TILAPIA LAKE VIRUS (TiLV)–A NOVEL ORTHOMYXO-LIKE VIRUS

PATHOGEN INFORMATION

1. CAUSATIVE AGENT

1.1. Pathogen type

Virus.

1.2. Disease name and synonyms

Tilapia lake virus (TiLV) disease.

1.3. Pathogen common names and synonyms

Tilapia lake virus (TiLV).

1.4. Taxonomic affiliation

The taxonomic affiliation has not been definitively concluded; however, TiLV has been described as a novel virus in the Family *Orthomyxoviridae* (Eyngor *et al.*, 2014).

1.5. Authority (first scientific description, reference)

The virus was first described by Eyngor *et al.* (2014).

1.6. Pathogen environment (fresh, brackish, marine waters)

Fresh and brackish water.

2. MODES OF TRANSMISSION

2.1. Routes of transmission (horizontal, vertical, indirect)

Co-habitation studies have demonstrated that direct horizontal transmission is an important route of transmission. There is no evidence of vertical transmission. The biophysical characteristics of the virus are not well characterised so it is difficult to determine the significance of indirect transmission by fomites.

2.2. Reservoir

Infected populations of fish, both farmed and wild, are the only established reservoirs of infection. The original source of TiLV is not known.

2.3 Risk factors (temperature, salinity, etc.)

Disease has been associated with transfer between ponds and thus may be associated with stress (Ferguson *et al.*, 2014, Dong *et al,* 2017). No other risk factors (temperature, salinity, etc.) have been identified as potential risk factors.

3. HOST RANGE

3.1. Susceptible species

Mortalities attributed to TiLV have been observed in wild tilapia *Sarotherodon (Tilapia) galilaeus*, farmed tilapia *Oreochromis niloticus* and commercial hybrid tilapia (*O. niloticus* X *O. aureus*) (Bacharach *et al.*, 2016; Ferguson *et al.*, 2014; Eyngor *et al.*, 2014). To date only tilapines have been shown to be susceptible. It is possible that other species will be found to be susceptible.

3.2. Affected life stage

In the outbreak reported by Ferguson *et al.* (2014) and Dong *et al.* (2017) fingerlings were mainly affected. Dong *et al.* (2017) reported approximately 90% mortality in red tilapia fingerlings within one months of stocking into cages. Mortality just over 9% in medium to large sized Nile tilapia was noted by Fathi *et al.* (2017). Other reports have not commented on different levels of mortality by life stage (Eyngor *et al.*, 2014),

3.3. Additional comments

There is some evidence that certain genetic strains of tilapia are resistant. Ferguson *et al.* (2014) noted that one strain of tilapia (genetically male tilapia) incurred a significantly lower level of mortality (10-20%) compared with other strains.

4. GEOGRAPHICAL DISTRIBUTION

TiLV has been reported in Colombia, Ecuador and Israel (Bacharach *et al.*, 2016; Ferguson *et al.*, 2014; Tsofack *et al.*, 2016), and most recently, Egypt (Fathi *et al.*, 2017) Thailand (Dong *et al.*, 2017) India (Behera *et al.*, 2018), Malaysia (Amal *et al*., 2018) and the Philippines (OIE, 2017). However, a lack of thorough investigation of all mortality incidents means that the geographic distribution of TiLV may be wider than currently. For example, reports of mortality in tilapia in Ghana and Zambia in 2016 have not been attributed to TiLV but the available information does not indicate that the presence of the virus has been investigated. A partial genome from Thailand showed relatively high variation to strains from Israel (around 97% nucleotide identity) (Dong *et al.,* 2017).

5. CLINICAL SIGNS AND CASE DESCRIPTION

5.1. Host tissues and infected organs

The main organs where pathology is observed are the eyes, brain and liver (Eyngor *et al.*, 2014).

5.2. Gross observations and macroscopic lesions

Gross lesions included ocular alterations, including opacity of the lens and in advanced cases ruptured lens. Other lesions included skin erosions, haemorrhages in the leptomeninges and congestion of the spleen (Eyngor *et al.*, 2014).

5.3. Microscopic lesions and tissue abnormality

Histologic lesions have been observed in the brain, eye and liver (Eyngor *et al.*, 2014). Lesions in the brain included oedema, focal haemorrhages in the leptomeninges, and capillary congestion in both the white and grey matter *and* neural degeneration. Foci of gliosis and occasional perivascular cuffs of lymphocytes have been detected. Ocular lesions included ruptured lenticular capsule and cataractous changes. Foci of hepatocellular swelling were observed. The spleen was hyperplastic, with proliferating lymphocytes. Melanomacrophage centres (MMCs) were increased in size and number in both the liver and the spleen. Transmission electron microscopy confirmed the presence of an orthomyxo-like virus within diseased hepatocytes and thus confirmed earlier reports of syncytial hepatitis (del-Pozo *et al.*, 2016).

5.4. OIE status

Under consideration for listing but currently does not meet all of the criteria for listing as described in Chapter 1.2. of the *Aquatic Animal Health Code* (OIE, 2016).

6. SOCIAL AND ECONOMIC SIGNIFICANCE

Tilapines, comprising more than 100 species, are the second most import group of farmed fish worldwide after carp. Global production is estimated at 4.5 million metric tons with a current value in excess of U.S.$7.5 billion (FAO, 2014). In some regions they are ecologically important (algae and mosquito control and habitat maintenance for shrimp farming) and an important wild capture species. Introduction of the virus has been shown to cause significant mortality (up to 90%) and thus result in serious economic losses to both farmers and fishers (Eyngor *et al.*, 2014; Dong *et al.*, 2017).

7. ZOONOTIC IMPORTANCE

None

8. DIAGNOSTIC METHODS

8.1. Definition of suspicion

High levels of mortality in tilapine species, associated with ocular alterations (opacity of the lens or more severe pathology), should be considered suspicious of TiLV. Skin erosions, haemorrhages in the leptomeninges and moderate congestion of the spleen and kidney may be observed on post-mortem.

8.2. Presumptive test methods

TiLV can be cultured in primary tilapia brain cells or in an E-11 cell line, inducing a cytopathic effect at 3-10 days (Eyngor *et al.*, 2014) (Liamnimitr *et al.*, 2017). Tsofack *et al.* (2016) describe optimal conditions for culturing TiLV.

8.3. Confirmatory test methods

A PCR primer set has been designed and a reverse transcriptase (RT) PCR has been developed (Eyngor *et al.*, 2014). A more sensitive, nested RT-PCR has been published and is suitable for the detection of TiLV in clinical cases (Tsofack *et al.*, 2016). Most recently a semi-nested RT-PCR with an improved analytical sensitivity (7.5 viral copies per reaction), has been published (Dong *et al.,* 2017) as has a real-time SYBR assay with analytical sensitivity of 2 copies of plasmid (Tattiyapong *et al*., 2017). All molecular tests require further validation.

9. CONTROL METHODS

Restrictions on the movement of live tilapines from farms and fisheries where the virus is known to occur will limit the spread of the disease. Generic biosecurity measures to minimise fomite spread via equipment, vehicles or staff (i.e. cleaning and disinfection) should also be implemented.

Currently, no published methods have been shown to be effective in limiting the impact of an outbreak on an infected farm. It has been suggested that breeding for resistance or the development of a vaccine may offer the long term prospects for managing the disease (Ferguson *et al.*, 2014). A breeding programme would need to select and test a range of different strains of tilapia with a view to finding those least susceptible.

10. TRANSMISSION RISK

As TiLV has been horizontally transmitted through cohabitation, disease transmission is likely with movement of live aquatic animals. There is limited information about TiLV biophysical properties and the risks associated with aquatic animal products. However, it may be assumed that it will share properties of other aquatic orthomyxoviruses, such as infectious salmon anaemia virus. Current evidence suggests that the eye, brain and liver are likely to contain highest concentrations of TiLV and thus solid and liquid waste is likely to be contaminated. However, it is possible that the pathogenic agent may also be found in musculature of infected fish. TiLV has been detected by real-time RT-PCR and virus isolation in mucous but not faeces (Liamnimitr *et al.*, 2017).

11. ADDITIONAL USEFUL INFORMATION

- CGIAR Research Program on Fish Agri-food Systems (2017). Tilapia Lake Virus (TiLV): What to know and to? Factsheet: FISH-2017-03
- Food and Agriculture Organization of the United Nations (FAO). (2017). FAO issues alert over lethal virus affecting popular tilapia fish. http://www.fao.org/news/story/en/item/888884/icode/
- Food and Agriculture Organization of the United Nations (FAO)/GIEWS Special Alert nr. 338. (2017). Outbreaks of Tilapia lake virus (TiLV) threaten the livelihoods and food security of millions of people dependent on tilapia farming. http://www.fao.org/3/a-i7326e.pdf
- Network of Aquaculture Centres in Asia-Pacific (NACA). (2017). Urgent update on possible wordlwide spread of tilapia lake virus. https://enaca.org/?id=870&title=urgent-update-on-possible-worldwide-spread-of-tilapia-lake-virus-tilv

REFERENCES

- Amal, M.N.A, Koh, C.B., Nurliyana, M., Suhaiba, M., Nor-Amalina, Z., Santha, S., Diyani-Nadhirah, K.P., Yusof, M.T., Ina-Salwany, M.Y. & Zamri-Saad, M. (2018) A case of natural co-infection of Tilapia Lake Virus and Aeromonas veronii in a Malaysian red hybrid tilapia (Oreochromis niloticus x O. mossambicus) farm experiencing high mortality. Aquaculture Vol 485, 12-16
- Bacharach, E., Mishra, N., Briese, T., Zody, M. C., Kembou Tsofack, J. E., Zamostiano, R., … Lipkin, W. I. (2016). Characterization of a Novel Orthomyxo-like Virus Causing Mass Die-Offs of Tilapia. mBio, 7(2), e00431-16. https://doi.org/10.1128/mBio.00431-16
- Behera, B.K., Pradhan, P.K., Swaminathan, T.T., Sood, N., Prasenjit Paria, Abhischek Das, Verma, D.K., Kumar, R., Yadav, M.K., Dev, A.K., Parida, P.K., Das, B.K., Lal, K.K. & Jena, J.K. (2018) Emergence of Tilapia Lake Virus associated with mortalities of framed Nile Tilapia Oreochromis niloticus (Linneaus 1758(In India. Aquaculture Vol 484, 168-174
- Del-Pozo, J., Mishra, N., Kabuusu, R., Cheetham, S., Eldar, A., Bacharach, E., Lipkin, W.I., & Ferguson, H. W. (2016). Syncytial Hepatitis of Tilapia (Oreochromis niloticus L.) is Associated With Orthomyxovirus-Like Virions in Hepatocytes. Veterinary Pathology . https://doi.org/10.1177/0300985816658100
- Dong, H.T., Siriroob, S., Meemetta, W., Santimanawong, W., Gangnonngiw, W., Pirarat, N., Khunrae, P., Rattanarojpong, T.,. Vanichviriyakit, R,. Senapin, S (2017), Emergence of tilapia lake virus in Thailand and an alternative semi-nested RT-PCR for detection. Aquaculture, advance online publication oi: 10.1016/j.aquaculture.2017.04.019
- Eyngor, M., Zamostiano, R., Tsofack, J. E. K., Berkowitz, A., Bercovier, H., Tinman, S., Lev, M., Huryitz, A., Galeotti, M., 7 Eldar, A. (2014). Identification of a novel RNA virus lethal to tilapia. Journal of Clinical Microbiology, 52(12), 4137–4146. https://doi.org/10.1128/JCM.00827-14
- FAO. (2014). The state of world fisheries and aquaculture. Food and Agriculture Oraganization of the United Nations (Vol. 2014). https://doi.org/92-5-105177-1
- 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. Journal of Fish Diseases, 37(6), 583–589. https://doi.org/10.1111/jfd.12142
- 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 Vol. 472, 430-432
- Liamnimitr, P., Thammatorn, W., U-thoomporm, S., Tattiyapong, P & Surachetpong, W. (2018) Non-lethal sampling for Tilapia Lake Virus detection by RT-qPCR and cell culture. Aquaculture Vol 486, 75-80
- OIE. (2016). Aquatic Animal Health Code (19th ed.). Paris: OIE. Retrieved from http://www.oie.int/international-standard-setting/aquatic-code/access-online/
- OIE (2017) Disease notification report 25278, 23/11/2017. ,[https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&reportid=25278]
- Tsofack, J. E. K., Zamostiano, R. Watted, S., Berkowitz, E., Mishra, N., Briese, T., Lipkin, W.I., Kabuusu, R.M., Ferguson, H., del Pozo, J., Eldar, A., and Bacharach, E. (2016) Detection of Tilapia Lake Virus (TiLV) in Clinical Samples by Culturing and Nested RT-PCR. J. Clin. Microbiol. JCM.01808-16; Accepted manuscript posted online 14 December 2016, doi:10.1128/JCM.01808-16
- Tattiyapong, P., Sirikanchana, K., & Surachetpong, W. (2017) Development and validation of a reverse transcription quantitative polymerase chain reaction for tilapia lake virus detection in clinical samples and experimentally challenged fish. Journal of Fish Diseases DOI: 10.1111/jfd.12708