Organisation Mondiale de la Santé Animale

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Organización Mundial de Sanidad Animal

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REPORT OF THE ELECTRONIC MEETING OF THE OIE *AD HOC* GROUP ON TILAPIA LAKE VIRUS[1]

November 2017–January 2018

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The OIE *ad hoc* Group on Tilapia lake virus (TiLV) was established in November 2017 to assess TiLV diagnostics and validation.

At their September 2017 meeting the Aquatic Animal Health Standards Commission (Aquatic Animals Commission) reviewed the assessment of tilapia lake virus (TiLV) against the new criteria in Chapter 1.2. *Criteria for listing aquatic animal diseases* of the *Aquatic Code* noting that revised criteria had been adopted at the 2017 OIE General Session. The Commission also considered new scientific information published since their last meeting in February 2017.

The Aquatic Animals Commission re-evaluated evidence for the third criterion for listing a disease by the OIE: “a precise case definition is available and a reliable means of detection and diagnosis exists”. The Commission considered information in a recent publication describing a new diagnostic assay for TiLV (Dong *et al.*, Emergence of tilapia lake virus in Thailand and an alternative semi-nested RT-PCR for detection. *Aquaculture*, doi: 10.1016/j.aquaculture.2017.04.019). The Commission agreed that with this additional information the criterion is still not met because of insufficient information concerning analytical and diagnostic specificity and sensitivity of the assay.

Because TiLV is continuing to spread around the world, the Aquatic Animals Commission decided to convene an *ad hoc* Group (AHG) to assess TiLV diagnostics and validation and report to the Aquatic Animals Commission before the February 2018 meeting.

Conclusions and recommendations from the *ad hoc* Group

Further evaluation and comparison of molecular tests is recommended. Real-time PCR methods usually have the highest sensitivity and specificity and should therefore be recommended for validation.

Material for interlaboratory comparability studies could be prepared at Australian Animal Health Laboratory (AAHL), which has expertise and is ISO 17043 accredited for producing, analysing and reporting quality-assured proficiency testing panels for national and international proficiency testing programmes. This activity would also fall within AAHL’s mandates as an OIE Collaborative Centre for Diagnostic Test Validation Science and for New and Emerging Diseases.

Once sufficient information is available about the comparative fitness of the tests under study, validation should also include results on recommended tissues and alternative samples such as mucous (Liamnimitr *et al.*, 2018) for sampling (including any variations for different life stages) and determine the appropriateness of pooling of samples.

More comprehensive validation studies to determine DSe and DSp for testing of clinically-affected and in particular apparently-healthy animals are needed, for example Senapin *et al.* (2018) reported about test results obtained for inapparent infected cases of TiLV.

[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 2018 report of the Aquatic Animal Health Standards Commission because this report provides its considerations and comments. It is available at http://www.oie.int/en/international-standard-setting/specialists-commissions-groups/aquatic-animal-commission-reports/meeting-reports/

The OIE Aquatic Animals Commission is requested to review the findings of this report and agree to the recommendations.

The **specific recommendations** of the *ad hoc* Group on Tilapia lake virus are:

1. OIE Headquarters contact the OIE Delegates of Member Countries where TiLV has been reported and request that positive control material is supplied for molecular test evaluation and inter-laboratory comparability studies to Dr Collin at the Collaborating Centre for New and Emerging Diseases, Australian Animal Health Laboratory (refer to Annex I for contact details).

2. The OIE Aquatic Animal Health Standards Commission revise the Terms of Reference of the *ad hoc* Group (TORs) to develop detailed work plans in order to undertake the work described in 3., 4. and 5. below. Expanded TORs could also include provision of the inter-laboratory comparability panels for TiLV to countries providing material when the *ad hoc* Group is satisfied the assays are performing in a suitable manner.

3. The OIE noted that the *ad hoc* Group will consider resourcing requirements to undertake the proposed work.

…/Annexes

Annex I

REPORT OF THE ELECTRONIC MEETING OF THE OIE *AD HOC* GROUP ON TILAPIA LAKE VIRUS

November 2017–January 2018

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Annex II

OIE *AD HOC* GROUP ON TILAPIA LAKE VIRUS

TERMS OF REFERENCE

Purpose of the *ad hoc* Group

The *ad hoc* Group on Tilapia lake virus (TiLV) will evaluate published and unpublished methods for detection of TiLV, describe the level of validation of each method and determine additional validation requirements, recommend any additional assays that may need to be developed and facilitate the sourcing and distribution of well-characterised positive control material for method evaluation, implementation and inter-laboratory comparability studies.

Background

At the September 2017 meeting of the Aquatic Animals Commission, the Commission undertook an assessment of tilapia lake virus (TiLV) against the criteria in Chapter 1.2. *Criteria for listing aquatic animal diseases* of the *Aquatic Code*.

The Commission reviewed available scientific information against the third criterion: “a precise case definition is available and a reliable means of detection and diagnosis exists” and agreed that the criterion is not met because of insufficient information concerning analytical and diagnostic specificity and sensitivity of the assay.

Terms of Reference

The *ad hoc* Group should:

1. Critically review the available literature regarding detection methods for TiLV and any unpublished methods that may also be available.

2. Provide recommendations on additional method development requirements.

3. Provide recommendations on method validation requirements.

4. Determine sources of well-characterised viable and non-viable positive control material for use in method evaluation and implementation in laboratories.

5. Develop a work plan for inter-laboratory comparability studies.

6. Draft a report by the end of January 2018 to be considered by the Commission when they meet in February 2018.

Ad hoc Group members should be familiar with Chapter 1.2. *Criteria for listing aquatic animal diseases* and the use of relevant glossary definitions in the *Aquatic Code*, and with the principles and methods of validation of diagnostic essays for infectious diseases in Chapter 1.1.2. of the *Aquatic Manual*.

ASSESSMENT OF THE *AD HOC* GROUP ON TILAPIA LAKE VIRUS

1. Critically review the available literature regarding detection methods for TiLV and any unpublished methods that may also be available

The literature has been reviewed[2] and three molecular methods have been identified which would be candidates for further validation studies:

a) Conventional semi-nested assay (RT-nPCR) described by Dong *et al.* (2017a). This uses primers designed by Eyngor *et al.* (2014) with modifications described by Tsofack *et al.* (2017).

b) Real-time SYBR assay (RT-qPCR) described by Tattiyapong *et al.* (2017b).

c) Real-time probe-based assay (RT-qPCR) which is unpublished and has been provided to the *ad hoc* Group by Dr Hong.

The applicability of the primer and probe for the molecular assays has been assessed and confirmed as appropriate for further validation work by *in silico* analysis with TiLV sequences available in the public domain that contain the binding sites for the primers/probes under investigation.

Virus isolation in E-11 cells using standard procedures has been described. The E-11 cell line is available from international cell culture collections including the European Collection of Authenticated Cell Cultures (ECACC).

Details of selected papers are provided in the attached Excel spreadsheet for a) purpose of study, b) source animals, c) PCR details, d-g) ASe, ASp, DSe, DSp, h) Tissue type (sample), i) Tilapia species (domestic or wild) and j) comments.

2. Provide recommendations on additional method development requirements

Dr Marshall has suggested a LAMP assay is developed to add to the suite of tests available to laboratories for molecular detection of TiLV and has offered to develop this assay.

3. Provide recommendations on method validation requirements

The molecular assays mentioned in 1.) above have had varying levels of validation undertaken. Generally, only small numbers of local samples from clinically affected Tilapia have been tested and determination of ASe, ASp, DSe and DSp for all assays are incomplete. A TiLV LAMP assay could also be included in initial evaluation described below.

Recommendations for further validation work could be undertaken in combination with inter-laboratory comparability studies described in 5.) below. An initial evaluation of the assays would be to:

a) Determine ASe for each of the molecular assays using 10-fold dilutions of quantified RNA transcribed from plasmids;

b) Determine the analytical comparability of the molecular assays using 10-fold dilutions of genomic TiLV RNA;

[2] Twelve papers were identified using online search engines such as “Web of Science” and “PubMed”. In addition ten peer-reviewed current papers and one FAO review were provided by some members of the *ad hoc* Group and the OIE.

c) Determine ASp for each of the molecular assays using finfish viruses available in *ad hoc* Group members' laboratories;

d) Determine ASp for each of the molecular assays for TiLV isolates obtained from different geographical locations, after preliminary characterisation of the isolates by conventional PCR and sequencing of segments 1, 5 and 9;

e) Determine initial preliminary estimates for repeatability and reproducibility.

If the above initial evaluation of the assays combined with the inter-laboratory comparability studies produce acceptable results, more comprehensive validation studies to determine DSe and DSp for testing of clinically-affected and apparently-healthy animals can be planned and conducted.

4. Determine sources of well-characterised viable and non-viable positive control material for use in method evaluation and implementation in laboratories

Dr Nadav has said that Israel can supply positive control material. Both researchers, Dr. Avi Eldar from Kimron Veterinary Institute and Prof. Eran Bacharach from Tel Aviv University, are working on the virus since its first description.

AAHL can receive viable, infectious material for *in vitro* and *in vivo* work with pathogens that are exotic to Australia. Dr Moody's laboratory is able to receive infectious TiLV and this can be amplified in E-11 cell cultures in his laboratory, rendered non-infectious and sent to *ad hoc* Group member's laboratories for use in evaluation and comparability studies. Some work will be required to determine the degree of degradation of the TiLV RNA by the gamma-irradiation but work done at AAHL with other finfish viruses suggests this will not render the material unable to be used.

A recommendation of the *ad hoc* Group is for OIE Headquarters to contact the OIE Delegates of Member Countries where TiLV has been reported and request that positive control material is supplied for molecular test evaluation and inter-laboratory comparability studies. Material Transfer Agreements would be set up to ensure any materials transferred are only used to support the activities of the *ad hoc* Group.

5. Develop a work plan for inter-laboratory comparability studies

Ideally, inter-laboratory comparability studies should be undertaken using material representative of tissues which would be sampled in the field and submitted to the laboratory for testing. However, given potential issues associated with the transboundary transfer of infectious materials and the risk of a lack of homogeneity when using infected fish, infected cell culture supernatant provides an alternative source of material for inter-laboratory comparability studies. Samples in the panel would be tested to ensure homogeneity and stability requirements are fulfilled. Enough material would be prepared to allow distribution to other laboratories wishing to implement the assays recommended by the *ad hoc* Group, pending endorsement by the OIE Aquatic Animal Health Standards Commission.

Comparability panels would consist of 20 positive and 10 negative samples that could include:

a) 10-fold dilution series (7 samples) to enable estimates of efficiency of real-time molecular assays;

b) Medium positive (2 samples);

c) Low positive (2 samples);

d) 10-fold dilution of medium and low positive (4 samples);

e) Positive samples with various viral concentrations (5 samples);

f) Negative samples consist of supernatant of uninfected cell culture (10 samples).

Participating laboratories would receive the samples as numbered tubes so would test them blind. Panels would be tested at least three times. Results would be reported back to the Chair of the *ad hoc* Group for collation and reporting back as uncoded results to the participating laboratories for discussion. Use of duplicate samples and 10-fold dilutions of samples enables statistical analysis which can be used to determine repeatability and reproducibility.

Results of the evaluation of the TiLV molecular assays, generation of preliminary validation data and inter-laboratory comparability studies could be presented at OIE conferences and/or published in the OIE *Scientific and Technical Review* or other relevant peer-reviewed journals.

References:

Dong *et al.* (2017a). Emergence of tilapia lake virus in Thailand and an alternative semi-nested RT-PCR for detection. *Aquaculture*, 476:111.

Eyngor *et al.* (2014). Identification of a novel RNA virus lethal to tilapia. *J. Clin. Micro*., 52(12): 4137.

Tattiyapong *et al.* (2017b). Development and validation of a reverse transcription quantitative polymerase chain reaction for tilapia lake virus detection in clinical samples and experimentally challenged fish. *J. Fish Dis.*, 12708.

Tsofack *et al.* (2017). Detection of Tilapia Lake Virus in clinical samples by culturing and nested reverse transcription-PCR. *J. Clin. Micro., 5*5(3): 759.

Liamnimitr *et al.* (2018). Non-lethal sampling for Tilpia Lake Virus by RT-qPCR and cell culture. *Aquaculture*, 486:75–80.

Senapin *et al.* (2018). Inapparent infection cases of tilapia lake virus (TiLV) in farmed tilapia. *Aquaculture*.