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Tilapia lake virus: An emerging viral disease of tilapia industry

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Abstract
Tilapines are the second most important group of farmed fish worldwide, and they serve as a primary protein source in the developing world. Tilapia lake virus (TiLV) has been recently described as a virus affecting wild and cultured tilapias. TiLV has been confirmed in some countries in Asia, Africa, and Latin America. It is likely that TiLV may have a wider distribution than is known today and its threat to tilapia farming at the global level is significant. Significant mortality of wild and cultured tilapia has been observed recently in many countries which poses a considerable risk, particularly to small-scale fish farmers. This article reviews the current status of the virus and also summarizes the published scientific information on its aetiology, genome, and susceptible species along with their clinical signs, diagnosis and recommended measures.

Keywords: Tilapia lake virus, tilapia, aetiology, genome, aquaculture

1. Introduction
Tilapia, also known as the aquatic chicken, is a freshwater fish that belongs to family "Cichlidae." In terms of volume, tilapias are the second most important aquaculture species providing food, jobs, domestic and export earnings for millions of people worldwide [1]. Tilapia culture offers a high possibility of commercialization due to its general hardiness, higher adaptability to a wide range of production systems and rapid growth, and also advancement in the genetic selection and targeted breeding further widens its scope for its culture. Tilapia has gained a prominent position in international fish trade [2]. The Nile tilapia (*Oreochromis niloticus*) was among the pioneer fish species cultured in the world. It is believed that tilapia culture originated more than 4000 years ago in Egypt [3]. In the 1920s, the first scientific trials of tilapia culture were recorded in Kenya. Tilapia culture has since then expanded enormously, and presently tilapias are being cultured in more than 100 countries all around the globe [4]. Interestingly, about 98% of the total tilapia production comes from countries which are not native of tilapia [1]. Estimated total global production of tilapia in 2015 was around 5.67 million mt and is expected to reach 7.3 million mt by 2030 [5]. At present, among the top three countries of tilapia aquaculture, People's Republic of China is leading in tilapia production (1.78mmt), followed by Indonesia (1.12 mmt) and Egypt (0.88mmt), and other leading producers include Bangladesh, Vietnam, and the Philippines [5].
Tilapia is considered to be relatively resistant to poor water quality and a number of diseases encountered in other farmed fishes but the emergence of Tilapia Lake Virus (TiLV) disease, the first significant disease epidemic reported in tilapia aquaculture, has put the global tilapia industry at risk [6]. Tilapia lake virus (TiLV) is a novel virus said to be the causative agent of Tilapia lake virus disease (TiLVD) which is emerging as a potential threat to the global tilapia industry [7]. The disease was first reported from the freshwater lakes of Israel in 2014 which led to the mass die-offs of tilapia in these lakes [8]. Subsequently, the virus was reported in Fresh and brackish waters of Ecuador, Colombia, Egypt, Thailand, and India [9, 10, 11, 12, 13]. Figure 1 represents the geographical distribution of Tilapia Lake Virus [14].
However, the geographic distribution of TiLV is expected to be broader than the current knowledge. The lack of proper investigations of mortality incidents is the primary reason for knowledge deficit regarding the geographic presence of the virus.

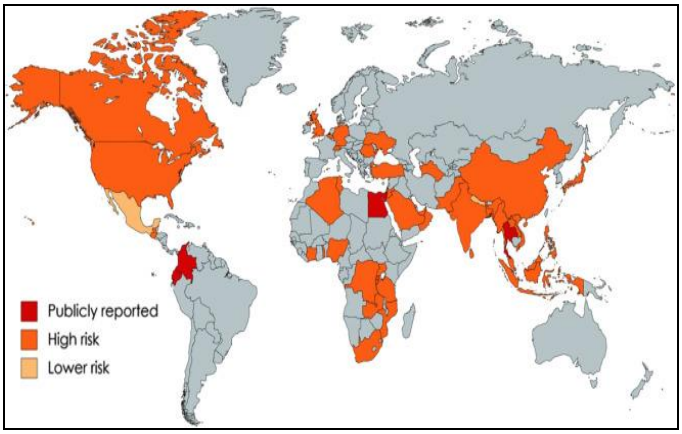

Fig 1: The geographical distribution map of tilapia lake virus [16]

2. TiLV genome

The aetiological agent of TiLVD is a novel virus belonging to Orthomyxoviridae family named as Tilapia lake virus (TiLV). The genome of the virus is enveloped, negative-sense, single-stranded RNA with ten segments encoding ten proteins (Table 1) [8]. The size of each segment ranges from 456 to 1641 nucleotides, and the total genome size is 10.3kb [9]. The diameter of the virion is between 75 and 80nm [8]. All 10 RNA segments contain an open reading frame (ORF), and ORF of the largest segment (segment 1) has weak sequence homology with the influenza C virus PB1 subunit. The rest of the nine segments show no homology to other viruses but contain conserved, complementary sequences at their 5′ and 3′ termini, as found in the genome organization of other orthomyxoviruses [9]. Recently, the complete genome sequence of a TiLV (Gen Bank accession no. MH319378 to MH319387) isolated from the infected Nile tilapia cultured in Thailand was also reported [14].

Table 1: Genomic characterization of 10 segments of TiLV isolated from tilapia in Israel

Segment no	Segment length (nt)	Gen Bank accession no	Predicted protein length (aa)
1	1,641	KU751814	519
2	1,471	KU751815	457
3	1,371	KU751816	419
4	1,250	KU751817	356
5	1,099	KU751818	343
6	1,044	KU751819	317
7	777	KU751820	195
8	657	KU751821	174
9	548	KU751822	118
10	465	KU751823	113

3. Susceptible species and life stages

TiLV has caused significant mortalities to wild tilapia *Sarotherodon* (*Tilapia*) *galilaeus*, farmed tilapia *Oreochromis niloticus* and commercial hybrid tilapia (*O. niloticus* X *O. aureus*) [8, 9, 10, 11, 12, 13, 15]. To date, only tilapias have been reported to be susceptible, but the susceptibility of other species to TiLV cannot be just ruled out.

Mortalities have been observed over a wide range of Tilapia in Israel [8]. The disease has been called "tilapia one-month mortality syndrome" as it is frequently reported within one month after fry or juvenile tilapias have been moved from hatcheries to the grow-out cages. The outbreak in Ecuador [15] and Thailand [12] showed that the fingerling stage of the fish was mainly affected. The huge mortality rate of around 90% has been reported in red tilapia fingerlings within one month of stocking into the cages. TiLV was even reported from hatcheries where fertilized eggs, yolk sac larvae, fry and fingerlings tested positive for TiLV [16]. On similar lines, the mortality range of 80 to 90% in fingerlings of Nile tilapia was observed in Egypt [10]. Successful experimental infection in red and Nile tilapia of about 30g weight has also been recorded. *In vivo* challenge studies showed that high mortality in the Nile (86%) and red tilapia (66%) occurred within 4-12 days post-infection [17]. In India, more than 85% mortality was revealed in farmed tilapia over a weight range of 20 to 80g [13].

4. Clinical signs associated with TiLVD

The principal organs affected by this virus are the eyes, brain, and liver. The most common microscopic lesions associated with TiLV infections comprise hepatitis and encephalitis lesions [7]. The reported symptoms of infected fish include skin redness, inflammation of the eyes and brain, liver damage, and eventual organ failure and death. The clinical signs associated with TiLV infection involve shrinkage of the eyes and loss of ocular functioning (Phthisis bulbi), multifocal to coalescing dermal erosions and ulcers, and the opacity of the lens (cataract). In advanced cases, the lesions include ruptured lenses with induced uveitis or endophthalmitis accompanied by the formation of a cyclitic membrane, followed by swelling of the eyeball (Buphthalmia) [8]. In the liver of naturally infected fish, syncytial giant cells along with multifocal areas of necrosis and the congestion of the blood vessels along with hemorrhages in sections of the brain have also been observed [13].

5. Risk factors for disease outbreaks

Currently, the epidemiological information available regarding TiLVD is limited. Outbreaks are reported to appear more during summer months. TiLVD has been stated to be associated with stress. In one study, the virus affected fingerlings were commonly detected within four to seven days post transfer to grow-out ponds [15]. Clinical outbreaks have also been observed during the hot season, May to October (water temperatures of 22-32 ˚C) in Israel [8] and Ecuador [15]. Another study reported the outbreaks in Egypt from May to November (water temperature 25-27 ˚C) and the risk factors concluded were high stocking densities and tilapia-mullet polyculture [10]. Other factors like temperature, salinity have not been yet identified as potential risk factors.

6. Route of transmission

The source of TiLV is not known yet. Direct horizontal transmission is an essential route of transmission [2]. It has not been reported, however, if TiLV is carried by non-tilapine species, piscivorous birds, mammals or frozen tilapia products. To determine the horizontal route of TiLV transmission, researchers performed a cohabitation experiment in which naive fish were cohabitated with experimentally fish infected with TiLV [8]. The experiments demonstrated that the naive fish developed a lethal disease, with a mortality rate similar to the one obtained by the intra peritoneal route, but with slower kinetics (2 to 3 days delay in reaching 50% mortality, providing proof of the ability of TiLV to spread through a waterborne course. Till now there are no reports of its vertical transmission.

7. Diagnostic techniques

7.1 Histopathology

The histopathological lesions observed in brain of the infected fish include edema, focal hemorrhages in leptomeninges, congestion in the white and grey matter [8], foci of gliosis and encephalitis [10, 13]. The liver of the infected fish is reported to have randomly distributed foci of hepatocellular swelling, accumulation of yellow to the brown pigment in cytoplasm [8], syncytial giant cells, multifocal areas of necrosis, pyknotic and karyorrhectic nuclei of hepatocytes [14, 16, 17] and syncytial cell formation, necrosis of gastric glands and diffuse congestion in multiple tissues [15]. Syncytial hepatitis has also been determined in the samples from Colombia [11]. The spleen of infected fish usually has eosinophilic intracytoplasmic inclusion bodies, increased melano-macrophage centers and dispersion of melanin granules. Additionally, the presence of multiple necrotic foci in the anterior kidney has been observed [17].

7.2 Cell lines developed

Often used in place of primary cells to study biological processes, cell lines offer several advantages, such as cost-effectiveness, ease of use, provide an unlimited supply of material and bypass ethical concerns associated with the use of animal and human tissue. Additionally, cell lines also offer a pure population of cells, which is valuable since it allows for a consistent sample and reproducible results. These advantages of cell lines have led to their active use in vaccine production against a specific pathogen, testing drug metabolism and cytotoxicity, antibody production, the study of gene function, and synthesis of biological compounds, e.g., therapeutic proteins. Viruses infecting fish are generally host-specific, which makes a cell line derived from a particular fish species more appropriate for studying the viruses reported from that species [18]. Therefore, the establishment of susceptible, homologous and tissues-specific cell lines is considered necessary for isolating viral pathogens.

In one TiLV-CPE study [8], eight established fish cell lines (CHSE-214, BF-2, BB, EPC, KF-1, RTG-2, FHM, and E-11) and primary culture of tilapia brain cells were used. Among them, only the E-11 cell line (from the striped snakehead *Ophicephalus striatus*) and the primary tilapia brain cells had consistently shown cytopathic effects (CPE) upon incubation when inoculated with TiLV homogenates. CPE in E-11 cell line became visible at 5 to 7 days post-inoculation (dpi), with the appearance of cytoplasmic vacuoles and plaque formation, and almost complete disintegration of the cell monolayer (9 to 10 dpi). Cell lines of primary tilapia brain post 10 to 12 days of inoculation showed swollen, rounded, granulated cells, monolayer detachment (14 to 19 dpi), but without plaque formation. Three additional cell lines derived from ovary [TO-2], brain [OmB] and bulbus arteriosus [TmB] were compared for TiLV CPE study along with E-11 cell line. The results revealed that E-11 cultures were superior because the CPE development was detected in a relatively short time. The cytopathic effects observed in CFF cell line derived from *pristolepis fasciata* include elongation of the cells followed by rounding, and CPE was found on 3 dpi and by 6-7dpi [13]. Recently, two more cell lines were successfully developed from the liver and brain of Nile tilapia, and these cell lines have been designated as OnlL and OnlB, respectively [19]. Like other cell lines, these will also be extremely useful as a sensitive *in vitro* tool for detection and further studies on TiLV.

7.3 PCR detection methods

The first diagnostic tool for TiLV detection was RT-PCR method [8]. A fast and sensitive nested RT-PCR reported to detect even few molecules of TiLV genome and can be applied in identifying TiLV RNA in fresh and preserved organs of diseased fish [11]. An improved detection method that is semi-nested RT-PCR has been developed to avoid false positive results [16]. The authors have reported this RT-PCR may be used freely for non-commercial applications to detect TiLV. Another sensitive, rapid and accurate diagnostic tool based on reverse transcriptase quantitative real-time PCR was developed for TiLV detection in field samples and experimentally challenged fish [17].

8. Recommendations

Many essential knowledge gaps exist presently in the TiLV disease which demands scientific documentation to increase the knowledge on TiLVD. More research is needed to demonstrate the susceptibility of fish species other than tilapia to TiLV infection and their possibility to act as carriers of the virus, to determine the likelihood of piscivorous birds, mammals, crustaceans, annelids mollusks to serve as carriers of TiLV. Efforts to study the ability of microorganisms and plankton to act as passive carriers must be carried out. The investigation into the ability of the virus to survive freezing and isolation from whole frozen tilapia and other frozen products needs detailed study. Acquiring information regarding the survival of the virus in fresh products like whole fish, gutted head and fillets will be helpful. Closing these knowledge gaps is the need of the hour and recommendations summarized for this include [7]:

- Need for more information on the real geographic distribution of TiLV.
- Encouragement of screening and surveillance programs in many tilapia producing countries.
- Increased awareness of the importance of reporting, recording, and mapping of any unusual mortality in tilapia.
- Need of more knowledge regarding the epidemiological aspects of TiLV thorough investigation of genetically improved strains of tilapia for TiLV susceptibility, urgent need to determine the possibility of vertical transmission of TiLV.

Besides this, FAO has also recommended biosecurity measures that countries need to follow when translocating live tilapias, in which TiLVis reported and for countries with an unknown TiLV status [6].

9. Conclusion

Tilapia, being a cheap source of protein, is being cultured widely all around the globe. TiLVD may cause significant risk to food security and international trade and is emerging as a potential threat to the global tilapia industry at a value of around millions of US dollars. Given the presence of TiLV in several countries across different continents there is a need for strong international collaboration to increase the diagnostic efficiency. New and improved diagnostic tests to screen the presence of the virus with increased specificity, efficiency and sensitivity is the need of the hour. The fact that the tilapia is cultured globally, the control of TiLVD can be further improved by efforts such as strict biosecurity programmes, development of effective vaccines and the selection and culture of resistant tilapia breeds to reduce the impact of this emerging viral disease.

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