2]: cohabitation with infected hosts; infection by immersion or feeding	*In vitro* experimental assays (contact between haemocytes and parasites) are not considered appropriate to answer the question of susceptibility or non-susceptibility.

[1] Note: This *ad hoc* Group report reflects the views of its members and may not necessarily reflect the views of the OIE. This report should be read in conjunction with the February 2021 report of the Aquatic Animal Health Standards Commission because this report provides its considerations and comments. It is available at https://www.oie.int/en/standard-setting/specialists-commissions-working-ad-hoc-groups/aquatic-animals-commission-reports/meeting-reports/

[2] Invasive experimental procedures including injection can only be used to demonstrate non-susceptibility.

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

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 used by the *ad hoc* Group including some considerations.

Stage 2: Pathogen Identification	Comment
Molecular sequence information (species-specific regions of 18S sequence) OR PCR-RFLP (as described in Cochennec *et al*., 2000) OR Species-specific Real-time or conventional PCR (for example Ramilo *et al*., 2013)	Molecular data should be associated with microscopical examination wherever possible to confirm the presence of the pathogen. ISH is currently not sufficiently specific to resolve species level identifications. For early studies without molecular information, corroborating evidence from later studies was considered. ITS rDNA sequence has a higher resolution than 18s rDNA and can add information about the intra-species diversity between populations.

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

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 in Article 1.5.6 were used to determine if there was sufficient evidence for infection with *B. ostreae* in the suspected host species. 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.

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

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.

The table below describes the criteria for assessment of Stage 3 to support susceptibility to infection with *B. ostreae*

Stage 3: Evidence for infection			
A: Replication	B: Viability / Infectivity	C: Pathology / Clinical signs*	D: Location
1) Presence of multiple intracellular cells or presence of multinucleated cells (including plasmodial stage) demonstrated **by:** Histopathology OR Cytology (usually gill or heart imprint or haemolymph smears) OR *In-situ* hybridization (ISH) OR TEM OR 2) Demonstration of increasing copy number over time with qPCR (targeting DNA) or reverse transcription qPCR (targeting RNA) in tissues	1) Transmission via co-habitation with uninfected individuals of a known-susceptible (e.g. *Ostrea edulis*) species OR 3) Demonstration of viability of cells isolated from tissues **by:** Flow cytometry OR Vital stains OR Successful infection of uninfected animals by inoculation	Mortality OR <u>Macroscopic lesions</u> such as - Discolouration of tissue - Gill ulceration OR Rapid loss of condition OR <u>Microscopic lesions</u> such as generalized haemocyte infiltration in connective tissues of several organs including gills and mantle	Within haemocytes circulating in the connective tissue in different organs, in particular gills** or heart (rarely extracellular)

* non-specific signs and inconsistent presentation

** inside gills, as opposed to potential external contaminant

An assessment of non-susceptibility was made when there was a ‘Yes’ for criterion D and a ‘No’ for other assessed criteria A, B, or C based on multiple sources with no conflicting results.

The table below describes the outcomes of the assessment undertaken by the ad hoc Group.

1.	Species that were assessed as susceptible (as described in Article 1.5.7) were proposed for inclusion in Article 11.3.2 of Chapter 11.3, Infection with *B. ostreae*, of the *Aquatic Code* and Section 2.2.1 of Chapter 2.4.3 of the *Aquatic Manual.*
2.	Species that were assessed as species for which there is partial evidence for susceptibility (as described in Article 1.5.8) were proposed for inclusion in Section 2.2.2, Species with incomplete evidence for susceptibility, of Chapter 2.4.3, Infection with *B. ostreae*, of the *Aquatic Manual*.
3.	Species that were assessed not to meet the criteria or for which there was unresolved conflicting information were not proposed for inclusion in either the *Aquatic Code* or *Aquatic Manual*. The exception were species where there had been reported pathogen-specific positive PCR results, but an active infection had not been demonstrated. These species were included in a separate paragraph in Section 2.2.2, Species with incomplete evidence for susceptibility, of Chapter 2.4.3 of the *Aquatic Manual.*
4.	Species that were assessed to have evidence of non-susceptibility were to be included in the revised Section 2.2.3 when applying the new template to Chapter 2.4.3 of the *Aquatic Manual*.
5.	Vector - at the time of the assessments, the *ad hoc* Group were waiting for a decision to be made by the Aquatic Animals Commission to determine/clarify the definition of 'vector'. Until this decision is made, the *ad hoc* Group did not consider 'vector' as an outcome.
NS	Not scored due to insufficient or irrelevant information.

Assessments of host susceptibility to infection with *B. ostreae*

Summary

The *ad hoc* Group found that of the six species currently listed in Article 11.3.2 as susceptible to infection with *B. ostreae*, three species, Australian mud oyster (*Ostrea angasi*), Argentinean flat oyster (*Ostrea puelchana*) and Asiatic oyster (*Ostrea denselammellosa*), did not meet the criteria for listing as a susceptible species and were proposed to be deleted from Article 11.3.2.

No new species were found to meet the criteria for listing as susceptible species to infection with *B. ostreae.*

The assessments, outcomes, and relevant references for host susceptibility to infection with *B. ostreae* conducted by the *ad hoc* Group are shown in the table below.

Family	Scientific name	Common name	Stages 1: Route of infection	Stage 2: Pathogen identification	Stage 3: Evidence for infection				Outcome	References
					A	B	C	D		
Score 1										
Ostreidae	*Ostrea edulis*	European flat oyster	ND	Yes	Yes	ND	Yes	Yes	1	Cochennec *et al*., 2000
			N	Yes	Yes	ND	Yes	Yes	1	Marty *et al*., 2006
Ostreidae	*Ostrea chilensis*	Chilean flat oyster	N	Yes	Yes	ND	Yes	Yes	1	Lane *et al*., 2016
			N	Yes[3]	ND	ND	Yes	Yes	1	Grizel *et al.,* 1983
Ostreidae	*Crassostrea ariakensis*	Suminoe oyster	N	Yes[4]	Yes	ND	Yes	Yes	1	Cochennec *et al.*, 1998
			E	Yes	ND	ND	No	Yes	3	Audemard *et al*., 2005 (conference abstract), and personal communication (R. Carnegie)
Score 2										
Ostreidae	*Ostrea puelchana*	Argentinean flat oyster	N	Yes[5]	ND	ND	Inconclu-sive[6]	Yes	2	Pascual *et al*., 1991
Score 3										

[3] Study sites referred in Grizel *et al*., 1983 were in areas known to be infected with *B. ostreae* (later characterized by molecular test in addition to histology or cytology).

[4] The parasite described by Cochennec *et al*., 1998 was later confirmed to be *B. ostreae* by DNA sequencing by the OIE reference laboratory as stated in Engelsma *et al*., 2014.

[5] Study sites referred in Pascual *et al.,* 1991 were in areas known to be infected with *B. ostreae* (later characterized by molecular test in addition to histology or cytology).

[6] Criterion C was considered as inconclusive because the cause of mortality was not clear (*B. ostreae* versus *M. refringens* and/or environmental.

Family	Scientific name	Common name	Stages 1: Route of infection	Stage 2: Pathogen identification	Stage 3: Evidence for infection				Outcome	References
					A	B	C	D		
Ophiotrichidae	*Ophiothrix fragilis*	Brittle star	N and E	Yes	ND	ND	ND	ND	3	Lynch *et al*., 2007
Actiniidae	*Actina equina*	Beadlet anemone	N	Yes	ND	ND	ND	ND	3	Lynch *et al.,* 2007
Ascidiidae	*Ascidiella aspersa*	European sea squirt	N	Yes	ND	ND	ND	ND	3	Lynch *et al*., 2007
		Grouped zooplankton	N	Yes	ND	ND	ND	ND	3	Lynch *et al*., 2007
Ostreidae	*Crassostrea gigas*	Pacific cupped oyster	N and E and EI	Yes[7]	No	No	No	No	4	Culloty *et al*., 1999
			N and E and EI	Yes	Yes	Inconclusive[8]	No	Yes	1	Lynch *et al*., 2010
			EI	Yes	No	ND	No	No	4	Gervais, 2016
Score 4										
Veneridae	*Ruditapes decussatus*	European clam	E and EI	Yes	No	No	No	No	4	Culloty *et al*., 1999
Veneridae	*Ruditapes philippinarum*	Manila clam	E and EI	Yes	No	No	No	No	4	Culloty *et al*., 1999
Mytilidae	*Mytilus edulis*	Blue mussel	E and EI	Yes	No	No	No	No	4	Culloty *et al*., 1999
Mytilidae	*Mytilus galloprovincialis*	Mediterranean mussel	E and EI	Yes	No	No	No	No	4	Culloty *et al*., 1999

[7] Study sites referred in Culotty *et al*., 1999 were in areas known to be infected with *B. ostreae* (later characterized by molecular test in addition to histology or cytology).

[8] Criterion B was considered as inconclusive because parasites *B. ostreae* detected in exposed *C. gigas* were detected in shell fluids and not in tissues.

Family	Scientific name	Common name	Stages 1: Route of infection	Stage 2: Pathogen identification	Stage 3: Evidence for infection				Outcome	References
					A	B	C	D		
Not scored (NS) because pathogen ID was inconclusive										
Ostreidae	*Ostrea angasi*	Australian mud oyster	N	No	ND	ND	Inconclu-sive[9]	Yes	NS	Bougrier *et al*., 1986
Ostreidae	*Ostrea denselamellosa*	Lamellated oyster	ND	No	ND	ND	ND	ND	NS	Le Borgne and le Pennec, 1983
Ostreidae	*Ostrea lurida* *(O. conchaphila)*	Olympia oyster	N	No	Yes	ND	Yes	Yes	NS	Farley, 1988
Ostreidae	*Crassostrea angulata*	Portuguese oyster	ND	No	ND	ND	ND	ND	NS	Katkansky *et al*.,1969, Engelsma *et al*., 2014

The scientific names of the species are in line with World Register of Marine Species (WoRMS) https://www.marinespecies.org/index.php (for *Crassostrea gigas* see explanatory note below).

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

[9] Criterion C was considered as inconclusive because reported mortality could possibly be due to an unidentified *Haplosporidium* parasite.

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

- The *ad hoc* Group decided to focus on studies published from the year 2000 onwards, when molecular testing was available. Papers published in earlier years were referred to where necessary to increase confidence of assessment or when no recent paper was available for the assessment of a specific host species.

- The *ad hoc* Group decided that either two papers with a score of '1', or a single study with a second study providing corroborative information, were enough to conclude susceptibility of a species. Additional studies were still checked and considered for conflicting evidence.

- The Brittle star only has a PCR positive and was thus scored as a "3" (Lynch *et al*., 2007). Although natural infection and feeding trials were carried out, information related to viability and pathology were inconclusive and information on location was not documented. *Actina equina, Ascidiella aspersa* and grouped zooplankton only have a PCR positive and were thus scored as a "3" Lynch *et al*., 2007.

- *Crassostrea ariakensis*: Cochennec *et al*., 1998, ID was based on histology and eccentric nuclei, but later confirmed by DNA sequencing (Engelsma *et al*., 2014). Limited corroborating evidence was provided by the Audemard 2005 abstract (and personal communication with co-author) regarding a cohabitation exposure trial (1/30 PCR positives following 6 mo exposure).

- *Ostrea puelchana* is currently listed as susceptible in the *Aquatic Code* but the *ad hoc* Group considered that it should be more accurately regarded as a species for which there is partial evidence for susceptibility (i.e., scored as a '2'). The study reporting this occurrence (Pascual *et al*., 1991) did not fulfil the criteria for evidence of infection (Stage 3) where only column D (Location) was scored as 'Y'.

- *Ostrea angasi* is currently listed as susceptible in the *Aquatic Code* but the *ad hoc* Group did not score this host species because pathogen identification was not provided unambiguously and it was not stated that experimental oysters were surveyed for existing infection prior to cohabitation in natural beds. Furthermore, experimental oysters were derived from an Australian locality that is now known to be endemic for *B. exitiosa*.

- *Ostrea denselamellosa* is currently listed as susceptible in the *Aquatic Code* but the *ad hoc* Group did not score this host species since the literature (Le Borgne & Le Pennec, 1983) provided no information with respect to infection with *B. ostreae*.

- *Crassostrea gigas* is currently listed as a 'carrier' in the *Aquatic Manual*, but the *ad hoc* Group found information regarding this host species to be conflicting and gave it a score of '3'. Two formal studies (Culloty *et al.*, 1999; Renault *et al*., 1995), in full or in part, met criteria for identifying a non-susceptible species. This was corroborated by the absence of detections by reference labs despite ongoing EU surveillance (extracted from EURL website, partial survey results show > 7200 animals tested from > 359 lots from areas known to be infected with *Bonamia* sp.). However, there have also been records that detect *Bonamia* sp. RNA (Gervais, 2016). Positive histology for three animals in one study (Lynch *et al*., 2010) clearly questions non-susceptibility. What is unclear is whether these histological findings reflect an early stage of phagocytosis by the host or indicate potential vector status. Consequently, further assessment of *C. gigas* is recommended pending additional information on the viability of detected organisms and/or a finalized definition for vector species.

- The *ad hoc* Group considered Article 1.5.9 in the *Aquatic Code* (Listing of susceptible species at a taxonomic ranking of Genus or higher) but felt that it was not applicable for the hosts of *B. ostreae* identified at this time.

- The *ad hoc* Group had difficulties with the current 'vector' definition and requested the Aquatic Animals Commission to discuss a new proposal and decide.

- The *ad hoc* Group noted that the inconsistency in the lists of susceptible species for infection with *B. ostreae* between Chapter 11.3 of the *Aquatic Code* and Chapter 2.4.3 of the *Aquatic Manual* should be addressed by the application of the recommendations of this *ad hoc* Group. For example, *O. denselamellosa* is currently listed as a susceptible species in the *Aquatic Code* but does not appear in the *Aquatic Manual*.

- According to WoRMS, the accepted name for *Crassostrea gigas* should be *Magallana gigas*. However, Bayne *et al.*, 2017, consider that the report by Salvi & Mariottini, 2017, is not sufficiently robust to support the proposed taxonomic change.

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

January–June 2020

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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 Member Countries 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 their September 2020 meeting.