

Emergency Response to Emerging Diseases: TiLV in Tilapia

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Abstract

Tilapia lake virus (TiLV) is a novel RNA virus resembling Orthomyxovirus. It has been recently re-classified to *Tilapia tilapinevirus* species, under *Tilapinevirus* genus, *Amnoonviridae* family (ICTV, 2018). Since the first discovery in Israel in 2014, so far TiLV has been reported from 14 countries in three continents (Asia, Africa, and South America). Thailand is one of the affected countries that reported emergence of this virus in 2017. Initially, we employed nested RT-PCR primer sequences previously published for TiLV diagnosis. However, the resulting amplification of nonspecific fish genes led us to modify the nested RT-PCR protocols into a semi-nested RT-PCR by omitting a non-specific primer to avoid false positive results. Subsequently, our molecular work together with histopathology and sequence analysis confirmed the presence of TiLV infection in Thailand. Prior to the publication of our manuscript, we informed the Thai Department of Fisheries of our discovery of TiLV in Thailand. Our publication was preceded by a brief article at the website of the Network of Aquaculture Centers in Asia-Pacific in which we warned of the spread of TiLV and offered free use of a newly improved, semi-nested RT-PCR method and positive control plasmid for detection of TiLV. To date, we have provided positive controls in response to 44 requests from 24 countries who have expressed their appreciation for our attempt to help in emergent controlling the spread of this fish pathogen. Our current study focuses on genetic diversity of TiLV and development of detection method that covers all genetic variants.

What has been done

Get ready for PCR diagnosis

Tilapia Lake Virus (TiLV) was considered as an emerging tilapia virus since the publication of studies by Eynogor et al (2014) and Ferguson et al (2014) have been published. When the partial viral genome sequence and PCR primers for detection become available (Kembou Tsofack et al, 2017), Centrex Shrimp starts synthesizing and had been optimizing the PCR conditions in the laboratory without any positive control or infected fish specimens. It was observed that some false positive results occurred due to non-specific binding of one primer pair. Thus, one of the primers was omitted and PCR conditions were modified from nested to become semi-nested PCR. Until December 2016, clinically sick tilapia specimens were obtained and was reported with over mortality of 20% and 90%. These two sets of the samples were all TiLV positive and sequence analysis revealed 96.28 to 97.52% nucleotide identity with the Israel isolate (Eynogor et al, 2014; Bacharach et al, 2016). With infected samples in possession, construction of the positive plasmid for use as positive control started and was used in detection sensitivity assay. Our modified semi-nested PCR protocol had the detection sensitivity of 7.5 copies per reaction (Dong et al, 2017a).

Announcements and offering positive control

Once the presence of TiLV by histopathology and sequence analysis was confirmed, Department of Fisheries in Thailand was informed prior to publication of results (Dong et al, 2017a). Subsequently, the group wrote a brief article which served as a warning of the spread of the virus. It was published at the website of the Network of Aquaculture Centers in Asia-Pacific (<https://enaca.org>). It also includes the Center's offering of the newly improved, semi-nested RT-PCR method and positive control plasmid for detection of TiLV (Dong et al, 2017b). Using the newly improved detection assay, it could reveal that some of the archived samples in our laboratory kept in 2012-2016 were tested positive for TiLV. This is an indication that the virus has already circulated in the country before it became known to science (Dong et al, 2017c, d). To date, positive controls have been provided in response to 44 requests from 24 countries. All of which have expressed their appreciation for the

center's attempt to help control the spread of this fish pathogen. Training courses on TiLV diagnosis based on molecular and histopathological analysis were also conducted upon request.

Way forward

There are still knowledge gaps, in many aspects, on TiLV (Jansen et al, 2018). Some massive mortalities were found to be associated with the virus while some TiLV-infected cases showed no abnormal mortality (Senapin et al, 2018). There are still problem areas that needed answer including if there are genetic variation types of this virus and whether it contributes to a difference in virulence and pathogenicity. The center's current studies focus on genetic diversity of TiLV and development of detection method that covers all genetic variants. Investigation of potential vertical transmission of TiLV is also in progress.

Acknowledgments

This research on TiLV has been financially supported by grants from Mahidol University, National Center for Genetic Engineering and Biotechnology (BIOTEC/NSTDA), and WorldFish CGIAR Research Program on Fish Agri-Food Systems.

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