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Tilapia lake virus - An update on an important pathogen for tilapia aquaculture - Literature Study

Diplomarbeit

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vorgelegt von

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List of abbreviations

TiLV: Tilapia lake virus

SHT: Syncytial hepatitis of tilapia

RNA: Ribonucleic acid

IPNN: Infectious pancreatic necrosis virus

NNV: Nervous necrosis virus

RT-PCR: Reverse transcriptase polymerase chain reaction

vRNA: Viral RNA

RT-qPCR: Reverse transcriptase quantitative polymerase chain reaction

NCBI: National center for biotechnology information

GC: Guanine-cytosine

Ig: Immunoglobuline

miRNA: Micro RNA

mRNA: Messenger RNA

KEGG: Kyoto encyclopedia of genes and genomes

PPAR: peroxisome proliferator activated receptor.

TCID: Tissue culture infective dose

1. Introduction

Tilapia is susceptible to infection by three main RNA viruses: Tilapia lake virus (TiLV), infectious pancreatic necrosis virus (IPNV) and nervous necrosis virus (NNV). TiLV, often referred to as syncytial hepatitis of tilapia (SHT), is a new and emerging virus that causes a disease in Tilapines that has led to serious concern for fisheries worldwide. All species and hybrids of Tilapia seem to be similarly at risk of infection with TiLV (Mugimba et al. 2019; Waiyamitra et al. 2021). The earliest documentations of outbreaks go back to only 2013 when affected fish were found in Ecuador. While its first detection was in 2013, it is believed that the first outbreak of TiLV was between 2003 and 2009 in Israel (Eyngor et al. 2014; Thawornwattana et al. 2021). Massive mortality in farmed Tilapia left scientists puzzled about the cause - outbreaks are documented as having caused between 9% and 90% mortality in studied populations, often affecting fingerlings and juvenile fish preferentially (Ferguson et al. 2013; Skornik et al. 2020a). Until 2020 the virus was detected in at least 16 countries (Thawornwattana et al. 2021). In comparison with other viruses, TiLV seems to have spread globally, and is suspected of being present even in countries that have not yet documented its presence (Skornik et al. 2020b).

Modern methods have been employed and countries are urged to make use of rapid detection RT-PCR methods in order to detect the presence of the virus early (Dong et al. 2017). This could lead to an improvement in the management of outbreaks. Samples sent in from Asia routinely test positive even in countries that have not yet reported outbreaks. Unpublished data from Thailand claims that most disease outbreaks in hatcheries have TiLV as the cause. The translocation of fry and fingerlings naturally leads to a spread of the disease (Debnath et al. 2020). TiLV outbreaks are probably underreported globally, but the potential spread from translocations is shown in Fig. 1 (Dong et al. 2017).

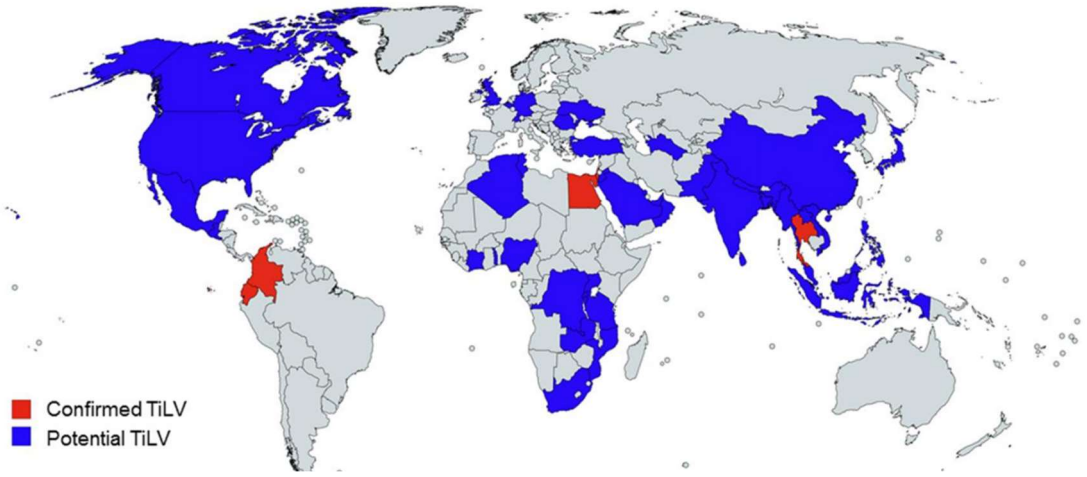


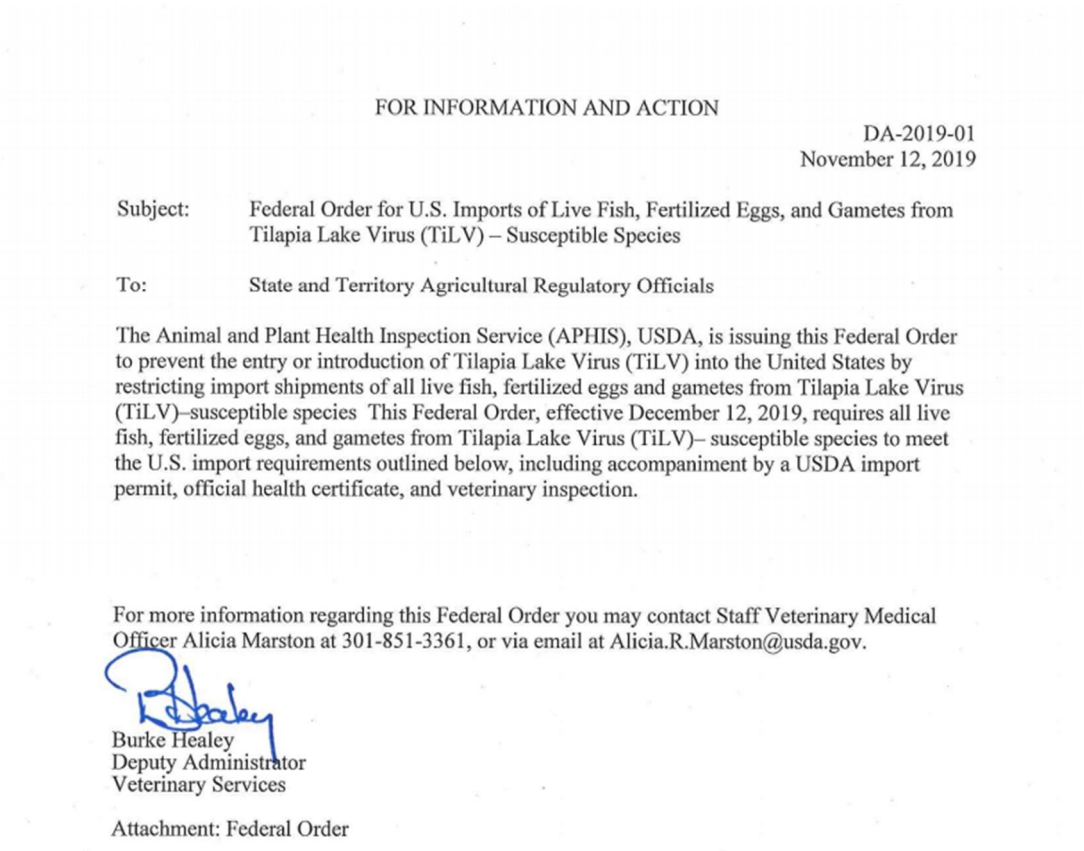
Figure 1 Map of globally affected countries in which Tilapia is either suspected or confirmed. (Dong et al. 2017)

Tilapia had been bred in Peru since the 70s and is important to the economy, as it is the second most cultivated fish after rainbow trout. As it is bred between some 668 fisheries in Peru, TiLV was not yet suspected as being present. Major outbreaks were taken as being either IPNV or NNV. A Peruvian team analyzed the genome of a suspected RNA virus during an outbreak in 2019 (Pulido et al. 2019). Although Peru had quite a low mortality, some symptoms of fish that were dying did not fit to the symptoms suspected for the aforementioned two viruses. NNV, for example, presents with lesions of vacuolation and necrosis of the central nervous system and retina. RT-PCR of infected fish showed that Peru had already infected Tilapia living in its ponds.

As genetic data exists on the early outbreaks, phylogenetic analysis led to the team being able to show where the virus was likely imported from. Israel had very early outbreaks of TiLV and published a lot of data. The viruses found in Peru were roughly 95% identical to those found in Thailand and 97% identical to those found in Israel. This may suggest that the virus was transferred with imported fish from Israel (Taengphu et al. 2020) This example shows the worldwide nature of the problem with Tilapia translocated between countries. The geographical separation between countries no longer serves as a natural barrier against global pandemic.

Political decisions and regulations followed to counteract the spread of TiLV. The United States of America reacted to a growing number of alerts from other countries with a federal order to prevent the entry or introduction of TiLV to the US. This order restricted the import or

introduction of all live fish, fertilized eggs and gametes from all TiLV susceptible species (Fig. 2).



FOR INFORMATION AND ACTION

DA-2019-01
November 12, 2019

Subject: Federal Order for U.S. Imports of Live Fish, Fertilized Eggs, and Gametes from Tilapia Lake Virus (TiLV) – Susceptible Species

To: State and Territory Agricultural Regulatory Officials

The Animal and Plant Health Inspection Service (APHIS), USDA, is issuing this Federal Order to prevent the entry or introduction of Tilapia Lake Virus (TiLV) into the United States by restricting import shipments of all live fish, fertilized eggs and gametes from Tilapia Lake Virus (TiLV)–susceptible species This Federal Order, effective December 12, 2019, requires all live fish, fertilized eggs, and gametes from Tilapia Lake Virus (TiLV)– susceptible species to meet the U.S. import requirements outlined below, including accompaniment by a USDA import permit, official health certificate, and veterinary inspection.

For more information regarding this Federal Order you may contact Staff Veterinary Medical Officer Alicia Marston at 301-851-3361, or via email at Alicia.R.Marston@usda.gov.

Burke Healey
Deputy Administrator
Veterinary Services

Attachment: Federal Order

Figure 2 Shows a part of the letter sent out to the state and territory agricultural regulatory officials in the US. This letter included the federal order. (USDA Animal and Plant Health Inspection Service 2019)

The pronounced Tilapia die-offs have found mention in the food chain crisis early warning bulletin of the FAO in the issue of July-September 2020 (No. 36), with specific mention of African regions. This illustrates some acute awareness on the part of the FAO as to the potential problems associated with tilapia shortages on the markets in question. Thawornwattana et al. (2021) however report that the virus reached its peak between 2014 and 2016 and has been in decline since. The authors suspect that herd immunity might have been reached in fish populations and that improved protocol in importing Tilapia as well as a generally better awareness have helped ease the situation overall.

Global tilapia aquaculture production has grown eleven percent annually from 0.3 million tons in 1987 to 5.9 million tons in 2017 according to the FAO (FAO 2020a). This represents a faster than average growth when compared to other species. In 1987 it made up 1.9% of global fish tonnage, in 2017 it made up 4.4% already. Tilapia is ranked fourth in the top 10 aquaculture species in terms of both quantity and value.

2. Method

The following literature review investigates current developments in combating the Tilapia lake virus. In the first part, it reviews literature on the species in question, its biology, current use and economic relevance. This provides an understanding on the relevance of the species around the world and informs on the social, ecological and economic environment that this crisis stems from.

This review presents a qualitative approach in which studies were chosen, that inform the reader on detections of the virus all over the globe, ongoing developments in methods for diagnosis, reverse-transcription polymerase chain reaction techniques and genotyping, vaccine development, other forms of treatment and prophylactic measures.

Literature was selected from the Pubmed.gov database and similar platforms by using search terms that include ‘Tilapia lake virus’, ‘TiLV’, ‘Tilapia pathogens’. All studies including these terms in the headings were analyzed in this review. Further studies were selected by searching for studies from the past two years that contain the following terms ‘TiLV diagnosis’, ‘TiLV genotyping’, ‘TiLV RT-PCR’, ‘TiLV antibodies’, ‘TiLV vaccine’, ‘TiLV prophylaxis’, ‘TiLV detection’, ‘TiLV impact’, ‘TiLV probiotics’. From the encountered literature, further literature was selected from papers cited in chosen literature. Any study reporting on the current status of the crisis or developments in its detection, treatment or any other related updates was analysed for this review. Only studies that were published in a scientific journal, either in print or online, were selected.

3. Species description

Tilapia fish are a species of African *Cichlidae*. The name "Tilapia" comes from the word "thlapi", a term used by the bantu people to describe fish in general (Fuchs et al. 2008). It was believed by biologists at the beginning of the 20th century that Tilapia fish made up the species with the greatest number of subtypes. Several revisions corrected this and led to all Tilapia that were mouthbrooders to be assigned to *Oreochromini*, leading to Tilapia to be reduced to a total of 40 species (Nagl et al. 2001). At the beginning of 2013, the subgeni of *Coptodon*, *Heterotilapia* and *Plematolapia* were elevated to the level of genus, and *Coelotilapia* were accepted as part of the genus of *Tilapia sjoka* (Dunz and Schliewen 2013). As an example, figure 3 shows a spotted Tilapia.

Tilapias are sometimes referred to as St. Peters fish in the English language. They should not be confused with the Saint-Pierre fish of the French.

China ranks first regarding Tilapia production while Egypt is the largest producer of Tilapia in Africa and the third internationally (Shaalan et al. 2017; Taha et al. 2020).

Tilapia farming was introduced in southern Congo basin between Kwango, Luvua and Luapula. South of this, the areas in which Tilapia may be found are between Kunene and Okavango, Sambesi to Limpopo. In the east, Tilapia populations may be found all the way to Lake Malawi. Lake Ngami, the Okavango delta, Lake Guinas and Lake Otjikoto have sizeable populations in Namibia, adding to the populations that may be found in the sidearms of the Oranje. Some populations have been described in Southern Gabon and the Congo (El-Sayed 2020).

Up to 200 eggs are fertilized at a time which are then safely guarded in the parent fish's mouth. Incubation may take up to two weeks. Between six and eleven cycles may be had per year. Sexual maturity time is variable, in the wild tilapia starts to reproduce at a total length of 20–30 cm while in farmed fish sexual maturity is reached at shorter lengths of 8–13 cm (Shoko et al. 2015). The largest Tilapia could grow to a size of up to 62 cm and can weigh 3.5 Kg (Bwanika et al. 2004).

An interesting side note to Tilapia is its introduction in Vietnam by rice farmers. As Tilapia eats the larvae of certain mosquitoes, it was hoped that Malaria could be combatted by introducing

Tilapia locally. That it was a fish that could be consumed easily was of some importance too, however (Petr et al. 2000) Kenya has followed the example of Vietnam in introducing Tilapia as a countermeasure to malaria (Howard et al. 2007). Certain types such as *Tilapia buttikoferi* which is also called Zebra Tilapia are kept in aquaria by hobbyists.

Tilapias have certain attributes that make them a great pick for aquaculture farming. These have led to them being introduced to some freshwater lakes across Central America and Southeast Asia. Tilapia can feed on a variety of food sources. They have been described as plankton-eating, or omnivore. This makes the raising of the fish easier compared to more specialized feeders. While other omnivores, given for instance carp or catfish, tend to have what is called a "muddy" taste, Tilapia meat is considered quite enjoyable and comparable to carnivorous fish in that sense (Gutierrez et al. 2013). This taste however, caused by musty odor due to geosmin and 2-methylisoborneol (MIB) in aquaculture ponds, mostly related to water management (Sompong et al. 2018).

Tilapia have been shown to grow fast relative to what they eat, making them efficient food convertors and thus more economically viable than many other species. They have also been shown to be exceptionally resistant to low oxygen environments, which makes the chance of death of illness considerably lower (Tsadik et al. 1987). What further increases their attractiveness for aquaculture is the fact that common diseases such as ectoparasites and the few bacterial pathogens of Tilapia species can be controlled well with pharmacotherapy. A low number of viral diseases that have been reported for Tilapia prior to TiLV are of limited impact (Kembou-Tsofack et al. 2017). This reflects the adaptability and resistance of the species that has led to their wide application.

The reproduction of Tilapia is quite simple compared to other species, and it generally breeds well in captivity. and it is quite resistant to high water temperatures. On a side note, Tilapias destined to be aqua farmed have been modified genetically, along with Atlantic salmon and pacific salmon (Maclean et al. 2002).

Figure 3 A spotted tilapia. From Tilapia, Spotted MNbowfinangler. 2017. (https://roughfish.com/content/tilapia-spotted-mnbowfinangler-0)

3.1 Tilapia as food

Tilapia can be processed into fillets with a yield of about 30%, some commercial strains even boast up to 47% yield (Janice 2014)

Tilapia have been described as having “muddy” flavors by chefs, usually only found in catfish or trout. These flavors may be caused by 2-methylisoborneol or geosmin, which come from the consumption of cyanobacteria that can be found in the same bodies of water that Tilapia inhabit. While being completely safe to consume, these tastes can sometimes take away from the enjoyability of the food (Robin et al. 2006).

Although Tilapia are omnivorous, the fish does have levels of mercury that are comparable to those of carnivorous fishes. They are typically quite healthy to consume and are low in saturated

fat, carbohydrates, and sodium for example. Experiments with flaxseed have been done to show that the Omega3/Omega6 fatty acids in the final product can be influenced. (Shapira et al 2009).

3.2 Other uses of Tilapia

As they are omnivorous, Tilapia have been used to control unwanted aquatic plants. Moreover, they readily consume algae. Their use makes the application of algaecides limited as they offer better alternatives to algaecides which often contain toxic chemicals and heavy metals (Lu et al. 2006).

As they are non-picky eaters, they can be cultured together with other fish in polycultures. They do not generally compete for food with the other fish and reduce oxygen depleting detritus. Adding Tilapia to other populations of fish can improve the population size, fish size and health of the other species (Shaalan et al. 2017).

The skin of Tilapia has been used in a clinical trial to treat burn injuries in human patients. (Lima-Junior et al. 2021). There have been some studies done on Tilapia skin grafts in veterinary trials (Ibrahim et al. 2020). A prospective, randomized phase 3 clinical trial has been published from the US in which 115 patients were either assigned Tilapia skin to treat their superficial partial thickness burns that affected under 15% of total body surface area or treated with silver sulfadiazine cream as control (Costa et al. 2019). The study is still ongoing, and results have yet to be published, but a mix of non-infectious microbiota, high amounts of type 1 collagen and a similar morphological structure to human skin suggest that this study may be of some promise. A study describing the use of Tilapia skin in the treatment of a 23-year-old male patient has been posted and the authors describe a promising result in a treatment that may be used as an easy to apply and highly available method to treat such patients (Lima-Junior et al. 2020).

3.3 Distribution of tilapia aquaculture across the world

After carp and salmon, Tilapia is the fish with the third largest production in the world, the annual production of Tilapia over time is presented in Fig. 4. Tilapias are comparatively large, deliver a high protein content and grow rapidly compared to other species. Tilapia fisheries originate from Africa and the Levant. As explained briefly above, Tilapia has been introduced to fight malaria in Vietnam (Howard et al. 2007). Apart from this introduction some populations have been introduced by accident and exist freely in parts of Asia. Outdoor aquaculture projects in tropical zones suit the natural environment of Tilapia. Examples of tropical fisheries are Honduras, Papua New Guinea, Indonesia and the Philippines. As the environment is close to the natural habitat, these fisheries have a high chance of being environmentally friendly. In colder climates, Tilapia needs a constant source of warm water for it to thrive. Belgium has used wastewater from nuclear power plants to supply fish farms with a constant stream of hot water (Tiews 1981). Economically, Tilapia is quite profitable for the reasons stated above. The young fish do not pass through a phase in which they feed on plankton which simplifies their raising considerably. The omnivorous diet in all stages of life makes for simple feeding strategies. Tilapia can generally be stocked densely and grow rapidly. This means that the fish can be delivered comparatively fast (Suresh and Kwei Lin 1992).

The Southeast Asians have perfected growing Tilapia in rice fields. Interestingly, the fish are ready for consumption once the rice is ready to harvest. Their inclination to eat larvae also make for a positive side benefit. Unfortunately, and due to their hardiness, Tilapia are highly invasive. Many species have been found in the wild in parts of the United States for example, where they do not belong (Martin et al. 2010). The shares of each country in Tilapia production are shown in Fig. 4 and 5.

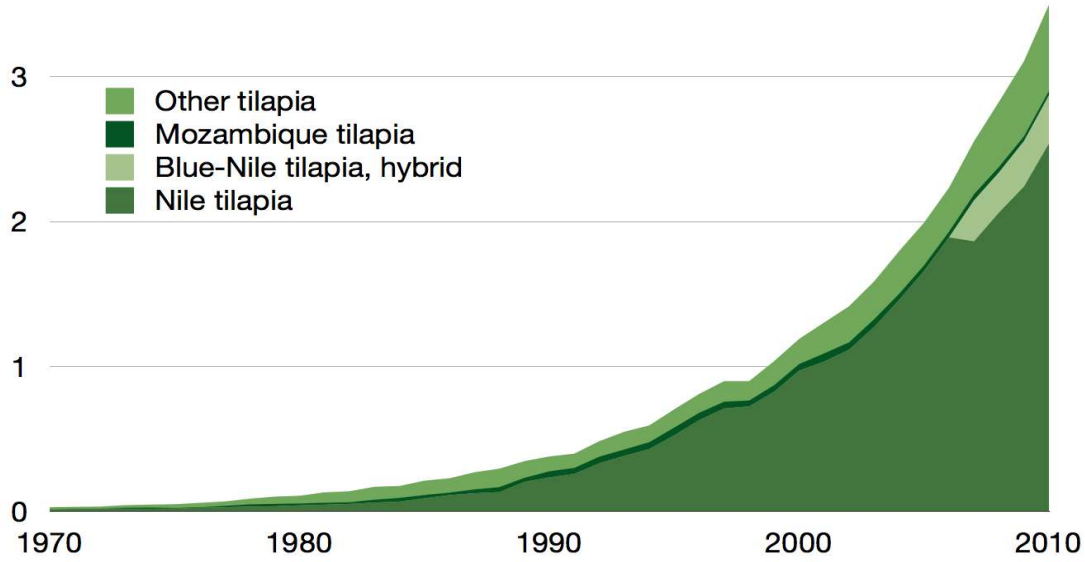


Figure 4 Aquaculture production of tilapia by species in million tons as reported by the FAO, 1950–2009. From Fishery and Aquaculture statistics 2021. FAO Fisheries Division. (fao.org/fishery/statistics/software/fishstatj/en)

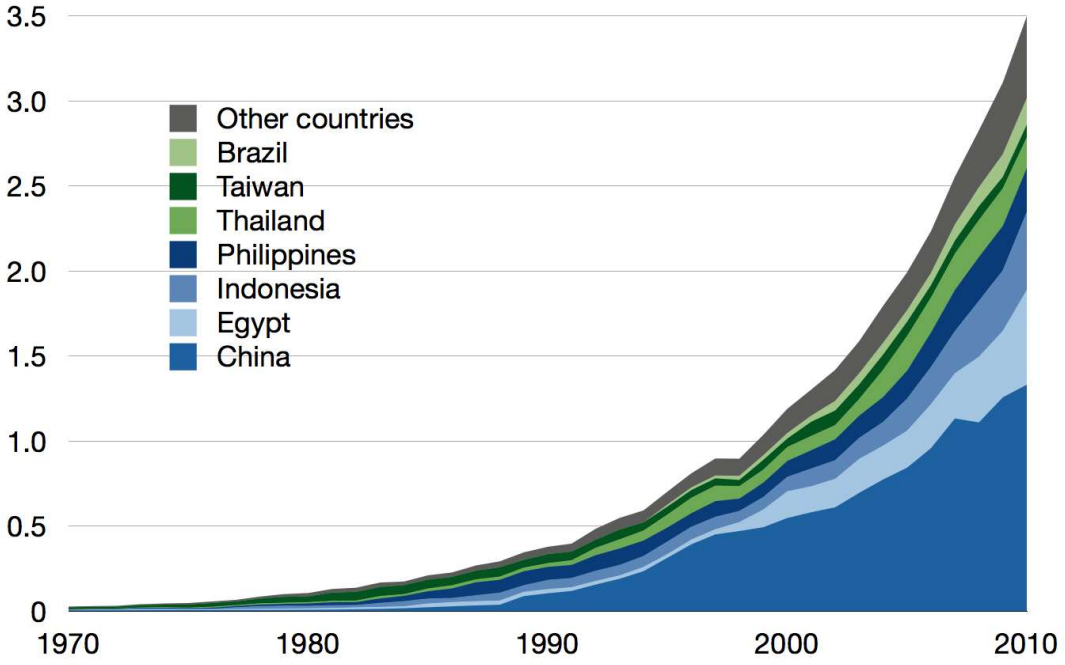


Figure 5 Aquaculture production of tilapia by country
in million tons as reported by the FAO, 1950–2009. From Fishery and Aquaculture statistics 2021. FAO Fisheries Division. (fao.org/fishery/statistics/software/fishstatj/en)

4. Developments in TiLV research

4.1 Taxonomy and description of TiLV

TiLV was first described in Israel where its genome has been sequenced (Eyngor et al. 2014). Further genomic investigation has been performed in Egypt (Fathi et al. 2017), in Ecuador and Colombia (Kembou-Tsofack et al. 2017) and in Thailand (Dong et al. 2017; Surachetpong et al. 2017).

The Virus itself is a negative sense, enveloped, single stranded RNA virus. The ten segments on its genome code for 10 proteins (Eyngor et al. 2014). Segment one of ten shows a weak homology to the PB1 subunit of the influenza C virus, while the other parts of the genome show no homology whatsoever to any known viruses (Wang et al. 2020, Bacharach et al. 2016). Electron microscopy has shown the structure of the TiLV to be an enveloped icosahedral particle that is between 50 and 100nm in diameter. The genome of the virus is 10323kb long with the ten segments ranging from 465 to 1641 nucleotides (Bacharach et al. 2016).

The TiLV has been shown to infect wild and aqua-cultured populations of Tilapia. It shares neither its genus nor its family with any other viruses. TiLV has been shown to have infected Tilapia species in Africa, Asia and South America. Scientists found the virus initially in the Kinneret lake in Israel following a suspicious decline in the population of Tilapia (Eyngor et al. 2014). Geographically distant populations of Tilapia have been infected by TiLV with unique genomes. These genomic differences are discussed in later chapters. TiLV has some similarity to other orthomyxoviruses, Thogoto and Isavirus in particular. This resemblance comes from 13 nucleotides present in 5´and 3´noncoding termini (Bacharach et al. 2016).

4.2 Diagnosis of TiLV

Infected Tilapia cells exhibit a cytopathic effect. This means that there are changes in the structure of the infected cell following the infection (Eyngor et al. 2014). Literature suggests that the E-11 cell line is particularly permissive to infection by TiLV. In Tilapia, cell lines of the brain and of the liver are prone to being infected too (Jansen et al. 2018). Syncytiae are formed by infected cells, which are the fusion of neighboring infected cells producing multi-

nucleated infected cells. The Syncytial cells have been shown to exhibit enlarged mitochondriae, with hepatocytes exhibiting hypertrophy (Dong et al. 2017). Pigmented cytoplasmic accumulation in the spleen and liver of infected fish has been noted.

The brain of infected fish shows morphological abnormalities too. Eyngor et al. (2014) described histological lesions of the brain including focal hemorrhages and edema in the leptomeninges and capillary congestion in experimentally infected fish. In natural outbreaks they described lethargy, ocular alterations, discoloration of the skin, patching of the skin and ulcerations of the digestive tract. They found that the main pathology stems from brain, eyes and liver with a pronounced exophthalmos and abdominal swelling.

4.2.1 Symptoms

The diagnosis of a TiLV infection can be considered if there is an increased mortality of the fish that follows the typical clinical signs of a TiLV infection. Clinical studies indicate that the fish display a range from lethargy, loss of appetite and decreased schooling behavior. The diseased fish can be seen to display exophthalmia, a darker than usual skin tone and ulcerated or bleeding skin (Nicholson et al. 2017; Tattiyapong et al. 2017). Other signs include pale gills, a swollen coelom and scale loss. The damaged skin is not due to the viral infection itself, but to a secondary bacterial infection (Al-Hussinee et al. 2019). Examples are shown in Figure 6 and 7.

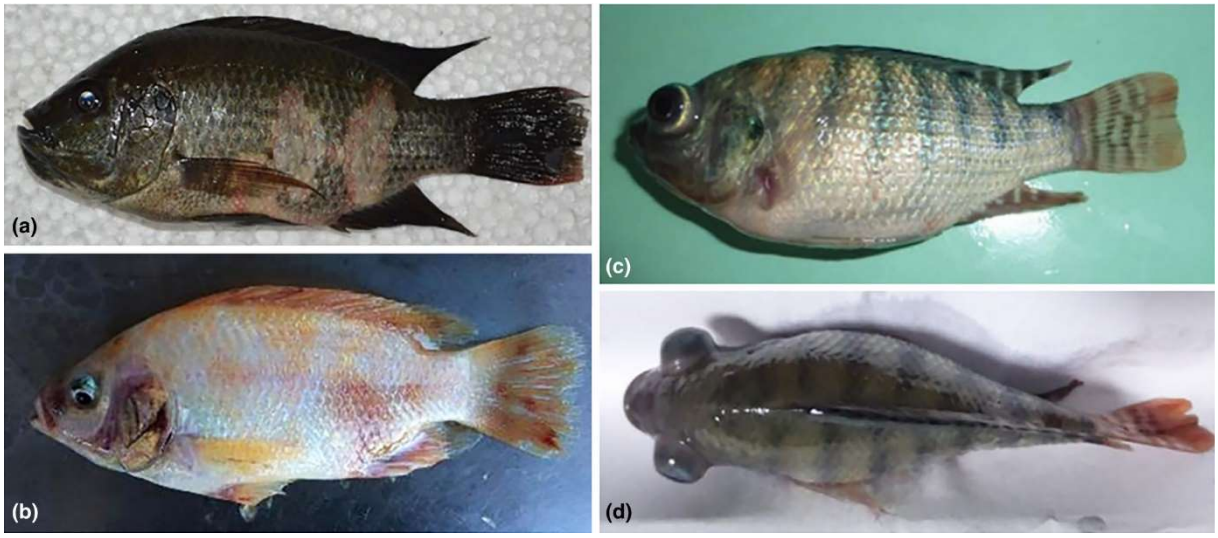


Figure 6 Clinical signs of TiLV-infected Nile Tilapia and Red Tilapia: discoloration, loss of scales and skin erosion (a), skin hemorrhages (b), exophthalmia, abdominal swelling and scale protrusion (c and d). (Jansen et al. 2019)

Figure 7 A TiLV-infected Red Tilapia having massive fluid in the coelomic cavity (arrow) and a pale liver (L). (Jansen et al. 2019)

4.2.2 Localization of the virus in the central nervous system

Studies report detection of the virus in multiple organs of the infected fish including intestines, gills, brain, liver, pancreas, spleen and kidkeys (Piewbang et al. 2021). The authors highlight intestines, gills and brain as common TiLV targets. They go propose the endotheliotropism and lymphotropism of this virus based on evident signals in the endothelial cells of various organs, the circulating leukocytes in the blood vessels and the areas of tissue inflammation.

Dinh-Hung et al. (2021) further studied the fish-virus interactions and neuropathogenesis. They conclude that the virus was broadly distributed throughout the brain, stating that it may productively enter into the brain through the circulatory system and widen broad regions, possibly through the cerebrospinal fluid along the ventricles, and subsequently induce brain dysfunction.

4.2.3 Reverse-transcription polymerase chain reaction (RT-PCR)

Beyond the in vivo examinations, biopsy and necropsy examinations of infected Tilapia, there have been a variety of published laboratory methods used for the detection, isolation, identification and quantification of TiLV. Once Tilapias show the clinical signs, a diagnosis can be confirmed via histopathological evaluation. Typical microscopic lesions (Fig.8) are found in many organs but are mostly found in the brain and in the liver (Fathi et al. 2017). Multinucleated giant cells have been shown to be present in the liver (Ferguson et al. 2014).

Several cell lines have been used to effectively isolate and propagate TiLV. Electron microscopes have been used to demonstrate TiLV directly. Reverse transcription PCR based techniques are the most promising and effective. Nested and semi nested methods have been used and have been developed to an extreme sensitivity and specificity - modern methods using SYBR Green 1 have been found to increase both (Tattiyapong et al. 2018).

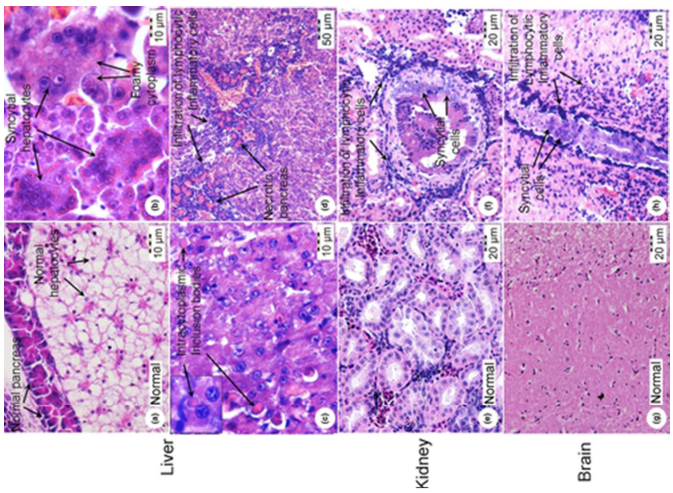


Figure 8 Sections of tissue from the liver, kidney, and brain of normal fish (a, e, g) and TiLV-infected fish (b–d, f, h). The infected liver tissue shows syncytial hepatocytes and foamy cytoplasm (b), intracytoplasmic inclusion bodies (c) and inflammation with pancreatic necrosis (d). Kidney tissue showed syncytial cells and severe infiltration of inflammatory

lymphocytes (f). Brain tissue also showed syncytial cells and severe infiltration of inflammatory lymphocytes (h) (Jansen et al. 2019).

If the virus is grown on the SSN-1 cell line, it will make holes in cell culture monolayers after five days and destroy the monolayer by day ten (Al-Hussinee et al. 2019).

Electron microscopy has revealed TiLV to look spherical to ovoid in shape, with diameters measured between 95-100 nanometers (Al-Hussinee et al. 2019). See figure 9 below.

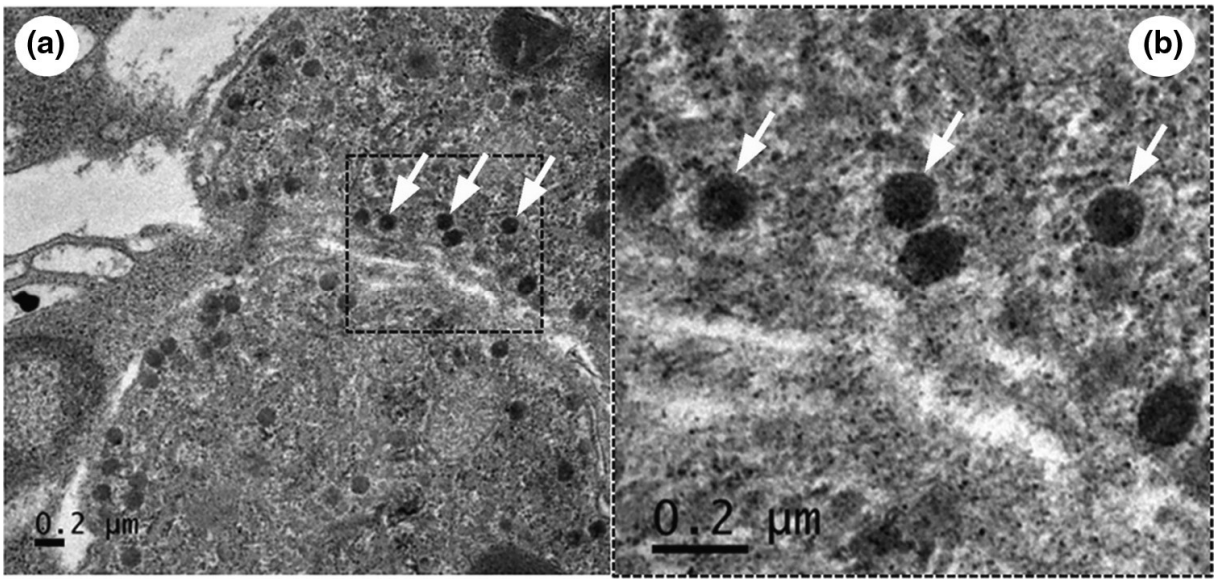


Figure 9 A picture of Transmission electron micrographs of TiLV-infected fish liver tissue showing cytoplasmic viral particles (white arrows) (Jansen et al. 2019).

PCR tests confirm the diagnosis definitively. The common standard of virus diagnostics includes virus isolation and complementary techniques for further verification. For example, reverse-transcription polymerase chain reaction (RT-PCR). So far for detection of Tilapia Lake Virus conventional RT-PCR was mostly used. Studies showed that to avoid false positive results semi-nested RT-PCR primer should be used instead of nested RT-PCR primer (Kembou-Tsofack et al. 2017).

Since conventional RT-PCR analysis is often very time-consuming, laborious and requires a severely infected tissue samples for detection, Nicholson et al. (2018) stress the importance to find other suitable, easy to use and fast protocols which can be used for biosecurity measures, surveillance programs and in TiLV research laboratories.

4.2.4 RT-qPCR

The gold standard in the detection of viruses is a simple RT-PCR assay. This method may be strong, but it is laborious, time consuming and not commonly available. In order to improve on these points, RT-qPCR methods have been developed.

The use of RT quantitative RT-qPCR for detection of TiLV is more beneficial because of its high sensitivity, quantitative nature, specificity, scalability, and quick results. It was found out that RT-qPCR for absolute quantification of TiLV only needed 2 copies which makes it exceptionally important for TiLV diagnosis also in sub-clinical cases. It showed that the RT-qPCR technique was 100 and 10,000 times more sensitive for virus detection compared to the RT-PCR and virus isolation in cell culture methods (Tattiyapong et al. 2018).

Besides the disadvantage of RT-qPCR initially being more expensive, it has even more advances in comparison to conventional RT-PCR. For example, a faster turn-around time from sample to results and it does not need any post-PCR steps. For generating precise results and prevent false positive results, it is very important due to the highly sensitive RT-qPCR, that accurate working is necessary as well as a precise understanding of quantification techniques (Nicholson et al. 2018).

Various fish tissues can be used for RT-qPCR TiLV detection including gills, liver, brain, heart, anterior kidney, and spleen (Tattiyapong et al. 2018; Nanthini et al. 2019).

In Tilapia, specific TaqMan 5′nuclease assays offer two important improvements in specificity: a probe and primers (Tattiyapong et al. 2018).

Two TaqMan primers were developed by Tattiyapong et al. (2018) (TiLV-93F 5’-AGCCTGCCACACAGAAG-3′) & TiLV93R 5’-CTGCTTGAGTTGTGCTTCT-3′) and a probe was developed (TiLV-93Probe 5’- FAM-CTCTACCAGCTAGTGCCCCA-Iowa Black-3′) that targets the most conserved region of TiLVs genome. These sequences were tested as to their melting point, GC contents and primer-dimer formation. The NCBI database was searched for similar sequences in order to exclude the possibility of false positives.

This development could be part of a greater puzzle in developing rapid, specific and sensitive high-performance tools that further the development in understanding the spread of TiLV. The

authors suggest that this simple method should improve the accessibility in developing nations in order to help in proving TiLV infections.

4.2.5 Reverse transcription loop-mediated isothermal amplification (RT-LAMP) as a timesaving and possibly non-lethal detection method

Success in reducing the time taken for TiLV detection is reported since 2019 in applying Reverse transcription loop-mediated isothermal amplification (RT-LAMP) (Yin et al. 2019). This technique, combined with colorimetric gold nanoparticle (AuNP), was previously successful in diagnosing the Nodavirus in another fish species (*Penaeus vannamei*) (Suebsing 2013).

Similarly, Yin et al. (2019) suggest an isothermal condition of 63°C for 45 min for their optimized RT-LAMP reaction to test for the detection of TiLV based on RT-LAMP. They designed six primers targeting two locations based on a highly conserved sequence in the segment 1 region of the TiLV genome. The amplifications could be verified by turbidity or a colour change by adding SYBR Green I after which the RT-LAMP products were observed by a ladder pattern following gel electrophoresis. Their method was sensitive enough to detect as low as 1.6 copies of viral particle. Further, their assay was highly specific since no cross-reactivity was observed with other pathogens. When TiLV-positive samples and non-target virus were tested, they achieved a diagnostic sensitivity and specificity of 100% (Yin et al. 2019).

Analogous to Suebsing et al. (2013), Kampeera et al. (2021) tested for the detection of TiLV based on RT-LAMP and gold nanoparticle (AuNP)-labelled oligonucleotide reporter probe. Targeting the viral genomic segment 9, their technique proved compatible with a rapid nucleic extraction method that does not demand centrifugation steps or any benchtop laboratory equipment. Albeit being completed within one hour, the validation with field-acquired Tilapia samples of their RT-LAMP-AuNP assay “exhibited a near-perfect agreement with the semi-nested RT-PCR assay recommended by OIE with Cohen's κ coefficient of .869” (Kampeera et al. 2021).

A third study recommends this detection technique as a screening tool on farms for the rapid diagnosis of TiLV due to its quick procedure and effectiveness (Phusantisampan et al. 2020). Their comparison of the RT-qPCR and RT-LAMP assays of infected samples revealed positive results in 63 (100%) and 51 (80.95%) samples, respectively. From their test for cross-reactivity with five pathogens in Tilapia, their evaluation using RT-LAMP showed negative results in all tests. They further suggest mucus to be used in RT-LAMP as a nonlethal assay to avoid killing fish, since both liver and mucus samples obtained from infected fish showed comparable results in their use of the RT-LAMP method (Phusantisampan et al. 2020).

4.2.6 Genotyping TiLV in poorly equipped laboratories

Sequence information used in genotyping TiLV is used for "epidemiological tracking and implementation of evidence-based biosecurity actions" (Delamare-Debouteville et al. 2021). This information can give some idea as to the evolutionary separation of the strains in question. Phylogenetic differences allow inferences to be made as to which viruses were traded between populations (Jansen et al. 2017).

Since there are few sequencing facilities in regions where Tilapia is mostly produced, this process usually takes several days from sample to sequence results. Due to lack of sequencing capacity or limited access to specialist laboratories, clinical samples from disease outbreaks in many low- and middle- income countries have to be sent overseas. The analysis via the current preferred sequencing platform Sanger is laborious and my require manual inspection of the chromatogram, which further takes time (Delamare-Debouteville et al. 2021). Delamare-Debouteville et al. (2021) also note, that currently used sequencing platforms such as Ion Torrent, Illumina and PacBio are very useful for genomic sequencing of aquatic pathogens "yet require even more substantial capital investment and major laboratory infrastructure" and have thus been seldomly used.

A method suggested for preliminary screening in remote locations without access to a well-equipped laboratory is real-time recombinase polymerase (real-time RPA) (Wang et al. 2021). Its sensitivity was tested to be 93.33% (compared to 100% for qPCR) while the assay takes only 30 minutes.

Another technique was reported in May 2021. Here Delamare-Debouteville et al. (2021) tested the sequencing platform (MinION/Flongle) from Oxford Nanopore Technologies (ONT) that was designed to reduce the need of time and laboratory equipment. They report successful use of the semi-nested RT-PCR for the diagnosis of TiLV coupled with Nanopore sequencing of amplicons for rapid identification and preliminary genotyping of TiLV. The authors further note the reduced time taken and the inexpensiveness of using the MinION/Flongle platform due to the portability and low cost of the device. The study concludes with stating its attractiveness for genomic sequence-informed management and mitigation of pathogens in low- and middle-income countries.

4.2.7 Similarity in gene sequences of different strains of TiLV

GenBank library is a collection of all publicly available DNA sequences. Table 2 shows the full GenBank accession numbers associated with the virus. It is part of the International Nucleotide Sequence Database Collaboration which is made up of the DNA DataBank of Japan, the European Nucleotide Archive and the GenBank at the NCBI. Data is exchanged daily between these databases. The accession numbers are formatted in such a way that they comply with an index.

The genomic sequences that have been found have been compared to the original sequence that was published (Eyngor et al. 2014). Chaput et al. (2020) note that whole genomes should be used when using phylogenetic analysis to track movement of TiLV. Table 1 shows the similarity in gene sequences of the different viral strains analyzed.

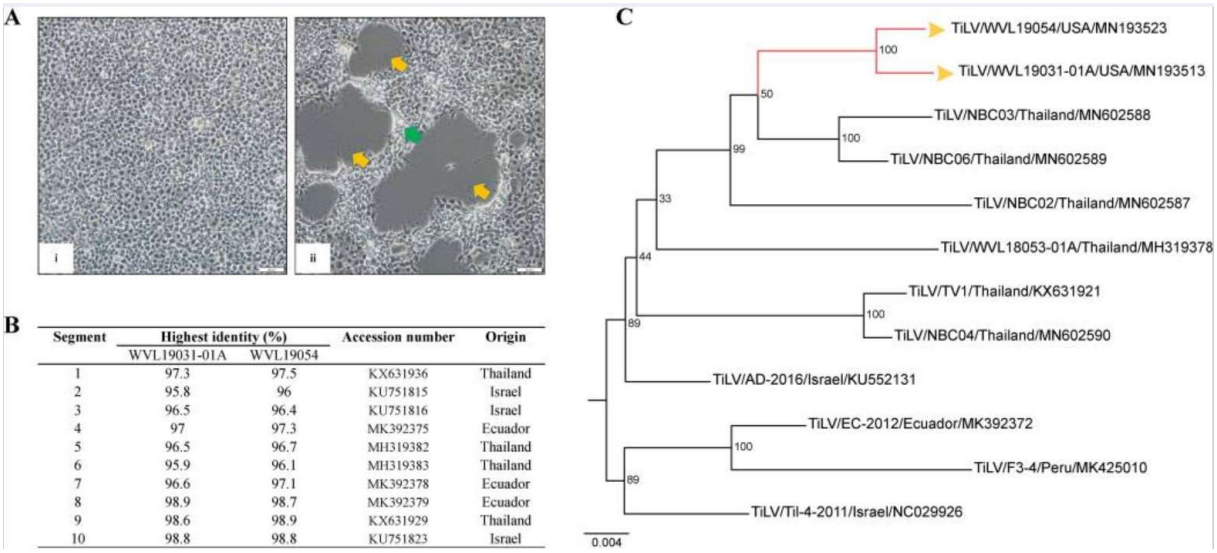
Source	GenBank accession no.	Identity to TiLV from Israel		Reference
		GenBank accession no. of TiLV Israel	% of identify	
Egypt	Not available	KU751816 (segment 3)	93%	Fathi et al. 2017
Egypt	KY817381–KY817390	Segments 3, 4 and 9 (accession numbers not specified)	93%	Nicholson et al. 2017
Ecuador	Not available	full genome sequences KU751814–KU751823	97.2% to 99.0% nucleotide identity; 98.7% to 100% amino acid identity	Bacharach et al. 2016
Ecuador	Not available	KJ605629 (ORF)	98% to 100%	del-Pozo et al. 2017
Thailand	KY615742	KU751814 (segment 1)	96.3% to 97.5% nucleotide identity; 97.4% to 98.8% amino acid identity	Dong et al. 2017a
Thailand	KY615743	KU751818 (segment 5)		
Thailand	KY615744–KY615745	KU751822 (segment 9)		
Thailand	KX631921 KX631930–KX631936	full genome sequences KU751814–KU751823	95.6% to 99.1% nucleotide identity; 96.7% to 99.5% amino acid identity	Surachetpong et al. 2017

Table 1 showing the similarities in the viral genomes of different strains. It includes the GenBank accession numbers and nucleotide and amino acid identities. Taken from Tilapia lake virus: Literature review (Jansen et al. 2017)

GenBank accession number	Segment	Originating country	reference
KY817381	Not specified	Egypt	Nicholson et al. 2017
KY817382	Not specified	Egypt	Nicholson et al. 2017
KY817383	Not specified	Egypt	Nicholson et al. 2017
KY817384	Not specified	Egypt	Nicholson et al. 2017
KY817385	Not specified	Egypt	Nicholson et al. 2017
KY817386	Not specified	Egypt	Nicholson et al. 2017
KY817387	Not specified	Egypt	Nicholson et al. 2017
KY817388	Not specified	Egypt	Nicholson et al. 2017
KY817389	Not specified	Egypt	Nicholson et al. 2017
KY817390	Not specified	Egypt	Nicholson et al. 2017
KJ605629	Clone 7450, ORF	Israel	Eyngor et al. 2014
KU751814	Segment 1	Israel	Bacharach et al. 2016
KU751815	Segment 2	Israel	Bacharach et al. 2016
KU751816	Segment 3	Israel	Bacharach et al. 2016
KU751817	Segment 4	Israel	Bacharach et al. 2016
KU751818	Segment 5	Israel	Bacharach et al. 2016
KU751819	Segment 6	Israel	Bacharach et al. 2016
KU751820	Segment 7	Israel	Bacharach et al. 2016
KU751821	Segment 8	Israel	Bacharach et al. 2016
KU751822	Segment 9	Israel	Bacharach et al. 2016
KU751823	Segment 10	Israel	Bacharach et al. 2016
KY615742	Segment 1	Thailand	Dong et al. 2017a
KY615743	Segment 5	Thailand	Dong et al. 2017a
KY615744	Segment 9	Thailand	Dong et al. 2017a
KY615745	Segment 9	Thailand	Dong et al. 2017a
KX631921	Segment 1	Thailand	Surachetpong et al. 2017
KX631922	Segment 2	Thailand	Surachetpong et al. 2017
KX631923	Segment 3	Thailand	Surachetpong et al. 2017
KX631924	Segment 4	Thailand	Surachetpong et al. 2017
KX631925	Segment 5	Thailand	Surachetpong et al. 2017
KX631926	Segment 6	Thailand	Surachetpong et al. 2017
KX631927	Segment 7	Thailand	Surachetpong et al. 2017
KX631928	Segment 8	Thailand	Surachetpong et al. 2017
KX631929	Segment 9	Thailand	Surachetpong et al. 2017
KX631930	Segment 10	Thailand	Surachetpong et al. 2017
KX631931	Segment 1	Thailand	Surachetpong et al. 2017
KX631932	Segment 1	Thailand	Surachetpong et al. 2017
KX631933	Segment 1	Thailand	Surachetpong et al. 2017
KX631934	Segment 1	Thailand	Surachetpong et al. 2017
KX631935	Segment 1	Thailand	Surachetpong et al. 2017
KX631936	Segment 1	Thailand	Surachetpong et al. 2017

Table 2 showing the full GenBank accession numbers associated with the virus. (Jansen et al. 2017)

By comparing the percentage of similarity between genomic sequences from viral outbreaks in different regions, their spread can be traced back. This was done for the first reported outbreak in farmed Tilapia in the United States (Ahasan et al. 2020). Figure 10 shows the maximum likelihood phylogram depicting the relationships of their TiLV isolates to ten other TiLV isolates.



Segment	Highest identity (%)		Accession number	Origin
	WVL19031-01A	WVL19054		
1	97.3	97.5	KX631936	Thailand
2	95.8	96	KU751815	Israel
3	96.5	96.4	KU751816	Israel
4	97	97.3	MK392375	Ecuador
5	96.5	96.7	MH319382	Thailand
6	95.9	96.1	MH319383	Thailand
7	96.6	97.1	MK392378	Ecuador
8	98.9	98.7	MK392379	Ecuador
9	98.6	98.9	KX631929	Thailand
10	98.8	98.8	KU751823	Israel

Figure 10 (A) Striped snakehead (SSN-1; E-11 subclone) cells inoculated with internal tissue homogenates. (i) Uninfected control. (ii) Infected SSN-1 cultures showing multiple plaques (yellow arrows) and associated vacuolated cells (green arrow) at the edge of the plaques. Bar, 50 µm. (B) The table represents the highest nucleotide identity for each gene segment of the U.S. TiLV isolates (WVL19031-01A and WVL19054) to TiLV strains present in the GenBank database. (C) Maximum likelihood phylogram depicting the relationship of the U.S. TiLV isolates (yellow arrowheads) to 10 other TiLV isolates based on the nucleotide sequences of the PB1 gene. Bootstrap values are given at each node, and the branch lengths represent the number (Ahasan et al. 2020).

4.2.8 mRNA and miRNA expression in infected Tilapia

Micro RNAs are 21-23 nucleotide long fragments of RNA that do not code for proteins. They regulate messenger RNAs post-transcriptionally and affect the stability of the mRNA and its translation. As they are involved in the immune response of organisms infected by viruses, these miRNAs have been studied in Tilapia in order to find out the mechanisms on a cellular level. High-throughput RNA sequencing is used to study differentially expressed mRNA and miRNA in organisms that have been infected, and this technique has been used to study infections of TiLV in Tilapia (Yang et al. 2020, Wang et al. 2020).

The study of Yang et al. found 26 122 genes that were differentially expressed, including 863 genes that had not been described prior. It is worth noting that this has been the largest genomic miRNA study of any fish to date. Most of the 5000 differentially expressed mRNAs were expressed due to the immune response caused by an TiLV infection. They included an interleukin-15-like isoform X1, four class II histo-compatibility antigens and two polymeric Ig receptor-like genes.

Analysis of the differentially expressed miRNA during a TiLV infection showed that about 200 miRNAs were involved in Tilapias immune response (Yang et al. 2020). This was proven via KEGG pathway analysis. A functional enrichment analysis showed differentially expressed mRNAs and miRNAs that took part in the immune system progress, behavior, biological adhesion, biological regulation, virion, virion part, cell junction, antioxidant activity, binding, and catalytic activity (Yang et al. 2020; Yadav et al. 2021). These were all shown to have been directly provoked by TiLV.

The study showed that virion and virion part were not involved in a genetic ontology in fish studied that had been analyzed in under 24 hours. This suggests that the virus takes longer than 23 hours to replicate.

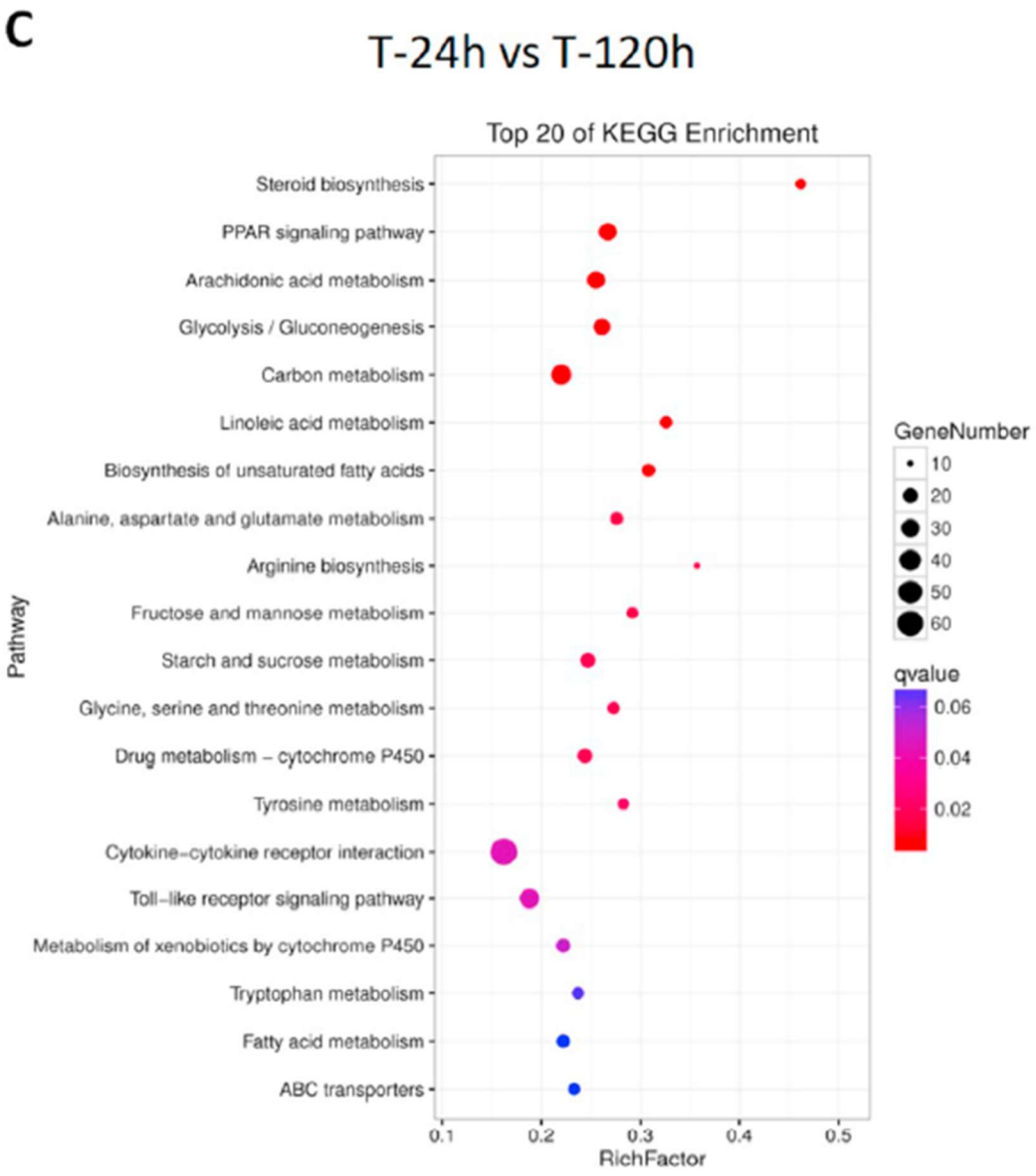


Figure 11 KEGG pathway enrichment analysis of the metabolism (Wang et al. 2020).

The KEGG (Fig.11) pathway enrichment analysis proved that the mRNAs analyzed were associated with PPAR signaling, phagosome starch and sucrose metabolism, drug and p450 metabolism and salmonella infection. In the case of the miRNAs, they were associated with melanogenesis, ErbB, Wnt, VEGF and MAPK signaling, lysosome, apoptosis, and endocytosis regulation.

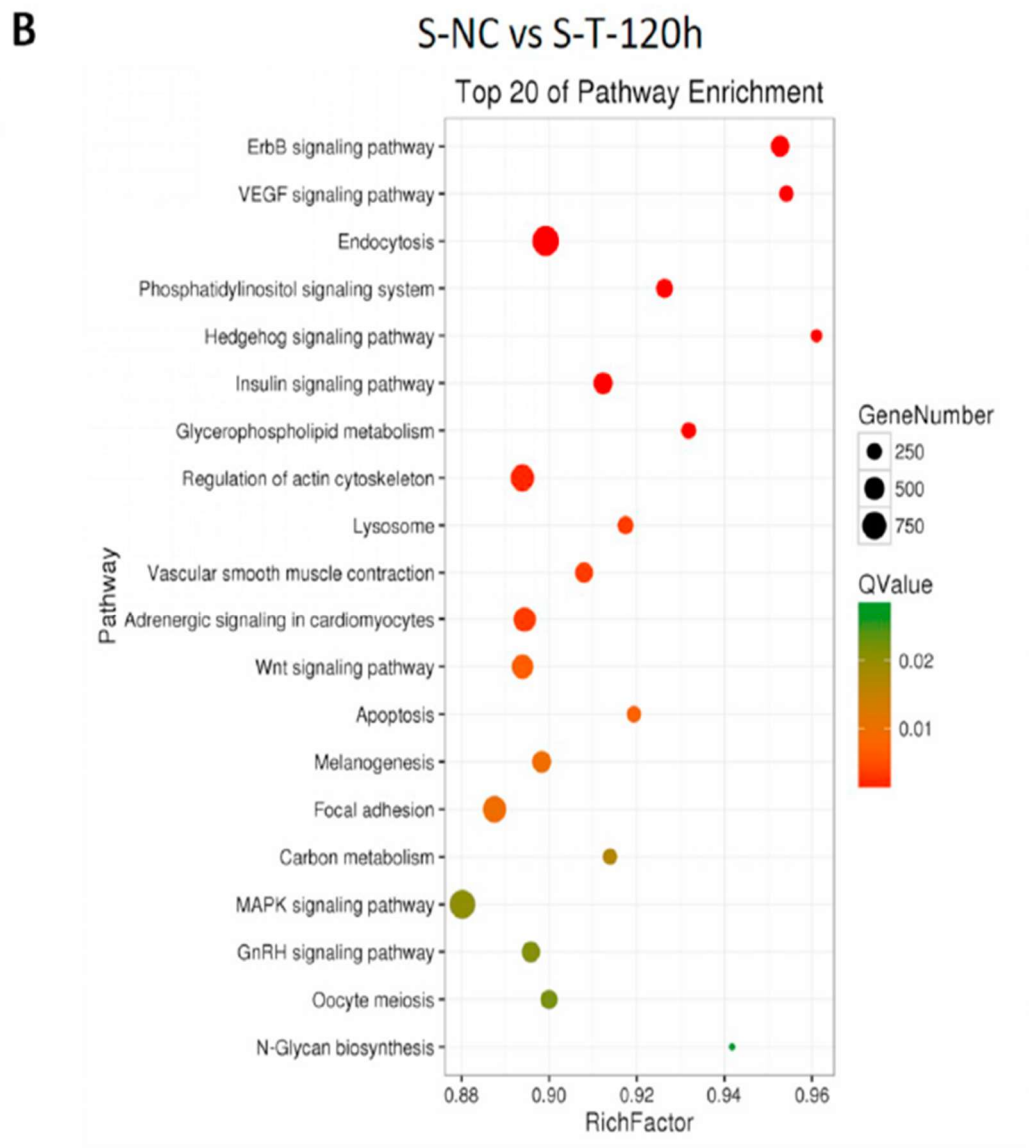


Figure 12 miRNA KEGG pathway enrichment 120 hours post infection (Wang et al. 2020)

Many of these pathways are not specific enough to credit them with being of importance though they were specifically expressed, but some have been found to mediate or take part in viral infections in other cases. The targets in this specific study that map with previously described pathways are melanogenesis, ErbB and Wnt pathways (Wang et al. 2020; Mugimba et al. 2020).

The graph above (Fig.12) illustrates the activation of immune-specific pathways in the infected Tilapia that were mentioned previously. It shows the upregulated pathways 120 hours post

infection. This gives a good general idea of the immune response of the Tilapia when faced with a challenge from a TiLV virus.

4.2.9 Transmission

The transmission dynamics have been modelled by Yang et al. in 2018 (Fig.13). The first epidemic of TiLV showed mortality levels between 9.2% to 90%. This unsettled many producers and made a concrete study of the mathematics behind the transmission important. Knowledge of the exact dynamics of the transmission may help hinder or limit future outbreaks. TiLV transmission have been shown as waterborne and horizontal in affected populations (Eyngor et al. 2014; Kenne et al. 2021). A study by Liamnimitr et al. (2018) showed that TiLV could persist in affected fish for up to 12-14 days post-infection in mucus, liver, and intestines. This realization allowed for inferences on management, those being on length of quarantine for example.

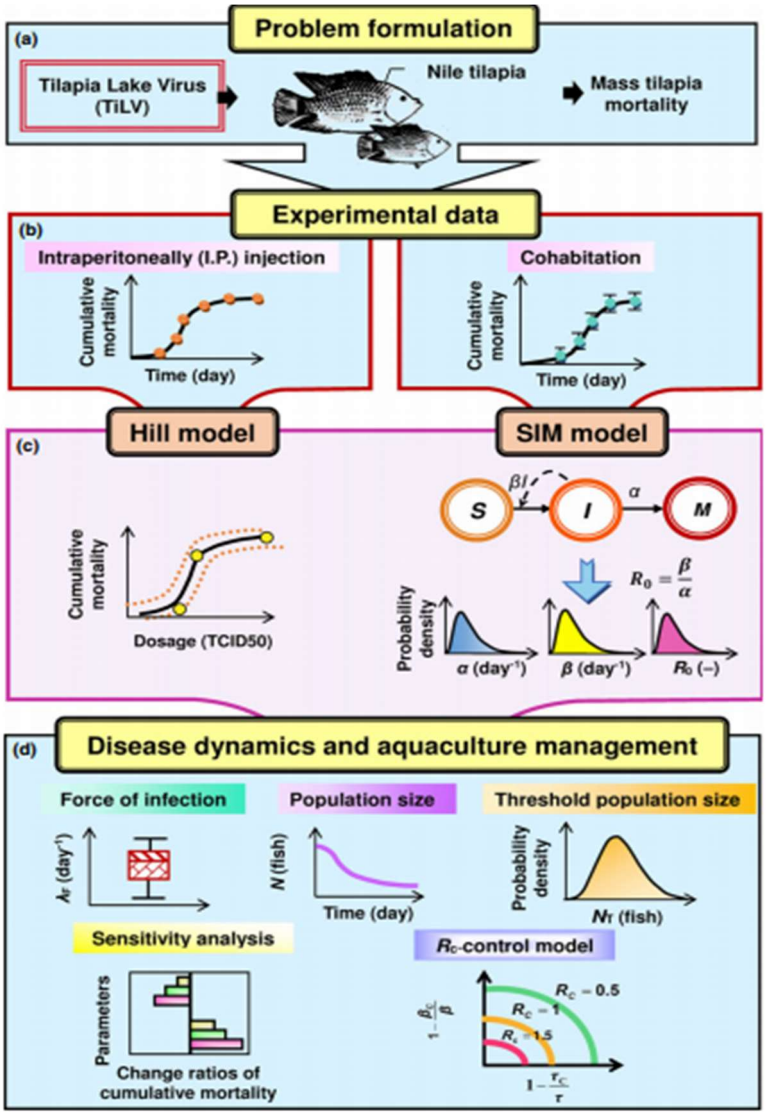


Figure 13 The steps that were taken by Yang et al. (2018) to model the infection rates.

The outbreak of TiLV in Taiwan in June 2017 inspired the study (Yang et al. 2018) to determine specifics. This was the first study to inquire into the mathematical mechanics of an outbreak, assessing the mechanistic relationship between virus dosage and TiLV-induced mortality.

Tilapia were infected intentionally with TiLV at specific doses intraperitoneally. These fish were studied in isolation or kept in water that was in contact with controls. Some infected fish were kept in 200L compartments with water permeable grids to determine the infection through water, together with a control group of uninfected fish. From the data collected, the α-value is

the mortality rate per day, the β-value the transmission rate per day, the R_0 -value can be calculated from the alpha and beta values. The R_0-value is the basic reproduction rate under the given circumstances. N (-) values are host population sizes and N_T (-) are threshold population sizes of Tilapia studied. If the number of Tilapia "N" is below the threshold N_T (-), the pathogen will not spread. The force of infection was also modelled as λ_F per day.

Two-parameters Hill models were calculated from the studies of Eyngor et al. 2014 and Tattiyapong et al. 2017.

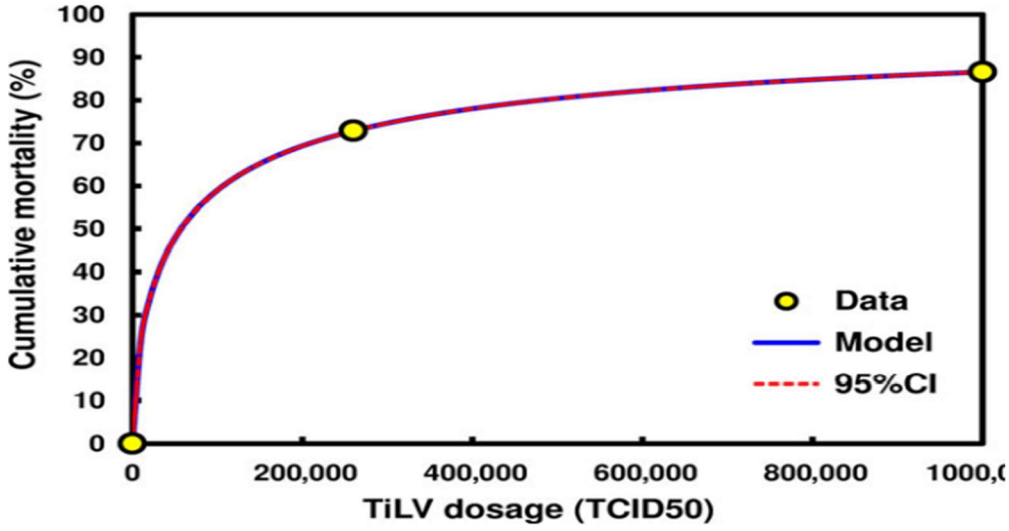


Figure 14 showing cumulative mortality with varying dose. (Yang et al. 2018)

In the group of Tilapia that were infected by peritoneal injection, the estimated exposure dose that caused the death of half of the Tilapia (TCID50) was 5.7 x 10^4. A cumulative mortality graph has been included and is shown in Fig. 14.

In the group of Tilapia that were subjected to cohabitation, the variables characterizing the infection discussed above were calculated. The force of infection λ_F were estimated as being between 0.79 and 1.03. The population of Tilapia dropped to 12% of the original population 16 days post-infection. Fig.15 shows the cumulative mortality of fish injected peritoneally. This serves to estimate mortality from viral load. Fig. 15 shows the normally distributed probability densities of key variables.

From the cohabitation numbers, a sensitivity analysis was performed to show which value was the most influential parameter for the outbreak. The transmission rate proved more sensitive to alternations of cumulative mortalities that the mortality rate itself. The implication of this is that the mortality rate plays a vital role in controlling TiLV outbreaks. A population size reduction in each volume of water may therefore lead to a reduced mortality in fish. Aquaculture management should aim to reduce populations under a critical threshold in order to manage an outbreak properly.

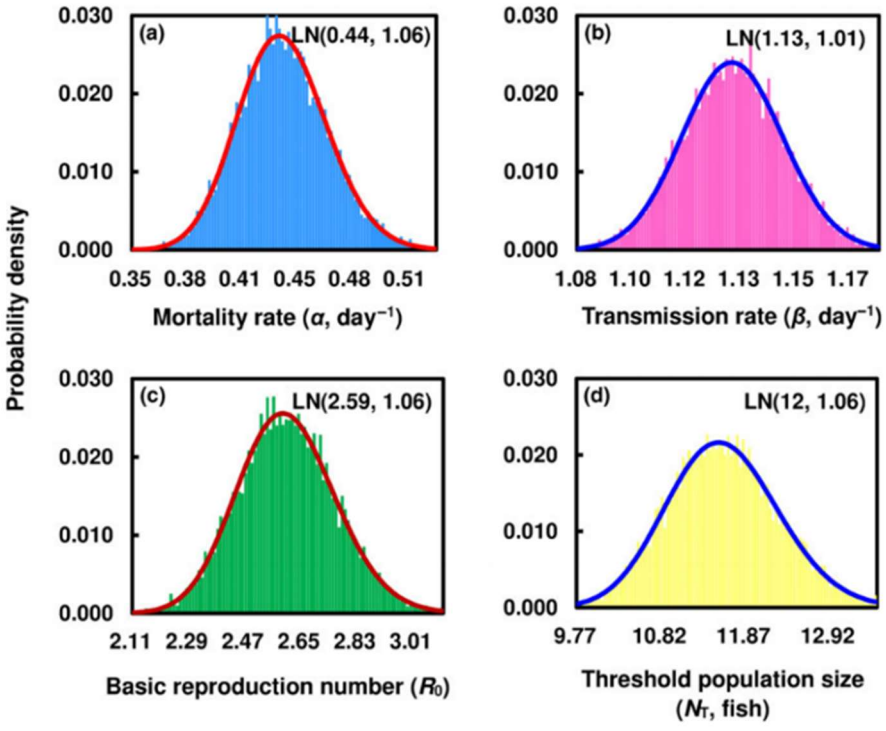


Figure 15 Characteristic numbers discussed earlier. (Yang et al. 2018)

4.3 Solutions

Currently no antiviral therapy or vaccines exist for TiLV (Zeng et al. 2021). Once a diagnosis is made, the protocol for dealing with an infected colony of fish is depopulation and disinfection (Jaemwimol et al. 2019). No vaccinations are currently available to pre-empt an infection. The following section provides an update on current research and developments in finding solutions against the spread of the virus.

4.3.1 Enhancing genetic resistance to TiLV in Tilapia

A suggested method of mitigating the high mortality and fast transmission of TiLV has been the selective breeding of Tilapia that have a genetic resistance to it. The combination of field data and genomic tools is expected to increase understanding the genetic architecture of disease resistance and inform disease control (Barría et al. 2021). As TiLV has been seen to affect fisheries to such an extent over several continents, and biosecurity measures taken may be dire, selective breeding could be a cost-effective measure.

In many currently farmed aquaculture species, selective breeding has proven to be a valuable tool to improve the resistance to specific pathogens in the past. *Dicentrarchus labrax* for example has been bred to develop a resistance to viral nervous necrosis (Palaiokostas et al. 2018). *Salmo salar*, the Atlantic salmon, was bred to be resistant to *Piscirickettsia salmonis* which has been proven to be effective (Correa et al. 2015). The rainbow trout has been affected by the same species as Atlantic salmon, *Piscirickettsia salmonis,* and its resistance to the infection proven (Bassini et al. 2019). Lastly, Coho salmon (*Oncorhynchus kisutch)* have been studied in the resistance they developed to *Piscirickettsia salmonis* (Barría et al. 2018).

Studies have shown that there may be a significant genetic variation for resistance to bacterial pathogens in controlled challenge experiments on Tilapia (Barría et al. 2020).

In a 2021 study, Barría et al. (2021) identified several candidate genes (including lgals17, vps52, and trim29) related to host response to viral infection. The authors highlight that the genetic markers identified “have potential in marker-assisted selection to improve host resistance, providing a genetic solution to an infectious disease where few other control or mitigation options currently exist” (Barría et al. 2021).

Tattiyapong et al. (2020) demonstrated that fish that survived either an infection through cohabitation or intraperitoneal injection developed a protective immunity. The authors further demonstrated that antibody responses against TiLV that supports protective immunity to subsequent TiLV disease. This was backed by testing antibody kinetics following a second exposure to TiLV which showed that humoral memory had developed upon re-infection. The authors consider their findings a first step in the development of an efficacious vaccine against TiLV.

4.3.2 Probiotics as means of improving immune response to TiLV

As an alternative to antibiotics and drugs, the use of probiotics was tested for their effects on mortality, viral load and expression of immune-related genes in infected Red Hybrid-Tilapia (Waiyamitra et al. 2020). Previous reports anticipate widespread use of probiotics in fish and shellfish cultures in reducing the negative impact of bacterial and viral infections (Wang et al. 2019, El-Saadony et al. 2021). In Tilapia, no improvements were documented in terms of weight gain, feed efficiency or feed conversion ratio (Waiyamitra et al. 2020). The authors however report a reduced mortality as well as a significantly reduced viral load in infected Tilapia that received a probiotic-supplemented diet compared to a control group. Differences were also stated for the expression patterns of immune-related genes, namely an upregulation upon probiotic treatment. The study concludes that the tested *Bacillus sspp.* probiotics could have beneficial effects on strengthening Tilapia immunity and resistance against TiLV infections and suggest their preventative use.

4.3.3 Prophylaxis – surveillance and biosecurity

Since Tilapia are frequently grown in polyculture systems with other aquatic species, the risk of other species acting as carriers was tested by Debnath et al. (2021). From 15 fish species and seven other invertebrates that were co-cultivated with infected Tilapia, none were tested positive for TiLV. Of six experimentally infected species, none showed any clinical signs of TiLV. The authors thus ruled out the possibility of other species being TiLV-carriers, however still stress the importance of ongoing surveillance as mutations or adaptions to new hosts are always possible.

Evidence from experimental transmissions has revealed that transmission is possible between Tilapia brood stock to the reproductive organs and fertilized eggs (Dong et al. 2019).

Tilapia seem to be most susceptible to infection during early life stage, at a weight of about five grams (Roy et al. 2021). This indicates special care to be taken when handling fingerlings.

It is therefore important for fish farms to buy only from TiLV free vendors. To prevent outbreaks, any fish that have been bought are to be quarantined and monitored for signs of TiLV before they may be mixed with pre-existing stocks. They should be monitored continuously for the symptoms described in the diagnosis section of this paper. It is of utmost importance that any equipment used shall be only for specific colonies to avoid cross contamination. Any fish showing signs of diseased must be analyzed histopathological to confirm or rule out a possible contamination. A reduction in stress that the fish are under can also play a role in reducing the risk of an outbreak, as stress negatively affects the immune system of the fish (Al-Hussinee et al. 2019).

Co-infection of virus and bacteria might pose problems in the future, as Basri et al. (2020) note.

4.3.4 Vaccine development

Tattiyapong et al. (2020) studied the adaptive immune response to exposure of Tilapia to TiLV as this is a critical step in the development of a vaccine. They demonstrated that fish that survived either an infection through cohabitation or intraperitoneal injection developed a protective immunity. Significant antibodies against the protein encoded by the TiLV segment 4 were further detected. The authors further demonstrated that antibody responses against TiLV that supports protective immunity to subsequent TiLV disease. This was backed by testing antibody kinetics following a second exposure to TiLV which showed that humoral memory had developed upon re-infection. The authors consider their findings a first step in the development of an efficacious vaccine against TiLV and suggest, based on their discovery of immunity after a single exposure to the virus, that a single vaccination might be adequate to protect Tilapia during the entire grow-out phase.

Lueangyangyuen et al. (2021) further identified two genomic segments (S5 and S6) as potential vaccine candidates for TiLV protection in Tilapias.

On the path to the development of an effective vaccine, Widziolek et al. (2021) suggest that zebra danios *(Danio rerio)*, that are easier and faster to study than Tilapia, could be a good model to study mechanisms of the TiLV infection and to follow antiviral responses. Also Mojzesz et al. (2021) used zebra fish to study effects of TiLV.

Zeng et al. (2021) report success in testing potential vaccines. They report on their "β-propiolactone-inactivated TiLV vaccine coupled with the adjuvant Montanide IMS 1312 VG and booster immunizations" that viral loads were significantly lowered and survival was significantly increased (Zeng et al. 2021). They deduce from their findings that the vaccine might also stimulate protective antibody response and inhibit viral proliferation, thus giving hope for the successful development of an effective and commercially available vaccine soon.

The most recent vaccine study was carried out by Mai et al. (2021). The authors tested heat-killed (HKV) and formalin-killed (FKV) vaccines and found relative percentage survival rates of 71.3% and 79.6% respectively. Their findings present another promising development in the availability of a vaccine.

4.4 Impacts

4.4.1 Economic impact

As the mortality in affected fish populations is so high, the economic impact can be quite severe. Fish farms regularly report mortality levels above 80% (Eyngor et al. 2014, Dong et al. 2017a, Surachetpong et al. 2017). Fathi et al. (2017) have estimated that the summer mortality syndrome linked to TiLV in 2015 led to a loss of production of 98,000 metric tons. This number of fish would be valued at around 100 million dollars in Egypt alone.

In Israel there is a considerable amount of Tilapia caught in the wild. In the Sea of Galilee (technically a lake) the annual wild catch figures were estimated by Eyngor et al. (2014) to have decreased from 316 metric tons in 2005 to 8 metric tons in 2009. It has not been determined however whether this decrease is due to TiLV, although positive samples of infected wild Tilapia have been identified in the affected areas (Eyngor et al 2014).

A continued growth in production of Tilapia coupled with a decreased demand in the United States of America has led to a drop in the price. China is currently the greatest producer of Tilapia. The ongoing trade-conflict between the US and China has led to a weak demand for Tilapia in the US and has been a strain on Tilapia traders and producers (FAO 2020a).

On import, Tilapia prices have fallen from 1,31 USD per kilogram to 0,79 USD per kilogram. This represents a fall in price of about 20%.

The world bank is actively lobbying for development in the Tilapia sector in India and other parts of Asia (FAO 2020a).

The recent COVID-19 outbreak led to an increased trend in small-scale backyard farming as global markets were slowing. Some countries have decided to cultivate Tilapia as an immediate response plan to this pandemic (FAO 2020b).

4.4.2 Socio-economic impact

According to an expert knowledge elicitation risk assessment for TiLV that was published by the FAO.

The TiLV may have already spread further than presently described. The risk of TiLV spreading within a country that already has infected colonies was described as very high; the risk of a regional spread was described as high. Naturally, this leads to a high level of care being needed. As the virus spreads in water temperatures between 22° and 32°, countries that have these temperatures occurring are particularly at risk. The food chain crisis early warning bulletin warns of spreads, specifically in Africa. Specifically, Tilapia-producing countries in North Africa include Egypt and Sudan. In East Africa Kenya, Uganda and the United Republic of Tanzania are named as countries at risk. In Southern Africa countries include Malawi, Mozambique, Zambia and Zimbabwe (FAO 2020c).

Africa has the added difficulty that it focuses on some species of Tilapia that are especially vulnerable to TiLV. These include *Oreochromis niloticus* x *O. aureus* hybrids, *O. niloticus* and *Oreochromis sp.*

If surveillance and preventative measures are not met, large scale mortalities may be expected. This may lead to a breakdown of Tilapia supply chains which may have severe effects on the affected fisheries (FAO 2020c).

The socio-economic impact of tilapia farming has been studied extensively in Brazil (Bertolini et al. 2019).

5. Conclusion

TiLV is a persistent problem in Tilapia fisheries. The implication of this is the importance that knowledge of the disease is increased and the methods of documentation improved. There is an urgent need to track the actual geographical distribution of the virus. It is likely that the virus has spread further than what the literature suggests due to the considerable international trade. 43 countries are unclear on the status of TiLV of their fisheries (Dong et al. 2017). However, it is reported that the peak of the virus' spread was reached between 2014 and 2016 (Thawornwattana et al. 2021). Co-infection of virus and bacteria might however pose a new threat (Basri et al. 2020).

Screening and surveillance programs are still lacking according to the FAO and should be actively encouraged in order to gather further knowledge on the spread of the problem and to effectively limit the spread. Several countries have initiated official programs that do just that (FAO 2017b). Translocation of Tilapia remains somewhat dangerous, and live Tilapia translocation must be accompanied by biosecurity measures explained in the respective chapter in this paper. International cooperation has shown to be effective in expediting this process to save time while local infrastructure and knowledge is built. Expertise in TiLV risk analysis should be encouraged in countries with significant Tilapia production. Local awareness can help to underline the importance of reporting, recording and mapping any unusual mortality in Tilapia populations (Jansen et al. 2017).

As Tilapia is an important source of protein in many countries around the world, the high mortality is a risk to food availability and security. Also, economic impact is considerable in weaker economies (Jansen et al. 2017).

The FAO has described some specific scientific goals that need to be researched. There are gaps in the knowledge in epidemiological aspects of TiLV. These include viral properties, methods of transmission, susceptible host life stages, survival of TiLV outside of the host in different mediums, risk factors for disease outbreaks and presence of non-tilapine hosts/carrier species. It is possible that due to different factors that may vary by country such as production methods and fish genetics, that regions should be evaluated in isolation (Jansen et al. 2017).

Special attention should be given to the possibility of vertical transmission of TiLV as well as a possible sub-clinical carrier status (Yamkasen et al. 2019). Descriptive, observational and experimental studies should be conducted in order to address the lacking knowledge in these areas. A possible vaccination in the near future may be key to solving the problem that most other measures may only limit. First successes on the development of effective vaccines have been reported recently (Zeng et al. 2021). Dissemination of information is vital in the fight against this multi-continent disease.

TiLV is an ongoing problem for Tilapia fisheries and aquaculture worldwide in safeguarding global food supply chains. Modern advances in PCR techniques have made detection of TiLV easier and have helped scientists around the world in proving infections as being caused by TiLV. Mathematical modelling of infection dynamics has helped understand the complexities in TiLV outbreaks and offer some insight into the methods with which outbreaks can be slowed, prevented or even stopped. More scientific work is needed in order to mitigate the danger posed by Tilapia outbreaks to developing nations both economically and regarding health.

6. Zusammenfassung

Das Tilapia-Lake-Virus ist ein ernstzunehmendes Virus, dass weltweit zu massiven Ausfällen in der Tilapia Aquakultur führt und es bestehen nach wie vor viele wichtige offene Wissenslücken. Die wissenschaftlichen Erkenntnisse über TiLV sind begrenzt und viele wichtige Fragen sind noch unbeantwortet. Bislang ist TiLV die verheerendste Krankheit für die Tilapia Aquakultursysteme. Obwohl das Virus erst vor einigen Jahren entdeckt wurde, wird angenommen, dass das Virus schon seit längerer Zeit in der Aquakultur vorhanden ist, aber nicht identifiziert wurde. Seit den ersten Berichten aus Israel haben viele andere Länder innerhalb kürzester Zeit ebenfalls das Auftreten des Virus gemeldet. Aufgrund der schnellen Verbreitung und des Vorkommens in verschiedenen Ländern kann die Krankheit als grenzüberschreitende Tierseuche angesehen werden. Da viele Länder Fischprodukte und Saatgut aus kommerziellen Brütereien importieren, in denen TiLV gemeldet wurde, ist zu erwarten, dass sich TiLV weiter global ausbreiten wird, wie auch die damit verbundenen schädigenden Auswirkungen auf Tilapia-produzierende Länder und die Reduktion der Tilapia-Produktion insgesamt.

Einer von vielen wichtigen Schritten, um eine weitere Ausbreitung einzudämmen, ist ein funktionierendes Surveillance Programm. Es sind zwar bereits Nachweismethoden vorhanden, jedoch wird die Suche nach spezifischeren und empfindlicheren Diagnosemethoden fortgesetzt. Weitere und genauere Studien zur Pathogenese und Prognose der Krankheiten helfen ebenfalls bei der Entwicklung geeigneter Präventivmaßnahmen. Da hauptsächlich Landwirte aus einkommensschwächeren Gruppen in der Tilapia Aquakultur tätig sind, liegt der Fokus darauf, kostengünstige und präzise Diagnoseverfahren zu entwickeln, für die keine umfangreichen Laboreinrichtungen notwendig sind. Die Entwicklung von selektiv gezüchteten resistenten Tilapias könnte ebenfalls eine Möglichkeit sein, um Ausfallsquoten zu senken.

Weiters wird daran gearbeitet, dass die Beschränkung oder das Screening von Lebendfischtransporten auf nationaler und internationaler Ebene strengeren Vorschriften unterliegen, um die Ausbreitung des Virus zu reduzieren oder im besten Fall zu verhindern. Ebenfalls notwendig für die Eindämmung der Virusverbreitung sind gute Betriebsführungspraktiken. Dazu gehören die Anwendung geeigneter

Biosicherheitsmaßnahmen wie die ordnungsgemäße Verwendung von Desinfektionsmitteln, und die schnelle Entfernung von sterbenden und toten Fischen aus betroffenen Teichen. Darüber hinaus spielen Stressfaktoren bei Fischen eine Rolle für die Schwere der Virusausbrüche und sind durch geeignete Managementmaßnahmen zu verringern.

Derzeit ist kein kommerzieller Impfstoff zur TiLV-Prävention verfügbar, es wird aber laufend zur Entwicklung eines wirksamen und erschwinglichen Impfstoffs geforscht. Ergänzend ist ein verbesserter Informationsaustausch oder umfangreiche Studien zu den mit den Feldausbrüchen verbundenen Faktoren hilfreich, die zu geeigneten Kontrollmaßnahmen führen. Dazu zählen die Wassertemperatur, der verwendete Teich, die Wasserparameter, die landwirtschaftlichen Praktiken und der Transport von lebenden Fischen.

7. Summary

The Tilapia lake virus is a serious virus that leads to massive failures in Tilapia aquaculture worldwide. The scientific knowledge about TiLV is limited and many important questions remain unanswered. To date, TiLV is the most devastating disease for Tilapia aquaculture systems. However, although the virus was only discovered a few years ago, it is believed that the virus has been present in aquaculture for a long time before being identified. Since the first reports from Israel, many other countries have also reported the occurrence of the virus within a very short period. Due to the rapid spread and occurrence in different countries, the disease can be viewed as a cross-border animal disease. As many countries import fish products and seeds from commercial hatcheries where TiLV has been reported, it is to be expected that TiLV will continue to spread globally as well as the associated damaging effects on tilapia-producing countries and the reduction in tilapia production.

One of many important steps to contain further spread is a functioning surveillance program. More accurate detection methods already exist, but the search for more specific and sensitive diagnostic methods continues. Further and more precise studies on the pathogenesis and prognosis of the diseases also help in the development of suitable preventive measures. Since it is mainly farmers from lower-income groups who work in Tilapia aquaculture, the focus is on developing cost-effective, precise diagnostic methods that do not require extensive laboratory facilities. The development of selectively bred resistant Tilapias could also be a way to reduce dropout rates.

Furthermore, work is being carried out to ensure that the restriction or screening of live fish transports at national and international level is subject to stricter regulations in order to reduce the spread of the virus or, in the best case, prevent it. Good operational management practices are also necessary to contain the spread of the virus. This includes the application of appropriate biosecurity measures such as the proper use of disinfectants, and the quick removal of dying and dead fish from affected ponds. In addition, stress factors in fish play a role in the severity of virus outbreaks and should be reduced through appropriate management measures.

No commercial vaccine for TiLV prevention is currently available, but research is ongoing to develop an effective and affordable vaccine. In addition, an improved exchange of information

or extensive studies on the factors associated with the field outbreaks, which lead to suitable control measures, are helpful. These include the water temperature, the pond used, the water parameters, agricultural practices and the transport of live fish.

8. List of Figures

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Acknowledgements

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10. List of References

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