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

September 2018–January 2019

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Activities, achievements and next steps

This interim report covers activities and achievement of the ad hoc group September 2018 – January 2019. In 2018 several TiLV endemic countries were approached by the Director general of the OIE, Dr Monique Eloit, to provide positive TiLV material, for example China, Israel, Malaysia, Peru, Philippines and Thailand.

Material has been obtained from Peru (8 x tissue samples) and Thailand (1 x virus isolate from red tilapia cultured in the SSN-1 cell line). Three of the most promising samples from Peru and the cell culture isolate from Thailand were assessed for infectivity by passaging on the E11 cell line. No isolates were obtained from the material from Peru, however, cytopathic effect was observed after passage of the isolate from Thailand.

The Thailand isolate (designated 18-03492) was confirmed as TiLV after testing with the TiLV RT-nPCR assay described by Dong et al. (2017), with modifications to be consistent with AFDL test protocols, and sequence analysis of the 415bp gel purified RT-PCR amplicon. By BLAST search through the NCBI database, the 375 bp primer-trimmed amplicon shared the highest nucleotide identity of 94.7% with two isolates from Israel; Tilapia lake virus isolate Til-4-2011 segment 3 (KU751816.1) and Tilapia lake virus clone 7450 (KJ605629.1). The isolate tested negative after adventitious agent testing for KHV, NNV and ISKNV/RSIV.

A preliminary evaluation has been conducted with Thai isolate 18-03492 using the 3 real-time PCR assays (described below) to compare limits of detection (LOD) using nucleic acid extracted from the sample. Results are still to be analysed. Included in this evaluation was an assessment of two master mixes for use in the SYBR assay. The analytical sensitivity (ASe) for the Hong Liu assay, determined using a positive control plasmid diluted in water, is 2 plasmid copies per reaction. As plasmids have not been prepared for the other real-time assays, their ASe has not been determined.

Real-time PCR – probe based

1. Hong Liu – China, personal communication to the ad hoc Group.
2. Waiyamitra et al., 2018.

Real-time PCR – SYBR

Isolate 18-03492 has been amplified in E11 cells and 100mL of clarified supernatant has been gamma-irradiated at 50kGy. This gamma-irradiated material will be tested by real-time RT-PCR to assess the effect of the gamma-irradiation on the viral genome. If results are as expected (i.e. minimal reduction in C_T value) the inter-laboratory comparability panels will be prepared as described in the “OIE ad hoc Group on tilapia lake virus (TiLV) November 2017-January 2018 report”. After homogeneity and stability testing, it is anticipated the panels will be sent to collaborating laboratories in April 2019 for testing and reporting of results by July 2019. After analysis of results and discussion by ad hoc Group members, the ad hoc Group report of the inter-laboratory comparability testing and recommendations on test performance will be submitted to the OIE Aquatic Animal Health Standards AAHSC for its meeting in September 2019.

References:

