Report of the WOAH *ad hoc* Group on susceptibility of fish species to infection with WOAH listed diseases

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1. Introduction

This report covers the work of the WOAH *ad hoc* Group on Susceptibility of fish species to infection with WOAH listed diseases (the *ad hoc* Group) who met virtually on 12, 13 and 19 April, 2023.

The list of participants and the Terms of Reference are presented in Annex 1 and Annex 2, respectively.

2. 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 WOAH *Aquatic Animal Health Code* (the *Aquatic Code*), to potential host species in order to determine susceptibility to infection with tilapia lake virus (TiLV).

A three-stage approach, as described in Article 1.5.3., was used to assess the susceptibility of a species to infection with TiLV and was based on:

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.);

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

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.):

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.

Details of the three-stage approach applied by the *ad hoc* Group for infection with TiLV, including any additional considerations are described below:

2.1. Stage 1: Criteria to determine whether the route of transmission is consistent with natural pathways for the infection:

Table 1 describes the route of transmission for infection with TiLV used by the *ad hoc* Group when applying Stage 1 to assess susceptibility to infection with TiLV, as well as some considerations.

Table 1: Route of transmission for infection with TiLV

Route of transmission	Considerations
1. Natural exposure including situations where infection has occurred without experimental intervention (e.g. infection in wild or farmed populations). OR 2. Non-invasive experimental procedures: e.g. cohabitation with infected hosts, infection by immersion.	Experimental infection via invasive routes (i.e. injection) was not considered a natural route of transmission and therefore such studies were only evaluated for conflicting evidence. References that reported co-infections or extreme stress conditions were noted as such and were interpreted with caution.

2.2. Stage 2: Criteria to determine whether the pathogenic agent has been adequately identified:

Table 2 describes the pathogen identification methods for infection with TiLV used by the *ad hoc* Group when applying Stage 2 to assess susceptibility to infection with TiLV, as well as some considerations. These criteria are consistent with identification methods for other listed diseases described in the *Manual of Diagnostic Tests for Aquatic Animals* (the *Aquatic Manual*), as well as consistent with the final report of the WOAH *ad hoc* Group on TiLV (https://www.woah.org/en/what-we-do/standards/standard-setting-process/ad-hoc-groups/#ui-id-3).

Table 2: Pathogen identification for infection with TiLV

Pathogen Identification (TiLV)	Considerations
Specific TaqMan RT-qPCR (e.g. Waiyamitra *et al*., 2018; Megarani *et al*., 2022) OR RT-PCR, SYBR green RT-qPCR, or nested RT-PCR, if followed by sequence analysis (e.g. Eyngor *et al*., 2014; Dong *et al*., 2017b) OR Positive results with more than one set of primers targeting different regions of the genome using RT-PCR, SYBR green RT-qPCR, or nested RT-PCR (e.g. Eyngor *et al*., 2014; Dong *et al*., 2017b) OR In situ hybridisation using a TiLV-specific probe (Dong *et al*., 2017a)	Nested RT-PCR is prone to contamination and sometimes difficult to interpret.

2.3. Stage 3: Criteria to determine whether the evidence indicates that presence of the pathogenic agent constitutes an infection:

Table 3 describes the evidence of infection with TiLV, used by the *ad hoc* Group when applying Stage 3 to susceptibility to infection with TiLV.

Table 3: Evidence of infection with TiLV

Evidence of infection			
A: Replication	**B: Viability / Infectivity**	**C: Pathology / Clinical signs****	**D: Location**
1. Sequential virus titration over time OR 2. Demonstration of increasing copy number over time by RT-qPCR with confirmatory PCR/sequencing OR 3. TEM showing virions in host cells OR 4. *In-situ* products (e.g. antigens) of virus replication detected (e.g. by IHC)*	1. Isolation by cell culture OR 2. Cohabitation with passage to a susceptible host	1. Mortality and/or abnormal behaviour such as: lethargy, loss of appetite AND Gross Pathology such as: • Exophthalmia • Changes in body colour • Skin erosion resulting in haemorrhagic dermal lesions Scale protrusion • Abdominal distension (due to ascites) • Enlargement of internal organs • Congestion of liver, kidney, spleen, brain and gills OR 2. Histopathological changes such as: • Lesions in the brain • Ocular inflammation • Syncytia and/or inclusion bodies in epithelial hepatocytes OR 3. Mortality in experimental virus-exposed group but not in negative control group	1. Infection found in gill lamellae or intestine***, or visceral organs OR 2. Pathogen identification in brain, eyes or visceral organs

* Considered evidence of replication due to the high load of antigen that would need to be present for detection

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

*** As demonstrated by histology, immunohistochemistry (IHC) or in-situ hybridisation (ISH).

3. Scoring and assessments

Table 4 describes the different scores and outcomes of the assessments undertaken by the *ad hoc* Group.

Table 4: Scores

Score	Outcome
1	Species assessed as susceptible (as described in Article 1.5.7.). These species were proposed for inclusion in Article 10.11.2. of Chapter 10.11., Infection with TiLV, of the *Aquatic Code* and Section 2.2.1. of Chapter 2.3.X., Infection with TiLV, of the *Aquatic Manual*.
2	Species assessed as having incomplete 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 2.3.X., Infection with TiLV, of the *Aquatic Manual*.
3	Species assessed as having unresolved or conflicting information. These species were not proposed for inclusion in the *Aquatic Manual*. Species assessed as having pathogen-specific positive PCR results but not having demonstrated active infection. These species were proposed for inclusion in the second paragraph in Section 2.2.2. Species with incomplete evidence for susceptibility of Chapter 2.3.X. Infection with TiLV, of the *Aquatic Manual*.
4	Species assessed as non-susceptible.
NS	Species not scored due to insufficient or irrelevant information.

Table 5 summarises the assessments for host susceptibility to infection with TiLV undertaken by the *ad hoc* Group together with the outcomes and relevant references. For Stage 3, as described in Chapter 1.5. of the *Aquatic Code*, 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 evidence of infection.

Table 5: Assessments for infection with TiLV

Family	Scientific name	Common name	Stage 1: Route of infection	Stage 2: Pathogen Identification	Stage 3: Evidence of Infection				Outcome	References
					A	B	C	D		
					Score 1					
Cichlidae	*Oreochromis aureus x O. niloticus*	blue-Nile tilapia hybrid	N	RT-qPCR, SYBR green RT-qPCR and sequence analysis	ND	YES	ND	YES	1	Abbadi *et al*., 2023
			N	nested RT-PCR and SYBR green RT-qPCR	ND	YES	ND	YES	1	Tsofack *et al*., 2016
			N	RT-PCR and sequence analysis	I[1]	I[1]	YES	YES	1	Eyngor *et al*., 2014

Family	Scientific name	Common name	Stage 1: Route of infection	Stage 2: Pathogen Identification	Stage 3: Evidence of Infection				Outcome	References
					A	B	C	D		
	Oreochromis mossambicus	Mozambique tilapia	N	RT-PCR and sequence analysis	ND	YES	YES	YES	1[2]	Suresh *et al*., 2023
	Oreochromis niloticus	Nile tilapia	N	RT-PCR and sequence analysis	ND	ND	YES	YES	1	Chaput *et al*., 2020
			N	RT-PCR and sequence analysis	ND	YES	YES	YES	1	Behera *et al*., 2018
			N	RT-PCR and sequence analysis	YES	ND	YES	YES	1	del-Pozo *et al*., 2016
	Oreochromis niloticus x O. mossambicus	red hybrid tilapia[3]	N	RT-PCR and sequence analysis	ND	ND	YES	YES	1[4]	Amal *et al*., 2018
	Sarotherodon galilaeus	mango tilapia	N	RT-PCR and sequence analysis	YES	YES	YES	YES	1	Eyngor *et al*., 2014
Score 2										
Cyprinidae	*Barbonymus schwanenfeldii*	tinfoil barb	N	RT-PCR and sequence analysis	ND	YES	I[5]	YES	1[6]	Abdullah *et al*., 2022
			N	RT-PCR and sequence analysis	ND	ND	ND	YES	3	Abdullah *et al*., 2018
Score 3										
Cichlidae	*Oreochromis aureus*	blue tilapia	N	RT-PCR and sequence analysis[7]	I[1]	I[1]	YES	I[1]	3	Eyngor *et al*., 2014
	Tilapia zillii	redbelly tilapia	N	RT-PCR and sequence analysis[7]	I[1]	I[1]	YES	I[1]	3	Eyngor *et al*., 2014
	Tristramella simonis	Tvarnun simon	N	RT-PCR and sequence analysis[7]	I[1]	I[1]	YES	I[1]	3	Eyngor *et al*., 2014
Latidae	*Lates calcarifer*	barramundi	N	RT-PCR and sequence analysis	ND	ND	NO	YES	3	Piamsomboon & Wongtavatchal, 2021
Osphronemidae	*Osphronemus goramy*	giant gourami	N	RT-PCR and sequence analysis	ND	ND	ND	ND[8]	3	Chiamkunakorn *et al.,* 2019

Family	Scientific name	Common name	Stage 1: Route of infection	Stage 2: Pathogen Identification	Stage 3: Evidence of Infection				Outcome	References
					A	B	C	D		
Not scored (NS) because pathogen ID was inconclusive										
Cyprinidae	*Cyprinus carpio*	common carp	N	Negative results by RT-PCR	ND	ND	ND	NO[9]	NS	Chaput *et al*., 2020
	Hypophthalmichthys molitrix	silver carp	N	Negative results by RT-PCR[10]	ND	ND	ND	ND	NS	Chiamkunakorn *et al.,* 2019
	Labeo rohita	roho labeo	N	Negative results by RT-PCR	ND	ND	ND	NO[9]	NS	Chaput *et al*., 2020
			N	Negative results by RT-PCR[10]	ND	ND	ND	ND	NS	Chiamkunakorn *et al.,* 2019
Danionidae	*Danio regio*	zebra danio	EI	stock virus (VETKU-TV01)[11]	N/A	N/A	N/A	N/A	NS	Widziolek *et al*., 2021
Pangasiidae	*Pangasius bocourti*	Basa catfish	N	Negative results by RT-PCR	ND	ND	ND	NO[9]	NS	Chaput *et al*., 2020
Salmonidae	*Oncorhynchus mykiss*	rainbow trout	EI	stock virus (VETKU-TV01)[11]	N/A	N/A	N/A	N/A	NS	Adamek *et al*., 2023
	Salmo trutta	sea trout	EI	stock virus (VETKU-TV01)[11]	N/A	N/A	N/A	N/A	NS	Adamek *et al*., 2023

[1] This study investigated several host species and not all results were clearly assigned to a specific host species.

[2] The *ad hoc* Group determined that the evidence in the paper scored '1' is sufficient for a final assessment of '1' as the study represents natural infections in wild fish from three different regions.

[3] No common name was available on FAOTerm or www.fishbase.se for hybrids of *Oreochromis niloticus x O. mossambicus* however the *ad hoc* Group proposed using red hybrid tilapia as this is the common name used in the region where these hybrids are predominantly cultured.

[4] The *ad hoc* Group determined that the evidence provided in the single paper scored '1' is sufficient for a final assessment of '1' for the following reasons. The *ad hoc* Group considered that both parent species were assessed as having a final score of '1' (Table 5) and that this should be considered as supporting evidence for the susceptibility of the hybrid species. As additional supportive evidence, the *ad hoc* Group considered studies where the species was identified as red hybrid tilapia but scientific name was only identified to the genus level (*Oreochromis* sp.) as it is a generally accepted common name for the species (Table 6).

[5] Clinical signs were observed; however these cannot be specifically attributed to TiLV as there were bacterial co-infections (*Aeromonas* spp., *Plesiomonas* spp., *Edwarsiella* spp.,) in these fish.

[6] The *ad hoc* Group determined that the evidence in the paper scored '1' was not sufficient for a final assessment of '1' as there were bacterial co-infections. The only other study for this species did not have sufficient evidence to corroborate susceptibility based on the criteria. The *ad hoc* Group assessed this species as an overall score of '2'.

[7] The authors of the study confirmed pathogen identification from this host species.

[8] Blood samples were screened for TiLV using RT-PCR and were found to be positive for the pathogen.

[9] Heart, liver, spleen, kidney, gill, gut, gonad and skin tissues were screened for TiLV using RT-PCR and were found to be negative for the pathogen.

[10] Blood samples were screened for TiLV using RT-PCR and were found to be negative for the pathogen.

[11] The study used a stock strain from Thailand (VETKU-TV01) described in Tattiyapong *et al*., 2017b.

Additional note regarding red hybrid tilapia

Table 6 summarises the assessments for host susceptibility to infection with TiLV undertaken by the *ad hoc* Group for studies that referred to 'red hybrid tilapia' without identification of the taxonomic name to the level of species of the animals used in the study. The *ad hoc* Group did not include these assessments in Table 5 as the taxonomic name of the species could not be confirmed however provided the assessments for information. The *ad hoc* Group did consider this as supportive evidence when assigning the final score for *Oreochromis niloticus* x *O. mossambicus* as the common name is generally accepted for this particular hybrid cross.

Table 6: Assessments for infection with TiLV in red hybrid tilapia

Family	Scientific name	Common name	Stage 1: Route of infection	Stage 2: Pathogen Identification	Stage 3: Evidence of Infection				Outcome	References
					A	B	C	D		
Score 1										
Cichlidae	*Oreochromis sp.*	red hybrid tilapia	N	RT-PCR and sequence analysis	YES	ND	YES	YES	1	Dong *et al*., 2017a
			N	RT-PCR and sequence analysis	ND	ND	YES	YES	1	Surachetpong *et al*., 2017
			N	RT-PCR and sequence analysis	ND	YES	YES	YES	1	Tattiyapong et al., 2017b

Assessment Table Key

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

I: Inconclusive
ND: Not determined
NS: Not scored
N/A: Not applicable

4. Results

The *ad hoc* Group agreed that five species, blue-Nile tilapia hybrid (*Oreochromis aureus x O. niloticus*), mango tilapia (*Sarotherodon galilaeus*), Mozambique tilapia (*Oreochromis mossambicus*), Nile tilapia (*Oreochromis niloticus*), and red hybrid tilapia (*Oreochromis niloticus x O. mossambicus*) meet the criteria for listing as susceptible to infection with TiLV in accordance with Chapter 1.5. and therefore should be proposed to be included in Article 10.11.2. of the *Aquatic Code.* All of these species are currently listed in Article 10.11.2. 'under study'.

Tinfoil barb (*Barbonymus schwanenfeldii*) which is currently listed in Article 10.11.2. 'under study' was assessed as having incomplete evidence of susceptibility and is therefore proposed to be included in Section 2.2.2. of Chapter 2.3.X., Infection with TiLV of the *Aquatic Manual*.

Two species, barramundi (*Lates calcarifer*) and giant gourami (*Osphronemus goramy*), were assessed as having pathogen-specific positive PCR results but not having demonstrated active infection. Therefore, these species were proposed to be included in the second paragraph of Section 2.2.2. of Chapter 2.3.X., Infection with TiLV of the *Aquatic Manual*.

Three species, blue tilapia (*Oreochromis aureus*), redbelly tilapia (*Tilapia zillii*) and Tvarnun simon (*Tristramella simonis*) which are currently listed in Article 10.11.2. 'under study' could not be assessed due to insufficient evidence and were not scored.

5. Naming convention for susceptible species

The scientific names of the host species are in accordance with www.fishbase.se.

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

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

'Inconclusive' was used to distinguish situations where more information was provided than would have been assessed as 'Non-determined' but the *ad hoc* Group could not conclude that the criterion was met. Each time inconclusive was used within the assessment table, the *ad hoc* Group provided additional information in a footnote. The *ad hoc* Group treated 'Inconclusive' as 'Non-Determined' when making their final assessment.

The *ad hoc* Group agreed that while the ideal situation was two papers with a score of '1', a single robust study scoring '1' was also enough to conclude susceptibility of a species in the absence of conflicting evidence. Where sampling strategy was distributed across seasons or locations, and/or where a single paper provided all evidence (molecular with corresponding evidence from histology within the same animals) the *ad hoc* Group considered that one strong paper was sufficient to conclude susceptibility of a species. Additional studies were still reviewed to check for any supporting or conflicting evidence. When additional papers were identified but the *ad hoc* Group did not feel that they were necessary to assess comprehensively because the species had already been determined as susceptible by other studies, these studies were retained in the list of references only.

A number of studies were unclear as to the species of fish used in their study; for example, authors referred to 'Tilapia' or 'red hybrid tilapia' without giving the scientific name of the species or species making up the cross. This made it difficult to assign these particular studies to host species. Some studies assessed multiple species without assigning results to specific species making it difficult to assess a single host species. Authors were contacted to determine if the identity of the fish used in these studies could be confirmed. Where identity could not be confirmed, these papers were not included in the assessments for susceptibility with the exception of those referenced in table 6.

7. Article 1.5.9 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 the *Aquatic Code*, and determined that it was not applicable for the susceptible host species for TiLV identified at this time.

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…/Annexes

Annex 1. List of Participants

MEETING OF THE WOAH *AD HOC* GROUP ON SUSCEPTIBILITY OF FISH SPECIES TO WOAH LISTED DISEASES

12, 13 and 19 April 2023 (virtual)

List of Participants

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Annex 2. Terms of Reference

MEETING OF THE WOAH *AD HOC* GROUP ON SUSCEPTIBILITY OF FISH SPECIES TO WOAH LISTED DISEASES

12, 13 and 19 April 2023 (virtual)

Terms of reference

Background

Chapter 1.5. Criteria for listing species as susceptible to infection with a specific pathogenic agent of the *Aquatic Code*, provides criteria for determining which host species are listed as susceptible in Article X.X.2. of each disease-specific chapter in the *Aquatic Code*.

Assessments for all of the WOAH listed diseases are being undertaken progressively by *ad hoc* Groups. Once completed, the revised list of susceptible species in the relevant Article X.X.2. of the *Aquatic Code* is circulated for Member comment and then presented for adoption.

Species, where there is some evidence of susceptibility but insufficient evidence to demonstrate susceptibility are included in the relevant disease-specific chapter in the *Aquatic Manual*.

The *ad hoc* Group on Susceptibility of fish species to infection with WOAH listed diseases has undertaken assessments for all of the WOAH listed diseases of fish, except for infection with tilapia lake virus and infection with *Aphanomyces invadans* (epizootic ulcerative syndrome).

Purpose

The *ad hoc* Group on Susceptibility of fish species to infection with WOAH listed diseases will undertake assessments for infection with tilapia lake virus in fish.

Terms of Reference

1) Review relevant literature documenting susceptibility of species for infection with tilapia lake virus and apply criteria, as outlined in Chapter 1.5. Criteria for listing species as susceptible to infection with a specific pathogen, to potential host species.

2) Determine susceptible species for infection with tilapia lake virus based on Article 1.5.7.

3) Determine species with incomplete evidence for susceptibility for infection with tilapia lake virus based on Article 1.5.8.

Expected outputs of the *ad hoc* Group

1) Propose a list of susceptible species for inclusion in the Article 10.X.2. of Chapter 10.X, Infection with tilapia lake virus, in the *Aquatic Code*.

2) Propose a list of species with incomplete evidence for susceptibility for inclusion in Section 2.2.2 and Section 2.2.2. of Chapter 2.3.X. Infection with tilapia lake virus of the *Aquatic Manual* (to be developed).

3) A report for consideration by the Aquatic Animals Commission at its September 2023 meeting.
