

# **DISEASE ADVISORY**



## Tilapia Lake Virus (TiLV) – an Emerging Threat to Farmed Tilapia in the Asia-Pacific Region

Network of Aquaculture Centres in Asia-Pacific, Bangkok, Thailand

- TiLV (an Orthomyxo-like RNA virus) is an emerging disease of cultured tilapia in the Asia-Pacific region;
- Originally observed and reported in Israel, Ecuador, Colombia and Egypt, TiLV is now confirmed in cultured tilapia in Thailand causing mass mortalities;
- At risk is here is the US\$7.5 billion global industry per annum, especially among the top tilapia-producing countries in the region including China, the Philippines, Thailand, Indonesia, Lao PDR and Bangladesh.

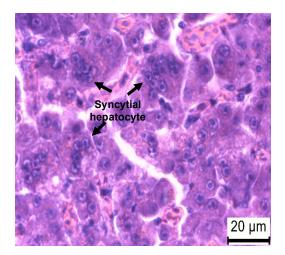
Tilapias are highly important (and inexpensive) source of fish protein in the world and are one of the most popular species for aquaculture in several regions including the Asia-Pacific. The top 10 producing-countries include China, Egypt, Philippines, Thailand, Indonesia, Lao PDR, Costa Rica, Ecuador, Colombia and Honduras. Since 2009, tilapia aquaculture has been threatened by mass die-offs of farmed fish in Israel and Ecuador (Bacharach et al., 2016). The aetiological agent causing this mass die-offs has been described and identified as a novel Orthomyxo-like (RNA) virus named as Tilapia lake virus (TiLV) (Eyngor et al. 2014; 2016; Bacharach et al., 2016). This has been reported as a newly emerging virus that causes syncytial hepatitis of tilapia (SHT). As of 2016, countries affected by this emerging disease of tilapia include Israel, Ecuador, Colombia and Egypt (Eyngor et al., 2014; Ferguson et al., 2014; Bacharach et al., 2016; Tsofack et al., 2016; Del-Pozo et al., 2017; Fathi et al., 2017).

Recently, disease outbreaks among cultured tilapias have occurred in Thailand, wherein high cumulative mortalities (20-90%) were observed and recorded (Dong et al., 2017a). Thirty-two outbreaks were investigated during 2015-2016 involving large number of deaths of unknown cause among farmed tilapia (*Oreochromis niloticus*) and red hybrid tilapia (*Oreochromis* spp.) (Suratchatpong et al., 2017). Histopathology (of the liver showing similar signs to SHT), transmission electron microscopy, in-situ hybridization and high nucleotide sequence identity to TiLV from Israel (Dong et al. 2017b) confirmed that these outbreaks were caused by TiLV.

## Signs of the Disease

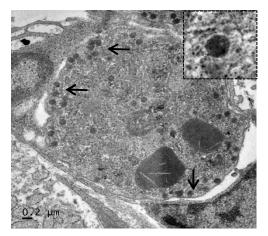
Mass mortality (20-90%) among cultured tilapias is an indicative sign of the disease. Gross signs include multifocal to coalescing dermal erosions and ulcers, ocular alterations including opacity of the lens and shrinkage of the eyes (Eyngor et al., 2014). Diseased fish also exhibit loss of appetite, pale color, gathering in the bottom, slow movement, and stopped schooling prior to death (Dong et al., 2017).

Histopathological lesions of the brain included edema, focal hemorrhages in the leptomeninges, and capillary congestion in both the white and gray matter (Eyngor et al., 2014). For the liver, histopathological changes include swollen and dissociated hepatocytes (Dong et al., 2017a) and typical feature of syncytial hepatitis as shown in Figure 1 (Dong et al., 2017b).



**Figure 1.** Photomicrograph of TiLV-infected liver tissue of tilapia revealed typical syncytial hepatitis (H&E). Photo courtesy of H. Dong.

Intracytoplasmic viral particles can be observed in the infected tissues using transmission electron microscopy (Figure 2).



**Figure 2.** Transmission electron micrograph of intracytoplasmic viral particles of TiLV (arrows) from liver tissues. Photo courtesy of H. Dong.

### **PCR Detection Methods**

Detection of the virus in infected tissues of tilapia is highly important in the confirmation of infection or the presence of the virus. Eyngor et al. (2014) developed an RT-PCR method for the detection of TiLV, while Tsofack et al. (2017) described a sensitive nested RT-PCR assay allowing the rapid detection of TiLV in fish organ. An improved PCR detection method for TiLV was published by Dong et al. (2017b) by modifying the nested RT-PCR protocols into a semi-nested RT-PCR by omitting the primer "Nested ext-2" to avoid false positive results. The semi-nested RT-PCR protocol may be used freely for non-commercial applications to detect TiLV. Heavily-infected samples will generate 2 amplicon bands of 415 bp and 250 bp while lightly-infected samples will generate a single 250-bp amplicon band. Please contact Centex Shrimp (saengchan@biotec.or.th) to obtain a free positive control plasmid (pGEM-415\_bp).

#### References:

- Bacharach, E., Mishra, N., Briese, T., Zody, M.C., Kembou Tsofack, J.E., Zamostiano, R., Berkowitz, A., Ng, J., Nitido, A., Corvelo, A., Toussaint, N.C., Abel Nielsen, S.C., Hornig, M., Del Pozo, J., Bloom, T., Ferguson, H., Eldar, A., Lipkin, W.I., 2016. Characterization of a novel Orthomyxo-like virus causing mass die-offs of tilapia. MBio. 7, e00431-00416
- Del-Pozo, J., Mishra, N., Kabuusu, R., Cheetham, S., Eldar, A., Bacharach, E., Lipkin, W.I., Ferguson, H.W., 2017. Syncytial hepatitis of tilapia (Oreochromis niloticus L.) is associated with Orthomyxovirus-like virions in hepatocytes. Vet. Pathol. 54, 164-170.
- Dong HT, Siriroob, S., Meemetta, W., Santimanawong, W., Gangnonngiw, W., Pirarat, N., Khunrae, P., Rattanarojpong, T., Vanichviriyakit, R. and Senapin, S., 2017a. 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
- Dong HT, Siriroob, S., Meemetta, W., Santimanawong, W., Gangnonngiw, W., Pirarat, N., Khunrae, P., Rattanarojpong, T., Vanichviriyakit, R. and Senapin, S., 2017b. A warning and an improved PCR detection method for tilapia lake virus (TiLV) disease in Thai tilapia farms. https://enaca.org/?id=858
- Eyngor, M., Zamostiano, R., Tsofack, J.EK., Berkowitz, A., Bercovier, H., Tinman, S., Lev, M., Hurvitz, A., Galeotti, M., Bacharach, E. and Eldar, A., 2014. Identification of novel RNA virus lethal to tilapia. J. Clinical Microbiology, 52:4137-4146.
- Fathi, M., Dickson, C., Dickson, M., Leschen, W., Baily, J., Muir, F., Ulrich, K., Weidmann, M., 2017. Identification of Tilapia Lake Virus in Egypt in Nile tilapia affected by 'summer mortality' syndrome. Aquaculture. 473, 430– 432.
- Ferguson, H.W., Kabuusu, R., Beltran, S., Reyes, E., Lince, J.A., del Pozo, J., 2014. Syncytial hepatitis of farmed tilapia, *Oreochromis niloticus* (L.): a case report. J. Fish Dis. 37, 583-589.
- Surachetpong, W., Janetanakit, T., Nonthabenjawan, N., Tattiyapong, P., Sirikanchana, K. and Amonsin, A., 2017. Outbreaks of tilapia lake virus infection, Thailand, 2015-2016. Emerging Infectious Diseases, <u>https://dx.doi.org/10.3201/eid2306.161278</u>
- Tsofack, J.E.K., Zamostiano, R., Watted, S., Berkowitz, A., Rosenbluth, E., Mishra, N., Briese, T., Lipkin, W.I., Kabuusu, R.M., Ferguson, H., Del Pozo, J., Eldar, A., Bacharach, E., 2016. Detection of tilapia lake virus (TiLV) in clinical samples by culturing and nested RTPCR. J. Clin. Microbiol. doi:10.1128/JCM.01808-16.