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## TiLV - A worldwide menace to tilapiine aquaculture

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#### Abstract

Tilapia is considered as a significant group of fish as it serves as a cheap protein source and livelihood for people. Recently, the significant mortality of wild and cultured tilapia has been observed in two continents. The etiological agent behind these outbreaks, a novel RNA virus (Tilapia lake virus) was described here and its pathogenesis and diagnosis have been revealed. This article creates awareness among the tilapia farmers to perform good aquaculture practices to prevent the catastrophic losses and also to control the spread of this deadly virus worldwide.

**Keywords:** Fish, tilapia, virus, orthomyxovirus, TiLV, PCR

#### Introduction

Tilapiines are the second most important group among fishes farmed worldwide with an annual production of 4.5 million tonnes<sup>[1]</sup>. The culture became popular due to the dwindling of capture fisheries stocks in the oceans. The current global tilapia trade is in excess of \$7.5 billion U.S. dollars (USD) and is estimated to increase by 2030<sup>[1, 2]</sup>. China, Ecuador, Egypt, Indonesia and Thailand are the largest producers while the largest importer is the United States<sup>[3]</sup>. Nile tilapia (*Oreochromis niloticus*) is the most predominant species cultured globally among the 100 species of tilapiines. Tilapia is increasingly important to domestic and global food security providing a major food source as well as a livelihood for people<sup>[4, 5]</sup>. Moreover, tilapia also has utility in algae and mosquito control irrespective of their value as a cheap source of dietary protein. Tilapiine aquaculture faces serious challenges from a wide range of bacteria, fungi, protozoa, and viruses<sup>[6-8]</sup>. Until 2009, viruses were not found to be a threat for tilapia, but started to have the footprints in massive losses of tilapia in Israel in the summer of 2009 and further in Ecuador in the recent years<sup>[9, 10]</sup>. However, no specific treatment has been described for viral infections of tilapia<sup>[11]</sup>.

#### 2. Milestone

Owing to their omnivorous diet, tolerance for high-density aquaculture, and relative disease resistance, tilapia is considered as an important global food source<sup>[12]</sup>. A series of huge losses of tilapia were recorded in fish farms all over Israel during the hot seasons of 2009 (R. Falk and N. Froiman, Department of Fisheries, Israel Ministry of Agriculture, personal communication). The culture has also been devastated by mass die-offs in Ecuador insisting a novel orthomyxo-like virus behind these scenes. The outbreaks were observed in summer leading to mortality of tilapia with different size ranges transmitting from one pond to the other<sup>[10]</sup>. The morbidity and mortality remained restricted only to tilapia. Surprisingly, in poly-culture systems, species other than tilapia showed no gross signs of the disease found in tilapia even after culturing for a long-term. In addition, no more outbreaks were noticed in the same pond once the initial mortality ceased. Instead, the apparent reason for the mortality was not identified<sup>[10]</sup>. Continuous monitoring for the availability of known viral and other pathogens did not reveal any abnormalities. Efforts to identify emerging viral pathogens such as the herpes-like tilapia larvae encephalitis virus (TLEV)<sup>[13]</sup> and the viral nervous necrosis (VNN) betanodavirus<sup>[7]</sup> associated with tilapiines in diseased fish were unsuccessful.

#### 3. Confirmation of TiLV

Symptoms comprising lethargy, endophthalmitis, skin erosions, renal congestion, and encephalitis were reported in Israel outbreaks and transmission of disease from affected to naive fish was demonstrated as well<sup>[10]</sup>. A team of scientists cultured a virus from infected fish in E-11 cells (cloned subculture of snakehead fish cell line), demonstrated sensitivity to ether

and chloroform and obtained a sequence specific for disease through cDNA library screening [12]. The sequence predicted a 420-amino-acid (aa) open reading frame (ORF) with no apparent similarity to any nucleic acid or protein sequence in existing databases [12]. The virus was tentatively named tilapia lake virus (TiLV); however, there was no possible taxonomic description. Further, a disease was described in farmed Nile tilapia in South America that differed from that reported in Israel. In this event, the pathology was mainly concentrated in the liver rather than in the central nervous system [9]. No causative agent was distinguished; however, PCR results denoted the presence of a similar virus isolated in Israel using primers and probes based on the sequence obtained from that of the Israeli virus (J. Del-Pozo, N. Mishra, R. N. Kabuusu, S. Cheetham Browns, A. Eldar, E. Bacharach, W. I. Lipkin, H. W. Ferguson, unpublished data).

#### 4. Clinical signs

Increased surveillance has led to the identification of weakened fish both in natural waters and in aquaculture farms with the following symptoms such as black discoloration, skin abrasions, and ocular degeneration. Also, the histological analysis of TiLV infected tilapias revealed the presence of the following pathology. Ocular alterations, including opacity of the lens (cataract), were found as gross lesions [10]. Ruptured lenses with endophthalmitis accompanied by swelling of the eyeball with occasional perforated cornea and shrinkage and loss of ocular functioning were observed in advanced cases. Other lesions included skin erosions and moderate congestion of the spleen and kidney. The histologic lesions of the brain included edema, focal haemorrhages in the leptomeninges, and capillary congestion in both the white and gray matter. MMCs were increased in size and number in both the liver and the spleen. In a variety of infections (especially viruses) or poor environmental conditions, as a response to severe tissue injury, MMC proliferation is observed in the late stages of chronic infection. Therefore, MMCs are considered indicators of fish population health [14-16].

#### 5. Detection of TiLV

Comprehensive analysis of the TiLV isolate from Israel using studies such as Unbiased high-throughput sequencing (UHTS), Northern hybridization, mass spectrometry (MS), in situ hybridization, and infectivity indicated that TiLV as a segmented, negative-sense RNA virus. The virus consisted of 10 genome segments, each with an open reading frame (ORF). Nine of the segments had no recognizable homology to other known sequences with one segment predicting a weak homology to the protein of PB1 subunit of influenza C virus, an orthomyxovirus [12]. The segment also had the major polymerase motifs [17]. The presence of complementary sequences in the other nine segments termini and identification of proteins in extracts of infected cells that correlate with the ORFs they carry provided evidence that represents gene segments of TiLV. In situ hybridization experiments indicated a TiLV replication and transcription at sites of pathology in the liver and central nervous system of tilapia with the disease. It is likely that TiLV will ultimately be classified as representing a new genus of the family Orthomyxoviridae. Recently, a sensitive nested reverse transcription-PCR (RT-PCR) assay [18] has been developed for the rapid detection of TiLV in fish organs.

#### 6. Conclusion

Virus propagated in cell culture implicated TiLV as the etiological agent of outbreaks of both viral encephalitis and

syncytial hepatitis [9, 19] in naive tilapiines in Israel and Ecuador due to the presence of viral nucleic acid at pathological sites of brain and liver [12]. There is no evidence of TiLV infecting other species. However, this infectious agent has been detected in massive losses of wild and cultured tilapia in two geographically disparate sites. The newly developed RT-PCR assay also disclosed the presence of TiLV in diseased Colombian tilapia [18]. Thus, TiLV represents a novel orthomyxo-like virus and confirms that it is an emerging global scourge to tilapiine aquaculture which is a multi-billion dollar industry.

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