Emergency Regional Consultation for Prevention and Management of Tilapia Lake Virus (TiLV) in the Asia-Pacific

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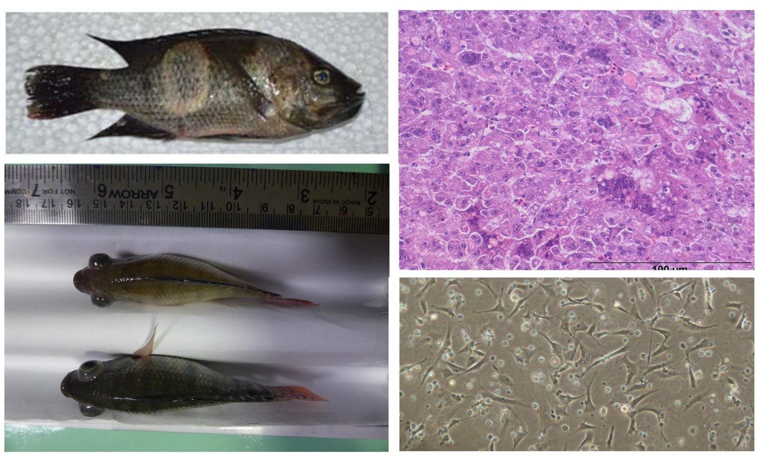

Published by:

Network of Aquaculture Centres in Asia-Pacific

Suraswadi Building, Department of Fisheries, Kasetsart University Campus, Ladyao, Jatujak, Bangkok 10900, Thailand

National Fisheries Technology Extension Center Ministry of Agriculture

No.18, Maizidian Street, Chaoyang District, Beijing P.R. China

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April 2018

COVER PHOTOS: Courtesy of the Director, ICAR-National Bureau of Fish Genetic Resources, Lucknow, India.

Top Left: *Gross sign of TiLV infected tilapia.*
Bottom Left: *Experimentally infected tilapia showing bilateral exophthalmia and scale protrusion.*
Top Right: *Photomicrograph of naturally infected tilapia showing syncytial giant cells in liver.*
Bottom Right: *Plaque formation along with elongation of cells in TiLV infected cell line.*

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Executive Summary

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Participants of the Emergency Regional Consultation on Prevention and Management of Tilapia Lake Virus (TiLV) in the Asia-Pacific
27-28 September 2017
Guangzhou, P.R. China

Since 2009, tilapia aquaculture has been threatened by mass die-offs in Israel and Ecuador, which is caused by a novel Orthomyxo-like (RNA) virus named as Tilapia lake virus (TiLV). This has been reported as a newly emerging virus that causes syncytial hepatitis of tilapia (SHT). As of 2016, countries affected by this emerging disease include Israel, Ecuador, Colombia and Egypt. In 2017, Thailand and Chinese Taipei confirmed the presence of the virus among farmed tilapia, which also caused mass mortalities. These are the first reports of the disease in Asia-Pacific Region. In response to this, NACA has released a Disease Advisory https://enaca.org/?id=864&title=tilapia-lake-virus-disease-advisory as part of the awareness programme in the region. The Advisory has been widely disseminated to all NACA member countries, partner institutes and other interested parties in the region and beyond. Moreover, FAO has circulated a Special Alert on TiLV http://www.fao.org/news/story/en/item/ 888884/icode/ , OIE published a Technical Disease Card http://www.oie.int/fileadmin/ Home/eng/Internationa_Standard_Setting/docs/pdf/ A_TiLV_disease_card.pdf , while WorldFish prepared a TiLV Factsheet https://www.worldfishcenter.org/content/tilapia-lake-virus-tilv-what-know-and-do and CGIAR published TiLV Literature Review https://fish.cgiar.org/publications/tilapia-lake-virus-tilv-literature-review .

As tilapia is a highly important aquaculture species in the region, it is highly necessary to contain the disease in affected countries, and to prevent its spread to major tilapia-producing countries in the region. As such, countries in the region should be able to harmonize efforts in preventing the entry of the pathogen through a more strict quarantine and biosecurity measures.

In this regard, this Emergency Regional Consultation for Prevention and Management of Tilapia Lake Virus (TiLV) in the Asia-Pacific was undertaken to discuss and plan actions on the overall prevention and management of the disease. The Consultation focused on the following:

- Implementation of proper quarantine and biosecurity measures, as well as responsible movement of live tilapias within the country and across the region;
- Strengthening of diagnostic capacities as well as active surveillance of the disease (to detect presence or absence of the virus).
- Formulation of recommendations on the sanitary measures for disease prevention
- Emergency preparedness for countries not yet affected by the disease highly considering the capacity of each country. As tilapia is a common food for many people in the region, especially among rural communities, emergency preparedness will make a big impact in the management of this emerging threat for tilapia aquaculture.

Keynote presentations cover overall status of tilapia aquaculture in the region, major diseases affecting cultured tilapia, overview and status of TiLV in the region (and the world), role of trade in the spread of transboundary aquatic animal diseases, and risk assessment and biosecurity as preventive measures in the spread of the disease. Status of and plan of actions on TiLV in eight countries in the region were also presented and discussed. These include China, India, Indonesia, Malaysia, Myanmar, Philippines, Thailand and Viet Nam.
The panel discussion dealt with relevant issues on the management, prevention and control of TiLV including: formulation of regional plan for prevention and control of TiLV; research gaps and priority; surveillance and reporting; listing in OIE; and, way forward.

Based on the presentations and panel discussions, important recommendations were made to further understand and manage TiLV in the region. For disease surveillance and diagnosis, a standard diagnostic test is still needed. There is also a need to set up two working groups to deal with TiLV investigations, one for national surveillance and another for the development of standard operating procedures (SOP) for biosecurity. Understanding the genetic variations of the virus, carriers (sub-clinically infected fish) and susceptibility of other fish species to the virus are also important. Similarly, research studies should focus on validation of diagnostic tests (study of coinfection of virus and bacteria; survival of virus in frozen sample; abundance of virus in mussel tissue; relationship between host and pathogen; phylogenetic analysis of different strains; susceptibility of marine ornamental fishes). It is also necessary to know whether the virus is already present in the natural aquatic environment, and to identify the root cause of the disease. As the clinical signs appear to be non-specific which causes confusion, it might be worthwhile to pay attention to several areas inclduing unbalanced “complete” feed which might cause nutrient deficiency, and environmental parameters that might trigger disease outbreak and proliferation of the virus.

KEYNOTE PRESENTATONS

Tilapia Aquaculture in the Asia-Pacific Region: Status and Trends

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Production of farmed tilapia reached 5.8 million tons in 2015, ranking the second most productive farmed finfish after carps. Asia produced more than 70% of the total and China was the top producer with the production of 1.8 million tons. Other major producers include Indonesia, Egypt, Bangladesh, Vietnam, the Philippines, Brazil and Thailand. Tilapia products are among most traded aquaculture commodities worldwide. China remains the major exporter while the United States, where tilapia consumption has being rapid increasing since 2000, is the major importer.

Tilapia is one of the major cultured finfish and most widely distributed species. They grow fast, feed on all kinds of food items available ranging from planktons, detritus, supplementary feed to nutritionally balanced commercial pellets. They can tolerate poor water quality and adapt well to various culture environments and systems ranging from extensive, semi-intensive and intensive operations. They also adapt well to different culture facilities such as earthen ponds, cages, tanks, and race ways. When co-cultured with other species, if properly stocked and managed, they produce production synergy and health benefits to other species, making them an ideal species for polyculture.

The technology for tilapia seed production is well-established. Mass production of mono-sex, all-male fry using hormonal sex-reversal techniques in hapa-in-pond based systems is widely practiced. This makes it possible and relatively easy to supply tilapia seeds of uniform size at large quantity. In the past three decades, genetic improvement has been made in some farmed tilapia species and strains. Some genitically improved strains are now commercially available with better growth rate in farming conditions than their natural counterparts.

Aquaculture is expected to continuously grow to feed the fast-growing world population. On the other hand, aquaculture practices are required to maintain environmental integrity and mitigate the impacts of climate change. Tilapia as a major aquaculture species meets most desirable biological criteria of species selection for sustainable farming systems. Tilapia culture is likely to play important roles in rural development, nutrition security, sustainable seafood supply, employment, and income generation.

Tilapia production is likely to increase continuously. Major contribution will be from Indonesia, Egypt and some new producers such as India in near future. The increase of production in China may slow down in coming 2-3 years, while Indonesia is likely to grow more tilapia and close the gap with China. It is also expected that more value added products will be developed to meet the dynamic demands of the consumer market.

The farming systems will remain diverse, yet sustainable intensification is seen as a current development trend which aims at better control of culture process, resource use efficiency and environmental friendliness. Better biosecurity mechanisms at farm level and vaccines are expected to be developed and applied. The industry will be better regulated wherein various production standards are in place and quality certification effectively implemented.

Important Diseases of Cultured Tilapia and Some Disease Prevention Strategies

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Tilapia is one of world's major aquaculture species with global production of cultured tilapia in 2015 of about 5.67 million tons. The top tilapia producing countries include China (31% of total world tilapia production), Indonesia (20%), Egypt (16%), Vietnam (5%), Philipines (5%), Brazil (4%) and Thailand (3%). Tilapia farming industry has suffered huge losses from a variety of diseases. Many diseases, including viral, bacterial and parasitic diseases, have been found in cultured tilapia. Viral pathogens of tilapia include tilapia lake virus, aquabirnavirus, betanodavirus, iridoviruses and herpesvirus. On the other hand, there are about 30 species of bacterial pathogens affecting cultured tilapia.

Viral diseases

The first RNA virus discovered in tilapia was an aquabirnavirus isolated from healthy Mozambique tilapia cultured in Chinese Taipei (Hedrick et al., 1983). In 2007, a betanodavirus, nervous necrosis virus (NNV), was detected by RT-PCR in Nile tilapia (*Oreochromis niloticus*) larvae following a mass mortality event at a western European fish farm (Bigarre et al., 2009). Ferguson et al. (2014) described a novel disease with suspected viral etiology, namely syncytial haptitis of tilapia (SHT) in fingerings of Nile tilapia in Ecuador. In Israel, massive mortalities of both wild and farmed hybrid tilapia (*O. niloticus* x *O. aureus*)were observed from all over country since 2009, and the etiological agent was subsequently identified as tilapia lake virus (TiLV) (Eyngor et al., 2014). Studies indicated that the virus causing SHT was genetically similar to TiLV and was classified as a novel orthomyxovirus–like (Bacharach et al., 2016). Later, TiLV was found in cultured tilapia in Colombia, Egypt and Thailand (Tsofack et al., 2016; Fathi et al., 2017; Dong et al., 2017).

The first DNA viruses discovered in tilapia was iridovirus. Paperna (1973) detected lymphocystis virus in tilapia from the Rift Valley Lakes Kitangiri and Victoria. A severe (100% mortality) epizootic among Mozambique tilapia in an Australian aquatic disease laboratory was tentatively attributed to a ranavirus, Bohle iridovirus (Arid and Owens, 1977). Smith et al. (1997) described an iridoviral-like infection in Nile tilapia fingerlings imported into Idaho from Florida that resulted in lethal systemic disease. A remarkably similar iridoviral-like infection was reported soon thereafter in Nile tilapia (Mc Grogan et al., 1998). Suhramanian et al. (2016) reported a disease caused by ISKNV in Nile tilapia cultured in the US Midwest. A novel herpesvirus, referred to as tilapia larvae encephalitis virus, has been detected in laboratory-reared blue tilapia larvae (Shlapobersky et al., 2010, Sinyakov et al., 2011).

The culture of tilapia will continue to face huge risks due to viral diseases. Betanodavirus, iridoviruses and herpesvirus found in cultured tilapia have extensive hosts that are highly pathogenic to some farmed fish, causing catastrophic losses. For example, NNV, a member of betanodavirus, could infect many fish species. From 2002 to 2003, we investigated NNV in wild and cultured marine fish in southern China. Sixty-nine species, composed of 892 wild marine fishes belonging to 20 families and 5 orders and 381 cage-reared marine fishes belonging to 3 families and 1 order, were sampled. In winter samples, NNV was detected by nested PCR in 32 species, 90% of wild fish and 100% caged-reared fish were found positive. The results showed that most of the sampled fish were asymptomatic contagious. ISKNV has been studied extensively due to their ability to cause significant disease across a wide range of cultured and wild freshwater and marine fishes (over 60 species). ISKNV caused serious disease in cultured mandarin fish, large mouth bass, grouper, large yellow croaker, red drum and turbot. From 2002 to 2003, we have investigated ISKNV in wild and cultured marine fish in Southern China. Eighty-six species composed of 1629 wild and cultured marine fishes belonging to 25 families and 6 orders were sampled. ISKNV was detected by nested PCR in 53 out of 86 species of the wild and cultured samples (Wang et al., 2007). KHV/CyHV-3 and CyHV-2, herpesviruses, were detected in cultured koi and crucian carp in China, respectively.

With the wide host ranges of some important viruses of finfish, the question is does TiLV has other hosts besides tilapia? If TiLV can infect a wide range of hosts, prevention and control of the disease in cultured tilapia will be a big challenge.

Bacterial diseases

There are about 30 bacterial pathogens that can infect tilapia. In China, the main bacterial disease of cultured tilapia is streptococcosis, caused by *Streptococcus iniae* and *S. agalactiae*, and has resulted in huge economic losses. In May to October 2011, the disease caused a loss of 7,127 tons of tilapia in Guangxi Province (Hu et al., 2013). Epidemiological investigation of tilapia streptococcosis showed that 35 kinds of aquatic animal were infected by the pathogen in China. With farming environment eutrophication and global climate change, bacterial diseases will seriously threaten the tilapia farming industry.

"Successive culturing obstacle" in aquaculture

Tilapia is threatened by various pathogens and diseases of farmed tilapia are becoming more and more serious. What could be the reason behind this phenomenon? To answer this question, it is time to rethink the root causes of aquatic animal diseases. Only by understanding the root causes of the diseases that we can effectively control the diseases in aquaculture. In fact, aquaculture is an efficient ecosystem management activity for producing more aquatic animals and plants. On the other hand, disease outbreak is a sign of imbalance in the aquaculture ecosystem. For example, in a particular area where intensive culture of shrimps was very successful at the beginning, a variety of serious diseases outbreaks often occur after successive culture cycles. I call it as "successive culturing obstacle (SCO)". SCO also happen in the culture of Tilapia.

Various factors resulting in occurrence of SCO in aquaculture

1. Threats of multiple diseases and cross-infection. An area that is used for aquaculture operations for long period will result to more serious diseases caused by various pathogens, and this is one of main reasons for SCO. Multiple pathogens may come from other cultured species. With longer aquaculture operations, multiple pathogens can infect one culture species, which can trigger the occurrence of SCO.
2. Problems of pathogens causing latent infection. For aquaculture animal, most viruses and bacteria can cause latent infection state, and can become an acute infection if triggered by environmental factors stress. Fry infected by pathogen has become a serious problem in aquaculture, as they can transmit the pathogen into the farm. Under stressful environmental factors, the infection can easily turn from latent to acute infection during culture.
3. Environmental eutrophication and climate change. Long-term aquaculture can result in eutrophication in ponds and farming area. More microalgae grow in eutrophic environment, and algal (including cyanobacterial) bloom often occurs, which are harmful for cultured animals. Calamities associated with climate change (typhoon, rainstorm, low or high temperatures) cause stress on farmed organisms, and result in aquaculture ecosystem change (algal die-off, ammonia increase, opportunistic pathogenic bacteria increase). These conditions can trigger viral and bacterial diseases outbreak resulting to mortalities among cultured organisms.

Prevention and control for disease of tilapia

1. Establish more fast, accurate and sensitive techniques for detecting tilapia diseases, especially for specific pathogen free (SPF) fry. This will eliminate one of the possible sources of infection.
2. Develop effective vaccines against the diseases.
3. Eliminate SOC by (1) reducing eutrophication in culture ponds and culture area, (2) reducing the stresses from physico-chemical factors and opportunistic pathogens, and (3) reducing the impact of climate change on aquaculture ecosystems.
4. One pathogen can infect multiple hosts, and to prevent the cross-infection, aquatic animals which can be infected by the same pathogen(s) should not be farmed in one area.

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Overview of Tilapia Lake Virus (TiLV)

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TiLV has recently been noted as an important infectious agent that may threaten the worldwide tilapia industry. According to the Food and Agriculture Organization of the United Nations (FAO), global production of tilapia is estimated at 6.4 million metric tons (MMT), with the top three producers in 2015 being the People's Republic of China (1.78 MMT), Indonesia (1.12 MMT) and Egypt (0.88 MMT) (FAO, 2017a). Bangladesh, Vietnam and the Philippines are other leading producers (FAO, 2017a). At the time of writing, TiLV has been reported to be present on three continents: Asia, Africa and South America.

With increased screening, the list of affected countries is likely to increase in the near future. Although the virus does not represent any direct risk to human health, its known distribution gives significant cause for concern regarding the potential impacts on livelihoods and food security. As a result, international organizations have released information to heighten awareness of the current situation. At time of writing, two countries (Israel and Chinese Taipei) have made an OIE notification of TiLV as an emerging disease to the OIE. A technical disease card for TiLV has been released by the OIE (OIE, 2017). In addition, a Network of Aquaculture Centres in Asia-Pacific (NACA) disease alert (NACA, 2017), a CGIAR Research Program on Fish Agri-food Systems (CGIAR, 2017) factsheet, an FAO Global Information and Early Warning System (GIEWs) special alert 388 (FAO, 2017b), as well as several website warnings, have been released. Together these publications highlight the urgent need for further knowledge regarding TiLV and its implications, as well as the importance of international collaboration.

The disease associated with TiLV infection is currently known under two different names, tilapia lake virus disease (TiLVD) as in the OIE technical disease card (OIE, 2017) and syncytical hepatitis of tilapia (SHT) as first referred to by Ferguson et al. (2014). It is caused by an enveloped, negative-sense, single-stranded RNA virus with 10 segments encoding 10 proteins (Eyngor et al. 2014; Bacharach et al. 2016; Surachetpong et al. 2017) and a diameter between 55 and 100 nm (Ferguson et al. 2014; Eyngor et al. 2014; del-Pozo et al. 2017; Surachetpong et al. 2017) and identified as Orthomyxo-like virus. Affected farmed species include hybrid tilapia (*Oreochromis niloticus* x *O. aureus* hybrids) in Israel (Eyngor et al. 2014); Nile tilapia (*O. niloticus*) in Egypt (Fathi et al. 2017), Ecuador (Ferguson et al. 2014) and Thailand (Dong et al. 2017a; Surachetpong et al. 2017); and red tilapia (*Oreochromis* sp.) in Thailand (Dong et al. 2017a; Surachetpong et al. 2017). Affected life stage is mostly the fingerlings, but fertilized eggs, yolk sac larvae, fries and fingerlings were found positive for the virus (Dong et al., 2017b).

Reported clinical signs include lethargy, ocular alterations, skin erosions and discoloration (darkening) in Israel (Eyngor et al. 2014) and exophthalmia, discoloration (darkening), abdominal distension, scale protrusion and gill pallor in Ecuador (Ferguson et al. 2014). In Thailand, loss of appetite, lethargy, abnormal behavior (e.g. swimming at the surface), pallor, anemia, exophthalmia, abdominal swelling, and skin congestion and erosion have been reported (Dong et al. 2017a; Surachetpong et al. 2017). In Egyptian farms experiencing summer mortality, affected fish showed haemorrhagic patches, detached scales, open wounds, dark discoloration and fin rot (Nicholson et al. 2017). Mortality levels of above 80% have been observed in affected farmed populations in Israel, while no such level of mass mortality has been reported in wild stocks from which positive samples have been obtained (Eyngor et al. 2014). In Thailand, mortality levels between 20% and 90% have been reported, with mortality usually seen within the first month after transfer to grow-out cages (Dong et al. 2017a; Surachetpong et al. 2017) and peak mortality rates observed within two weeks of onset of mortality (Surachetpong et al. 2017). Similarly, the case in Ecuador showed onset of mortality from four to seven days post transfer to on-growing ponds, with mortality ranging from a low level of 10–20% to a high level of 80%, depending on the fish strain (Ferguson et al. 2014).

For disease diagnosis, gross pathology, histopathology, cell culture (using E-11 cell lines) and molecular diagnostics (RT-PCR, semi-nested RT-PCR and real-time PCR) have been used in the different investigations and confirmation of the disease. With the on-going investigations and surveillance of the disease among tilapia-producing countries, close international collaboration to improve the collective knowledge and understanding of TiLV and its consequences is urgently needed. Specifically, understanding should include the needs for: knowledge on the real geographical distribution of TiLV; instigating screening and surveillance programs in many of the tilapia producing countries should be actively encouraged; socio-economic impact assessments should be encouraged in order to quantify the current or expected impact of disease as a result of infection with TiLV; epidemiological aspects of TiLV; and, regional capacity building within all stakeholder groups.

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Update on TiLV Research in Thailand and Possible Strategies for Control

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Tilapia lake virus disease (TiLVD) is a newly emerging disease caused by tilapia lake virus (TiLV), a novel segmented RNA virus resembling *Orthomyxovirus* (*Orthomyxo*-like) (Eyngor et al. 2014; OIE 2017). The disease is also known as syncytial hepatitis of tilapia (SHT), summer mortality syndrome (SMS) or tilapia one month mortality syndrome (TOMMS) (Ferguson et al. 2014; Fathi et al. 2017; Surachetpong 2017a; Tattiyapong et al. 2017). Although TiLV became known to science in 2014, the virus was suspected to be responsible for massive mortalities of tilapia in Israel and Ecuador since 2008-2009 (Eyngor et al. 2014; Bacharach et al. 2016; FAO 2017). Occurrences of TiLV in the Colombia (Ecuador's neighbor), Egypt (Israel's neighbor) and recently in Thailand, Chinese Taipei and India suggest serious transboundary spread of the emerging virus in tilapia aquaculture industry at global level (FAO 2017; OIE 2017; CGIAR 2017; NACA 2017).

In Thailand, unexplained mortalities of tilapia fry, fingerlings and juveniles have been observed for years. In April 2017, natural infections of TiLV associated with 20-90% mortalities in farmed tilapia have been confirmed by two independent research groups (Surachetpong et al. 2017b; Dong et al. 2017a). Histopathological features, viral morphology and its genetic characterization as well as molecular detection methods (PCR and *in situ* hybridization-ISH) were also described. After confirmed the presence of TiLV in the country, we immediately reported the case to the national competent authority. Subsequent study from Tattiyapong et al. (2017) using experimental infection was demonstrated to fulfill Koch's postulates. Relatively high sensitive PCR detection protocols developed based on initial primers published by Eyngor et al. (2014) have been available for disease investigation (Kembou Tsofack et al. 2017; Dong et al. 2017a,b). A warning of TiLV in the region together with our detailed detection protocol was also publicly accessible from the website of the Network of Aquaculture Centres in Asia-Pacific (NACA). To assist tilapia culturing countries to initiate surveillance program for the emerging virus, up to date, we have provided free positive control plasmid to 29 requests from 19 countries. We hope that wider surveillance for TiLV in the tilapia industry will help reduce the impact and spread of the disease. Moreover, an investigation using archived samples collected from unexplained mortalities of tilapia fertilized eggs, fry and fingerlings during 2012-2017 unexpectedly revealed that majority of samples have tested positive for TiLV, indicating the presence of TiLV in Thailand even before the virus became known to science in 2014 (Dong et al. 2017c). Unbeknownst to us, SHT histopathological feature resembling TiLV infection was previously described in an MSc. student thesis of Chulalongkorn University, Thailand (Weerapornprasit et al. 2014), possibly representing an earliest known case of SHT in Thailand. It is of concern that movement of live tilapia fry and fingerlings for aquaculture during that period without knowing the existence of TiLV may have contributed to unexpected widespread of the pathogen (Dong et al. 2017c,d).

Since TiLV is a newly discovered virus, current understanding of the pathogen and its impact on tilapia aquaculture remains unclear despite the fact that active surveillance has been performing by competent authorities and research organization. Most recently, we experienced two cases of inapparent infection of TiLV in adult and fingerling tilapia without abnormal mortality. Similar cases were notified to our lab from an unofficially reported country. In addition, attempts of challenge assays using viral preparation obtained from heavy infected tissues originated from three different sources failed to produce disease or mortality in experimental fish. Further analysis of samples from a single farm experienced high mortality (~90%) revealed presence of several pathogens, not just TiLV, and novel histopathological features which were previously overlooked. Thus, further studies to uncover non-TiLV pathogens, genetic diversity of TiLV and their pathogenicity as well as synergistic effects among them are urgently needed. If virulence depends on specific viral strains, molecular detection for differentiation of high- and low pathogenic types should be a priority that requires international collaborations. In this presentation, possible strategies for control of TiLV in tilapia including transparency for the status of TiLV, specific pathogen free (SPF) program, affordable vaccines and antiviral therapies will be discussed.

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Virus characterization, Clinical presentation, and Pathology of TiLV

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Tilapia Lake Virus (TiLV) is an emerging viral disease affecting both wild and farm-raised tilapia . The virus has been first reported in Israel in 2014 [1] and subsequent reports of TiLV infection in many parts of the world [2, 5]. In Thailand, high mortality of Nile and red hybrid tilapia due to an unknown etiology was examined since 2015 . The disease has been called "Tilapia One-Month Mortality Syndrome" (TOMMS). The clinical representations of infected fish included skin erosion, skin redness, exophthalmos, abdominal distension, scale protrusion, pale body color (red tilapia), multiple hemorrhages on the skin and fin base, pale and liver contraction . From 2015 to 2016, the investigation of 32 outbreaks of TOMMS confirmed that 22 outbreaks were PCR positive to the novel orthomyxo-like virus, TiLV [5]. The virus was isolated and propagated in the permissive E-11 cells . The infected cells showed round shaped, aggregate cells, cell shrinkage and cell detachment suggesting cytopathic effect (CPE) within 3 to 5 days post-inoculation . Electron micrographs of infected cell culture and fish tissues revealed round enveloped virions (60-80 nm) with characteristics very similar to those of *Orthomyxoviridae* [6]. Whole genome sequencing of ten segments of Thai TiLV showed high sequence identity to TiLV isolated from Israel (95-100%). Notably, multiple alignments of segment 1 of TiLV indicated that TiLV isolated in Thailand are grouped to other viruses in the family *Orthomyxoviridae* .

To demonstrate that TiLV isolated from moribund tilapia cause disease and mortality in susceptible fish, we intraperitoneally injected red hybrid tilapia and Nile tilapia with TiLV (KU-TV01) isolated from diseased fish in Thailand. Our results fulfilled Koch's Postulates concept that TiLV could cause disease in susceptible fish, the re-isolation of TiLV from challenged fish in E-11 cells, and confirmation of TiLV using RT-PCR and electron micrographs . Additionally, the histopathological findings of infected fish revealed inflammatory cells infiltration in the brain and massive degeneration of liver, syncytial hepatic cell formation and eosinophilic intracytoplasmic inclusion body in infected hepatocytes . The highly sensitive and accurate reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) protocol has been developed for the detection of TiLV in field and experimental challenged fish [7]. The virus has been detected in many organs of infected fish including gills, spleen, anterior kidney, liver, brain, and heart using the newly developed RT-qPCR .As the qPCR assay allows quantitative and qualitative measure of TiLV, the protocol could be applied as a standard method for epidemiological survey or other experimental study in the future . Taken together, reports of TiLV in different continents emphasize the important of this emerging viral disease. Additional studies to gain better understanding of the TiLV will limit the impact of TiLV on worldwide tilapia aquaculture **.**

Figure 1. Red hybrid tilapia (*Oreochromis* spp.) shows clinical signs of TiLV infection including skin redness, skin erosion, multiple hemorrhages on the skin. The clinical signs of TiLV infection in Nile tilapia (*Oreochromis niloticus*) include opacity of the eyes, and skin erosions.

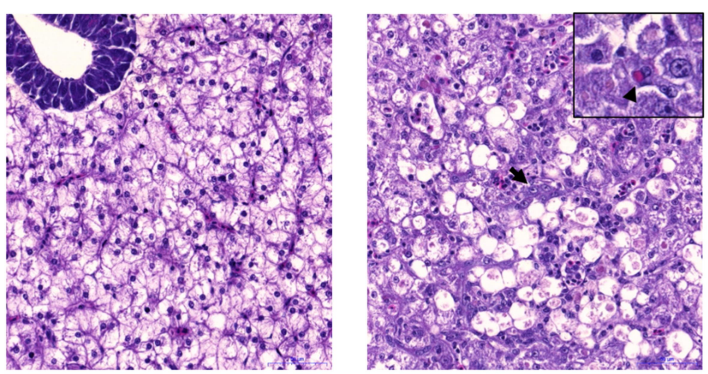

Figure 2. Histopathological findings of infected red hybrid tilapia (*Oreochromis* spp.). Left; normal liver, hepatocytes, the exocrine pancreas surrounds the hepatic portal vein. Right; infected liver, multiple necrosis and degeneration of hepatocytes. The presence of syncytial hepatic cells in the liver (arrow) and eosinophilic intracytoplasmic inclusion body in hepatocytes (inlet, arrow head). H&E stain.

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The Role of Trade in the Spread of Transboundary Aquatic Animal Diseases

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The Asia-Pacific region produces around 90% of the world aquaculture production, with seven countries among the top 10 producers in the world (China, India, Vietnam, Indonesia, Bangladesh, Thailand and Myanmar; Table 1). In general, aquaculture in the region is a highly significant food production sector that provides many livelihood opportunities, and contributes to food security, nutrition and poverty alleviation. Being the major aquaculture producers, Asia-Pacific is also the major consumers of seafoods accounting to 70% of global consumption. Despite the significant developments in the aquaculture sector of the region, the industry faces many challenges and problems that affect sustainable production. Disease outbreaks, specifically transboundary aquatic animal diseases, are one of the serious causes of production losses in many aquaculture operations around the region. Most are mainly a result of transboundary movement (trade) of live aquatic animals, which is a necessity for economic, social and aquaculture development purposes. Both live and frozen aquaculture products are widely moved/traded across boundaries that may result in the spread of aquatic animal diseases (Fig. 1). The risk os spread is also higher in live aquatic animal movements than the frozen or processed aquatic animal products.

Table 1. Top aquaculture producers in the world (FAO, 2014)

Rank	Country	Production (T)	Percent
1	China	41,108,306	61.69
2	India	4,209,415	6.32
3	Vietnam	3,085,500	4.63
4	Indonesia	3,067,660	4.60
5	Bangladesh	1,726,066	2.59
6	Norway	1,321,119	1.98
7	Thailand	1,233,877	1.85
8	Chile	1,071,421	1.61
9	Egypt	1,017,738	1.53
10	Myanmar	885,169	1.33

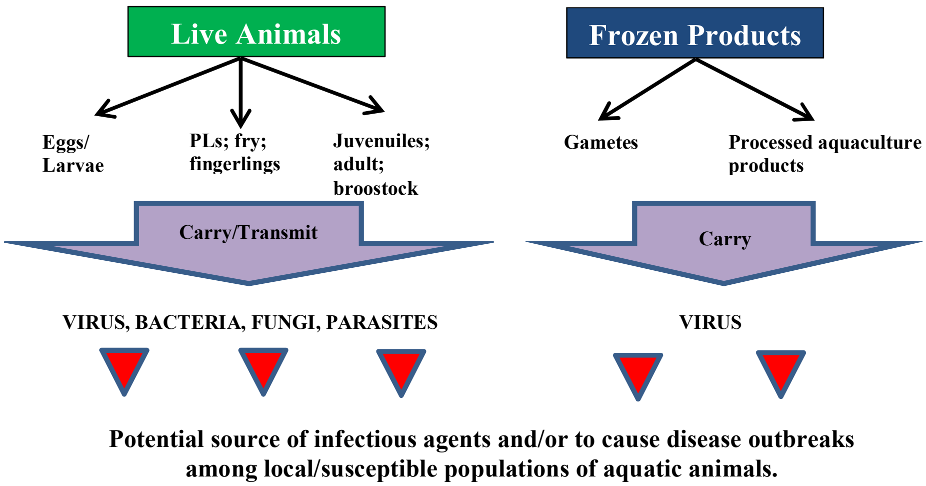

Figure 1. Potential pathway of pathogen spread through movement of live aquatic animals and aquatic animal products.

In the current state of aquaculture development, more disease outbreaks and disease emergencies will continuously occur as culture systems are intensified. The continuous diversification of cultured species will also increase the risk of pathogen introduction and spread, while diversification of farming systems will result in the potential spread of pathogens to a wider geographical area. The economic and social impacts of aquatic animal diseases are enormous, causing direct lossen of around US$6 billion annualy.

Aquatic Animal Health Management and Transboundary Movement of Live Aquatic Animals and Aquatic Animal Products

For transboundary movement of live aquatic animals and aquatic animal products, aqutic animal health management (AAH) should encompass the following (FAO-NACA, 2000):

- Pre-border (exporter), border and post-border (imprter) activities;
- Relevant national and regional capacity building requirements;
- Development and implementation of effective national and regional policy frameworks.

AAH management measures should be practical, cost-effective and easy to implement, and shall be based on assessment of the risk to animal, plant and human life or health. Risk assessment shall include the prevalence of specific pathogens in both regions of origin and destination, and the likelihood of new or emerging pathogens becoming established in the region of destination. Therefore, all movements should be within the provisions given in existing international agreements and instruments. Consequently, the risks associated with emerging and transboundary diseases are shared – shared water bodies and epidemiological links through trade (especially live movement) – thus, collaborative approach (in dealing with these diseases) is therefore warranted and necessary.

Transboundary Aquatic Animal Diseases and TiLV

Several transboundary aquatic animal diseases have swept the region over the past 25 years causing massive economic and social losses. These include spread and outbreaks of Infection with *Aphanomyces invadans* (EUS) in freshwater fish, viral nervous necrosis (VNN) in marine fish, viral haemorrhagic septicaemia (VHS) in marine and freshwater fish, and several viral diseases in shrimps (white spot disease [WSD], white tail disease [WTD], yellow head disease [YHD]) (Rogers et al., 2011). Recently, acute hepatopancreatic necrosis disease (AHPND) are seriously affecting shrimp aquaculture in China, Malaysia, Philippines, Thailand and Viet Nam (Flegel, 2012; Leaño and Mohan, 2012; Dabu et al., 2015). Moreover, a new disease (Viral covert mortality disease; CMVD) has been reported to be widespread in China (Zhang et al., 2014). For AHPND in particular, the spread was attributed to trading of live shrimp postlarvae and live polychaetes used as natural food for shrimp broodstocks (NACA, 2016). Thus, the spread of these transboundary aquatic animal diseases clearly demonstrates the vulnerability of the aquaculture industry, as well as the wild fish populations, to disease emergence where impacts have been aggravated by the lack of effective preparedness and response when diseases emerge.

Since 2009, tilapia aquaculture has been threatened by mass die-offs of farmed fish in Israel and Ecuador (Bacharach et al., 2016). The aetiological agent causing this mass die-offs has been described and identified as a novel Orthomyxo-like (RNA) virus named as Tilapia lake virus (TiLV) (Eyngor et al. 2014; 2016; Bacharach et al., 2016). This has been reported as a newly emerging virus that causes syncytial hepatitis of tilapia (SHT). As of this writing, countries affected by this emerging disease of tilapia include Israel, Ecuador, Colombia, Thailand and Chinese Taipei (Eyngor et al., 2014; Bacharach et al., 2016; Fathi et al., 2017, Dong et al. 2017, Suratchepong et al., 2017; OIE, 2017). As tilapia is one of the highly-traded commodities both in live (fry, fingerlings, broodstock) and processed (frozen fillets) forms, there is a very high concern on the possibility of the virus to become widespread among many tilapia-producing countries in the world.

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Import Risk Assessment: Role in Prevention of Transboundary Aquatic Animal Diseases

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With the expansion of global aquaculture, the trade of live aquatic animals and their products has significantly increase. This has inevitably led to the introduction and spread of live animals and pathogens. Import risk analysis (IRA) is of great benefit to preventing the spread of the aquatic animal diseases and the disruption of aquatic environmental. It is also easier to prevent the introduction of new pathogens than to eradicate them after introduction. IRA also provides references for trading of national aquatic products.

In this presentation, the import trade of aquatic animals in China from 2011 to 2014 was introduced. A dominant increase can be seen not only for the list of exporting countries but also on the amounts and values of imported live aquatic animals: 51.8, 63.3, 92.2, 97.8 thousand tons (in 2011, 2012, 2013 and 2014, respectively) with respective values of 348, 618, 1113, 1269 million US dollars. The commodities of live aquatic animals include broodstock, eyed-eggs, seeds, ornamental aquatic animals, and live aquatic animals for human consumption. It is predicted that 7 million tons of aquatic animals and products will be imported in China in 2020, which corresponds to 10% of the total aquaculture products in the country. South-east Asia, America and Canada, Europe, and Australia and New Zealand became the main export regions, occupying 66%, 23%, 5%, 5% and 1% respectively. The imported live aquatic animals pass through 60 checkpoints in 21 CIQs located in 18 provinces/ municipalities/autonomous regions.

Since 1990, some aquatic animal diseases have became serious threat to the aquaculture of China including WSSV, IPNV and IHNV (1990), TSV and VNNV (2000), IHHNV and KHV (2005), GFHNV, anguillad herpesvirus, AHPND-VP (2010), and carp edema virus (2015). Eighteen (18) out of 29 diseases listed in OIE Aquatic Code are still considered exotic to China. With the big amount of international trade of live aquatic animals, the risk assessment on imported live aquatic animals becomes very urgent and very important.

Based on OIE Aquatic Code, the main contents and components of Import Risk Assessment (IRA) are hazard identification, risk assessment, risk management and risk communication. For the international trade of aquatic animals and products, hazard means diseases or infection of some pathogens. The OIE-listed diseases should be considered a priority. Qualitative risk assessment is widely used in the risk analysis of imported aquatic animals and products. It is flexible and can incorporate diverse sources and types of information or data, especially in the new research field. However, this method is often influenced by group cognitive bias. Risk assessment should be flexible in order to deal with the complexity of real-life situations. No single method is applicable in all cases. Risk assessment should be able to accommodate the variety of aquatic animal commodities, the multiple hazards that may be identified with an importation, the specificity of each disease, detection and surveillance systems, exposure scenarios and types and amounts of data and information.

The steps included in IRA are entry assessment, exposure assessment, consequence assessment and risk estimation. It is very necessary and important to collect information (including properties of the agents, genotype of host, commodity status, quantity of commodity to be imported, ease of contamination, effect of the various processing methods on the pathogenic agents, effect of storage and transport on the pathogenic agents), as these are the raw materials for risk assessment. Risk estimation consists of integrating the results of the entry, exposure, and consequence assessments to produce overall measures of risks associated with the hazards identified at the outset. Thus risk estimation takes into account the whole of the risk pathway from hazard identified to unwanted outcome.

Some cases of import risk assessment in some member countries of OIE and WTO were also presented. In 1990s, a trade dispute over fresh salmon arose between Canada and Australia. That was a famous dispute based on risk assessment, which ended after WTO ordered Australia to lift its ban and increase quarantine requirements not only for salmon, but for imports of other species of fish as well. Australia carried out the risk assessment on CyHV2 in ornamental fish and implemented the quarantine policy to revoke veterinary certification for imported goldfish to be free of CyHV2. Australia also assessed the risk of Ranavirus and Infectious spleen kidney necrosis virus carried by ornamental fish, based on which, a risk management policy on Ranavirus was required on imported ornamental fish. For shrimps, products imported with the risk of carrying WSSV and YHV-1 was assessed and the new inspection and quarantine measures were put into effect since July, 2017 after the ban on imported shrimp products for half a year. Japan updated the regulations for imported aquatic animal based on import risk assessment, adding new diseases in the list and prolonging the isolation period.

Since Tilapia Lake Virus is an emerging disease and will become a very serious threat to tilapia culture and international trade, implementation of import risk assessment is very urgent. However, information especially scientific evidence are not enough for entry assessment, exposure assessment, consequence assessment and risk estimation. As such, the following are recommended:

1. Collaboration on validation of diagnostic tests for TiLV, not only cell lines but also nucleic acid test;
2. Collaboration on the study on the virus abundance in various tissues in tilapia;
3. Study on the virus subduction mode in tilapia fillet under different storage conditions;

4. Study on the phylogenetic analyis on TiLV strains isolated from Asia;
5. Study on the potential carrier and susceptible hosts of TiLV in ornamental Cichlidae;
6. Member countries in the region start the investigation projects on TiLV.

Biosecurity System for Aquatic Animal Health

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Aquatic animal diseases not only result in losses in production due to mortality and inhibited growth, but also bring about other consequences that have negative effects to the industry and the general public. These include public health risks of antimicrobial resistance and possible zoonosis, risks to product safety due to drug residue, risks to trades due to transboundary spread of diseases and degrading of product quality, wild ecological risks due to spreadof pathogen into wild and pathogen mutation, and environment risks due to drug residue in environment. It also cause risks to sustainability of the industrial development by increasing prevention costs, negative impacts to the industry, and investment depletion. For prevention and control of diseases, biosecurity concept was introduced in aquatic animal health. Comparing with the concepts of disease control and health management, the concept has a risk based ideology with a final goal to reach disease-free status through the practical pathway of biosecurity plan and SOPs. It highly emphasizes the integration of different technologies such as disease surveillance, environmental and ecological managements, immunological control, microbiological control, as well as good aquaculture practice and decelopment of disease resistant strains.

Country and zone level biosecurity plan is highly related to the aquatic animal health services. We suggested farm level biosecurity plan can be evaluated to five biosecurity grades based on implementation of biosecurity measures, which include:

- BSG1 (Biosecurity grade 1): diagnosis based treatment;
- BSG2: surveillance based prevention;
- BSG3: risk analysis based control;
- BSG4: systemic disease freedom;
- BSG5: official certificated compartment.

Each biosecurity grade is a standalone system and can be upgraded to the next grade, which is distinct from Palić's biosecurity steps to reach a disease free compartment (BSG5). Farms can consider to achieve whichever biosecurity grade they desire based on their background, conditions, management, capability, and investment. Biosecurity plans for breeding centers, hatcheries or nursery farms is in developing based on practices in the collaboration of some hatcheries. Twelve principle measures for biosecurity plan in shrimp farms were recommended, which can also be applied to other cultured aquatic animal species:

- Build a farm with well layout for prevention of risks;
- Prepare the farming with efforts on biosecurity plan;
- Recondition ponds by well dredging and disinfection;
- Flood ponds and reservoirs for early disinfection and fertilization;
- Select postlarva by pathogen detection to avoid infection;
- Use nursery culture for stocking and quarantine;
- Determine stocking density according to stages and health conditions;
- Use polyculture with fish for ecological prevention;
- Feed Precisely with little feed and many meals;
- Monitoring and control water and bottom quality regularly;
- Examine survival and growth, treat according to diagnosis;
- Use probiotics dominated biofloc.

Acknowlegements

This work was supported by the projects of China ASEAN Maritime Cooperation Fund Project (2016-2018), the Program of Introducing International Advanced Agricultural Science and Technology (948 Program, 2016-X56), the Aoshan Sci. & Tech. Innovation Program of Qingdao National Laboratory for Marine Science and Technology (2015ASKJ02), the China Agriculture Research System (CARS-47).

COUNTRY PRESENTATIONS

Health Management of Aquatic Animals in China

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China has a big aquaculture industry, with various cultured species and multiple aquaculture systems, that health management of aquatic animals is a challenging task. The Chinese government values the healthy and sustainable development of aquaculture industry, and has laid out the development goals of fishery with Chinese characteristics, which are to improve quality and efficiency, to reduce amount and increase profits, to pursue green development and to make fishermen better off. The biosecurity of aquatic animals, quality safety and ecological safety have increasingly drawn the attention of relevant government departments. At present, things have been improved greatly in terms of aquatic animal epidemic prevention and management mechanism, aquatic animal epidemic prevention system and team building. The achievements on Health Management of Aquatic Animals in China are summarized as follows:

- <u>Aquaculture industry in China.</u> In 2016, a total of 69 million tons of aquatic products were produced in China. Among which, 51 million tons were from the aquaculture industry accounting for 74.5%. These were produced from 8.35 million hectares of aquaculture area. The ratio of mariculture area to freshwater aquaculture area was around 1 to 2.8. Pond culture is the most popular system which produce around 49% of the total aquatic products. Fish, crustaceans and shellfish are the three major groups of cultured species. Fish species include black carp, grass carp, silver carp, bighead carp, crucian carp, bream, tilapia, yellow croaker, flatfish and trout. Crustaceans include white shrimp, freshwater prawn, crayfish and hairy crab, while shellfish include oysters and scallops.
- <u>Health management of Aquatic Animals in China.</u> Jurisdictional responsibility for animal health services is under the Ministry of Agriculture (MOA) through the Bureau of Veterinary (BOV) and the Bureau of Fisheries (BOF). The national aquatic animal health management is being implemented by the BOF, composed 1) Disease prevention and control system, 2) Research institutions, 3) Fishery industrial technology system, 4) Higher education and 5) Technical committees. Under the disease prevention and control system, National Fisheries Technology Extension Center (NFTEC) is in charge of the state-level management, and is responsible for national aquatic animal disease monitoring, surveillance, forecast and prevention. In the fishery industrial technology system, there are 6 groups according to cultured species: Conventional Freshwater Fish; Characteristic Freshwater Fish; Crustaceans; Shellfish; Marine Fish; and, Algae. Each industrial technology system contains institutes and specialists for related disease research which play important supporting roles in both research and prevention of aquatic diseases.

Veterinary Practitioners and Village Veterinarians are key components. Statistics in 2016 suggested that there were 3644 Veterinary Practitioners and 16183 Village Veterinarians. Official Veterinary Fishery System is under establishing.

Progress and Achievements

- Monitoring and Early Warning. Since 2000, national monitoring and forecast on aquatic plant and animal diseases have been carried out. At present, the national surveillance system spanning five jurisdictional levels "State - Province - City - County - monitoring points" have been established since 2005. There are more than 4000 monitoring points, 8000 people involved in monitoring and reporting work and more than 300,000 hectares of aquaculture area being monitored. Data collected are updated yearly and since 2013, the Aquatic Animal Health in China and Analysis on Epidemiology of Major Aquatic Animal Diseases in China have been published yearly, which is based on the data collected from National Surveillance Plan.
- Personnel capacity and capability. Capability test on Epidemic Prevention System for aquatic animals was organized to improve the diagnostic capacity of laboratories. Training courses on Official Veterinarian and Aquatic Offspring Quarantine is also held on a regular basis.
- Standardization of aquatic animal health. A working group on standardization of aquatic animal health has been established in 2000. As of 2016, there are 125 standards established or updated, of which 13 national standards and 69 industry standards have been published and implemented.
- Remote diagnostic network. In 2012, the remote diagnostic network has been established, which takes advantage of computer technology and aquatic animal disease diganostic techniques to provide real-time online technological information services on aquatic animal disease prevention and control. This is supported by 18 experts at national platform and 184 experts at provincial platform. Presently, this network has more than 600,000 views and more than 6,000 consultations in total, and has become a popular platform for public service.
- Establishment of specific disease free compartment. To control the spread of diseases and clear the potential of pathogen proliferation, the establishment of specific disease free compartments were carried out in Beijing, Tianjin, Hebei, Zhejiang and Qinghai since 2014. A series of good management measures and institutions have been summarized by the try-out trials and a code for constructions of specified epidemic disease free hatchery was drafted. To strengthen international cooperation, China actively participates in international affairs, and pays high attention to join and support works with OIE, FAO and NACA.
- National Surveillance of TiLV. Tilapia culture is mainly in Guangdong, Guangxi, Hainan and Fujian provinces of China. In 2016, there were around 1,870,000 tons of tilapia produced in China, which accounted to 6.3% of the total aquaculture production. The trade exports of tilapia was around 390,000 tons with an output value around US$1.2 billion, which represents 8% of the total export of aquatic products.

China quickly responds to outbreaks of new emerging disease. For TiLV in Asia, as example, the disease advisory issued by NACA prompted China to take immediate actions at national level. A series of actions were immediately implemented, including notification on prevention and control of TiLV in major tilapia-culture areas, national monitoring of TiLV, and epidemiological investigation. As of September 2017, there was no TiLV reported in China.

Fish Health Management in Egyptian Aquaculture

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Aquaculture is currently the largest single source of fish supply in Egypt, accounting for almost 65% of the total fish production of the country with over 99% produced from privately owned farms. The development of aquaculture, particularly tilapia farming, has put Egypt in number one position in Africa in this sector (FAO). With the rapid growth and success of aquaculture sector, challenges have also increased especially with competition for water over the last five years. Fish diseases are among the major challenges that hinder the progress of aquaculture and usually difficult to treat or control. Thus, there is an urgent need to clearly understand the causative agents and risk factors for unusual mortalities in order to develop risk reduction interventions in the form of better management practices.

In recent years, Egyptian fish farms have faced unexplained mortalities in tilapia during the summer months. In 2015, epidemiological surveys indicated that 37% of fish farms were affected with an average mortality rate of 9.2% which have negative economic impact as farmed tilapia represents more than 70% of the total aquaculture production in the country. Tilapia mortalities were reported up to September 2017. The disease, described by farmers as "red patch disease" (Fig. 1), results to severely distended gall bladder, liver necrosis and congested kidney of the infected fish during post-mortem examination. Several bacterial isolates were identified, however, potential causes for such losses are not clearly known.

Tilapia Lake Virus (TiLV) was reported to be a global threat to the tilapia farming industry. Therefore, Egyptian authorities have investigated TiLV as a suspected cause for the reported tilapia mortalities. In August 2017, Egyptian authority has confirmed that Egypt is TiLV free.

Fig. 1. Fish showing hemorrhagic red patches on the abdomen (left) and tail (right).

With the continuity of tilapia losses, WorldFish Research and Training Center in Egypt will work closely with national institutions to run an epidemiological study to assess risk factors associated with the emergence of summer mortality in tilapia in order to have better insight to the disease control strategies. Regarding the prevention of TiLV, the use of vaccines seems to be an impractical option as RNA genome viruses (e.g. Orthomyxoviruses) possess a high mutation and recombination rate giving rise to new strains. Therefore, the new viral strains continually escape the immune response produced by the vaccine. For this reason new solutions for prevention are being looked into.

Globally, no aquaculture system is free from the risk of diseases. Biosecurity is emerging as one of the most indispensable topics in disease prevention and control. Therefore, Abbassa "WorldFish Research and Training Center" has implemented strict biosecurity plan including periodical testing of tilapia seeds for TiLV before disseminating them to broodstock multiplication centers. So far, all tested samples showed negative results for TiLV. Biosecurity measures initiated for our works will be extended to fish farmers in the near future.

Reuse of agricultural water for fish culture and water flow system from one pond to another have led to significant losses in some fish farms. Recently, WorldFish has tested an Intensive Pond Aquaculture (IPA) technology that requires no water exchange during the entire production season. In this system, the risk of pathogen spread from affected pond will be reduced. Early stocking and harvest of fish have been proposed as precautionary measure to avoid summer associated mortalities, however, winter associated aquatic animal diseases should also be considered.

Tilapia Health Management in India with Focus on Status of and National Action Plan on Tilapia Lake Virus

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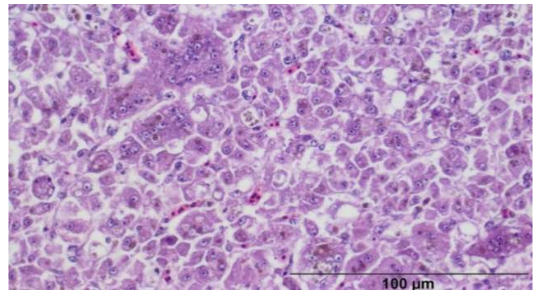
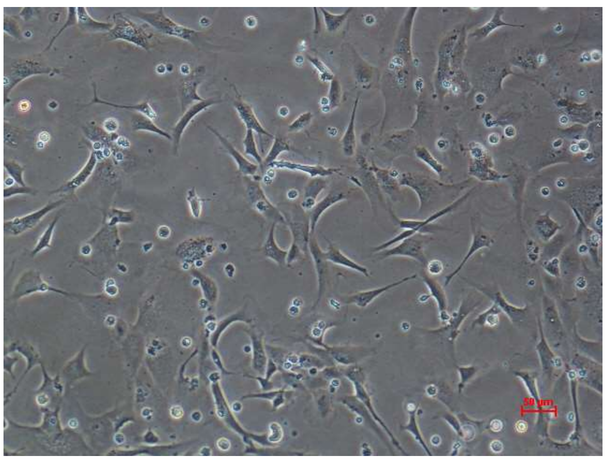
Indian major carps, catla, rohu and mrigal contribute about 60% of the total aquaculture production in the country. However, in the recent years, under the scope of diversification, process is on to bring newer indigenous and some fast growing exotic species into the ambit of culture system in order to improve the fish yield as well as the farm income. Among the fast growing potential exotic fish species, tilapia are considered to be one of the most important fish species to meet the growing demand for protein. Hence, Government of India is promoting culture of genetically improved farmed tilapia, *Oreochromis niloticus* (through all-male seed production) in the country. Importantly, tilapia are considered to be relatively resistant to a number of diseases encountered in other farmed fishes. However, over the last few years, large-scale mortalities have been reported in tilapia due to infection with Orthomyxo-like virus i.e. Tilapia Lake Virus (TiLV) which is considered as a threat to global tilapia industry.

In the present study, we report outbreak of TiLV disease in farmed tilapia in two states, West Bengal and Kerala from India. Diseased fish exhibited lethargy, inappetance and skin erosions with >85% mortality. TiLV infection was confirmed on the basis of amplification and sequencing of segment 3 of TiLV, histopathology, infection of fish cell line and bioassay. Phylogenetic analysis of the partial sequences of segment 3 of the TiLV revealed that North 24 Parganas (MF502419) and South 24 Parganas (MF582636) of West Bengal showed maximum similarity (97.2%) with Israel strain (KJ605629.1), whereas TiLV from Ernakulam, Kerala, India (MF574205) showed 96.4% similarity with the Israel strain. In histopathology, typical syncytial giant cells in liver and congestion of the blood vessels along with haemorrhages in sections of brain tissue of the affected tilapia were observed. The filtered tissue homogenate prepared from liver and brain of affected tilapia produced cytopathic effects in CFF cell line derived from *Pristolepis fasciatus*. The disease was successfully reproduced in naive tilapia following injection of culture supernatant from infected cell line and TiLV was successfully reisolated from experimentally infected tilapia.

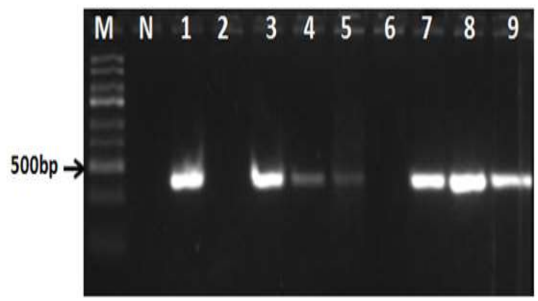
Gross sign of TiLV-infected tilapia.



Photomicrograph of naturally infected tilapia showing syncytial giant cells in liver.

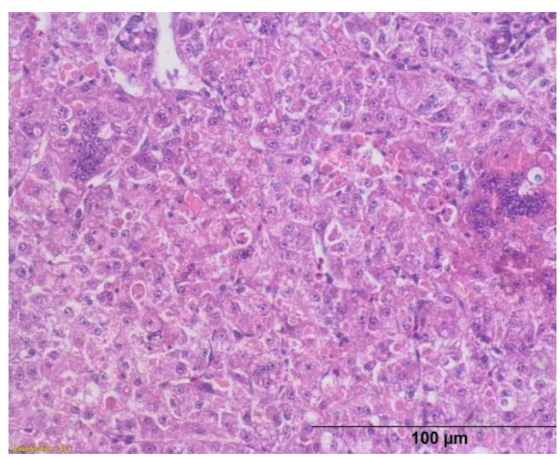


Elongation along with detachment of cells in CFF cell line (derived from *Pristolepis fasciatus*) at 6 days post infection following inoculation of filtered tissue homogenate of TiLV infected fish.



Detection of Tilapia Lake Virus in Tilapia samples by RT PCR.

Bilateral exophthalmia and scale protrusion in experimentally infected tilapia.



Syncytial giant cells in liver of experimentally infected tilapia.

This is the first report of TiLV from India and adds to the reports of TiLV outbreaks in five countries across three continents.

The work was carried out under the "National Surveillance programme for Aquatic Animal Diseases (NSPAAD)". As this forms first report of an emerging pathogen from the country, the presence of TiLV was cross-validated in two other collaborating centres of NSPAAD. After confirmation, a detailed report was submitted to the Competent Authority (CA) of the country i.e. Department of Animal Husbandry, Dairying and Fisheries (DADF); Ministry of Agriculture and Farmers Welfare, Government of India, and following actions plans have been suggested for management of TiLV in the country;

- All the state Fisheries Departments (SFD) and registered tilapia hatcheries/farms have been sent disease advisories prepared by NACA, World Fish, FAO and OIE.
- All the collaborating centers of NSPAAD have been asked to conduct awareness to educate the farmers about the clinical signs and symptoms of TiLV infection and share the contact details of the health experts with the stakeholders for intimating cases of suspected mortalities in tilapia.
- The tilapia hatcheries/farms have been asked to maintain record of daily mortalities. They have also been requested to report unusual mortalities to State Fisheries Departments/NSPAAD collaborating centre.
- Import of tilapia only should be permitted from TiLV free hatcheries/farms.
- The SFDs have been requested to intimate suspected cases of TiLV infection to the nearest collaborating centre of NSPAAD with intimation to the coordinating institute, i.e. ICAR-NBFGR.
- All the collaborating centers of NSPAAD should investigate suspected cases of TiLV disease using at least 2 diagnostic techniques including RT-PCR, sequencing of PCR products, histopathology and bioassay.
- In case of confirmation, a report must be sent immediately to the coordinating institute. Following confirmation of TiLV outbreak, the fish should be disposed of by incineration/buried and the farm must be disinfected.
- The hatchery owners should include screening of tilapia seed for TiLV as a routine test and sell only TiLV-free seed for culture.
- Under NSPAAD, it is proposed to carry-out a cross-sectional study to determine the distribution and prevalence of TiLV in the country.

Tilapia Health Management on TiLV in Indonesia: Status and National Action Plans

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Indonesia is rich in potential fisheries resources in freshwater, brackishwater, and marine. This potency is supported by wide aquaculture area and variety of fish species (Rukmana, 1997). B ased on Center of Information and Statistic Data Ministry of Marine Affairs and Fisheries on 2015, there are more than 5 billion freshwater aquaculture areas and 12 billion mariculture areas in 15 provinces in Indonesia, which are not at their optimum development yet (Darmanti and Nirmalanti, 2015). The wide area of freshwater can be used for freshwater aquaculture development such as for tilapia. Tilapia culture in Indonesia has high potential. The market was opened for consumption and hatchery operation. Tilapia is very popular among fish farmers because of its high selling price, easy to culture, high productivity, and it can be polycultured with other species (Khairuman and Amri, 2013). Tilapia is also resistant to disease and have high tolerance to below optimum environmental quality.

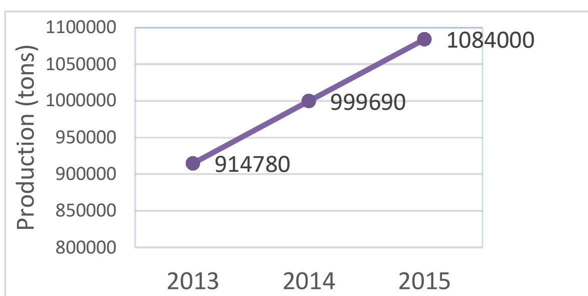

Figure 1. Tilapia Production in Indonesia from 2013 to 2015 (Source: Indonesian aquaculture statistic, 2016)

Tilapia is not endemic to Indonesia, it was introduced from Chinese Taipei in 1969 (Rusito, 2015). Most farmers in Indonesia are familiar with only two tilapia strains: red tilapia and black tilapia. They are cultured in ponds (freshwater and brackishwater), cages, floating net cages, and rice fields. The culture systems also vary including extensive and intensive, traditional and modern (Sonatha and Puspita, 2016). Tilapia became the most valuable commodity in Indonesia besides seaweeds and milkfish (Darmanti and Nirmalanti, 2015). In 2015, tilapia production reached 1,084 billion tons which is equivalent to US$1,594 billion. Indonesian tilapia production ranked third after China and Egypt (Fitzzimmons, 2016). In tilapia culture, Indonesian farmers face some obstacles such as production cost, overstocking, and mortality. The mortality in tilapia is not only related with aquaculture management problems, but also with the pathogens. The common diseases found in tilapia are Streptococcosis and Fracissellosis (Seafood watch, 2015), Aeromoniasis, and Iridovirus.

A new disease threat for tilapia, Tilapia Lake Virus (TiLV), has been reported in some countries such as Ecuador, Colombia, Israel, Egypt and Thailand. At the time of writing, Indonesia is still free from TiLV. The government is undertaking investigations in various tilapia-producing provinces through sample collection and laboratory analysis. Most of the samples were taken from mass mortality cases with or without clinical signs, and from different culture systems.

The spread of TiLV issue urged the government (Ministry of Marine Affairs and Fisheries Republic of Indonesia) to do follow up actions which involved Directorate General of Aquaculture (DGA), Fish Quarantine and Inspection Agency (FQIA), Marine and Fisheries Research and Development Institution, Fisheries Agency, Fish Service Integrated Post, Instructors, Stakeholder, Farmers, and Colleges/Universities. DGA as the authority for the formulation of aquaculture policies have released: 1) Letter of DGA no 3975/DJPB/VII/2017: Prevention and Monitoring of Tilapia Lake Virus (TiLV) in tilapias; 2) Prohibition of any importation of brood, broodstock, seed from TiLV infected countries; 3) Restriction on importation of brood, broodstock, seed from TiLV uninfected countries (the import permit, health certificate and laboratory tests must show TiLV-free); 4) Use of only TiLV-free seeds; 5) Supporting application of good aquaculture practices and good hatchery practices; 6) Limitation on inter-area transport from a location which has problem in tilapia culture; 7) Biosecurity and sanitary measures in hatchery and farm; 8) Active surveillance and monitoring from hatchery to grow-out; 9) Temporary restriction on restocking of tilapia in open inland waters (any restocking must be TiLV-free); 10) Laboratory testing in both entry and exit zones; 11) Maintainance of propeer stocking density and optimum water quality; 12) Increasing laboratory capacity on TiLV testing; 13) Increasing broodstock quality by genetic engineering to get best seeds and broods; 14) Public awareness about TiLV; 15) Increasing awareness and attention between fish farmers.

(a)

(b)

Figure 2. Seminar (a) and leaflet (b) as public awareness programme on the threat of TiLV in tilapia.

Currently, DGA is in progress to plan TiLV surveillance programs. This will involve 10 Technical Implementation Unit from DGA: Freshwater Aquaculture Development Center (FADC/BBAT) in Mandiangin, Tatelu, Jambi, Sukabumi; Station of Investigation for Fish Health and Environment (SIFHE/LP2IL) in Serang; Mariculture Development Center (MDC) in Lombok; Brackishwater Aquaculture Development Center (BADC/BBAP) in Situbondo and Jepara; Center of Shrimp and Oyter Broodstock Production (CSOBP/BPIUUK) in Karangasem; and, Center for Aquaculture Production Business Services (CAPBS/ BLUPPB) in Karawang. These Technical Implementation Unit will conduct the surveillance in 20 provinces. FQIA through Fish Quarantine Center has released letters in April 2017, which instruct the Technical Services Unit under FQIA to reject tilapia (live or frozen) importation from or transit in any country positive for TiLV, to increase control for tilapia importation from uninfected country, to monitor tilapia by histopathology and refer any positive result to Fish Quarantine and Inspection Examination Standard Laboratory (BUSKIPM).

As follow up from the DGA policies, FQIA have released decree no 303/KEP-BKIPM/2017 on avoidance of TiLV entry to Indonesia, with instructions to reject and eradicate tilapia (live, frozen, or its viable tissues) from countries with reports of TiLV, monitor TiLV in fish farms, open inland water, and importer farms which have imported tilapia from TiLV infected countries, detection of TiLV in tilapia which is transported to another region; to develop laboratory testing for TiLV; to coordinate actions (including publications on TiLV) with regional government and fish health units, DGA, and research institutions; to assess the social and economic impacts of TiLV.

The laboratories involved in TiLV testing in Indonesia always try to follow the recent method available for TiLV detection, i.e. semi-nested RT PCR develop by Dong et al. (2017) and supported by histopathological examination. Fish sampling by designated testing laboratories will be conducted together with Fisheries Agency and Fish Service Integrated Post.

As for emergency response and preparedness, Indonesian government established TiLV task force and prepared emergency preparedness document. TiLV task force will be led by DGA with FQIA, Marine and Fisheries Research and Development Institution; College/ University, Private Sector, and Association as members. For the fastest response, any indication of TiLV cases or outbreaks should be rimmediately eported to the nearest Fisheries Agency, Fish Health Integrated Service Post, Fish laboratory, and TiLV task force. The information about the disease must be disseminated to surrounding fish farmers followed by restriction of people from exposed area and prohibition from using water, equipment, and tools from infected area. Fish from affected area must be clinically examined and sampled for laboratory testing.

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Status of and National Action Plan for Prevention and Control of Tilapia Lake Virus in Malaysia

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Tilapia (*Oreochromis mossambicus*) is a non-native fish of Malaysia which was introduced in 1952 into earthen pond and ex-mining pool. Tilapia has a potential for primary and cheaper protein source and have higher consumer demands worldwide compared to other freshwater species. In 1980's, the small production was turned into commercial scale with the main species cultured being the hybrid red tilapia. The culture system was then extended to concrete tanks or floating net cages at the river and lakes throughout Malaysia. The major production sites are located at Temenggor Lake in Perak, Kenyir Lake in Terengganu, Batang Ai River and Bakun Lake in Sarawak. Besides red tilapia, Department of Fisheries (DOF) Malaysia, in collaboration with World Fish Centre in Penang, has developed the Genetically Improved Farmed Tilapia (GIFT) in early 2000's, through long term selective breeding and has been kept at the Freshwater Aquaculture Centre at Jitra, Kedah (Azhar et.al, 2014). This fast growing, hardier and more disease resistant strain has been disseminated to at least 16 countries including Myanmar, Timor Leste, China, Thailand and Vietnam, among others (source: Worldfish Website).

Currently, tilapia is listed as one of the Malaysian National Key Economic Area (NKEA) project, which promotes higher value and production. The current production of tilapia was approximately 36,000MT (2015) and the supply was mainly from freshwater culture while small percentage from brackishwater. These productions are mainly for local market. The supply from commercial farms, however, have been exported as frozen or tilapia fillet. The difficulties to obtain tilapia fry is one of the major obstacle for the industry. Currently, the supplies are mainly from government and private hatcheries located at Kedah, Perak and several other states, which is insufficient for the local industry. Therefore, for a long time, tilapia fry has been imported from Asian countries and regions such as Thailand, Vietnam, China, the Philippines and Chinese Taipei (source: Selangor Biosecurity Centre). In 2015, a total of 6,706,200 fry worth approximately USD95,000 were imported from Chinese Taipei, Thailand & China. The importation of live tilapia has exposed Malaysia to high risk of transboundary diseases (e.g: Tilapia Lake Virus, TiLV). Apart of the transboundary diseases, common reported diseases in red tilapia are bacterial infection (Streptococcosis) and parasitic infestation (Trichodiniosis, *Gyrodactylus*, Ich disease). Following the release of TiLV status by NACA in April 2017, the DOF Malaysia was on high alert to any tilapia mass mortality reported by the stakeholders. Several series of discussions with the stakeholders (tilapia farmers association, importers, hatcheries operators, fisheries officers) have been conducted at multiple levels. The farmers have been encouraged to report any mass mortality of tilapia in their farms. They also being educated on how to identify the clinical signs caused by TiLV. Any suspected cases should be reported directly to the nearest Biosecurity Division, DOF for diagnostic purposes.

In Jun to July 2017, DoF Malaysia had received two reports of tilapia mass mortality involving 2 states in northern region of Peninsular Malaysia. Mortality reported were 0.71% and 15% for wild black tilapia and farmed red tilapia, respectively. In all the cases, sampling and diagnosis were done by National Fish Health Research Division (NaFisH) together with Biosecurity Division and other DOF related Divisions. The common clinical signs observed in all the cases were skin erosion, exophthalmos, ulceration and haemorrhage of the diseased tilapines body, enlarged spleen, pale liver, severe congestion, necrosis and haemorrhage of the internal organs such as liver, spleen, brain & kidney (Fig. 1). All the required data were analysed and samples were subjected for water quality, parasitology, bacteriology, virology and histopathology analyses. Detection of TiLV was conducted using conventional PCR method and followed by sequencing using OIE recommended primers.

Results from the 2 reported cases showed occurrence of co-infection of multi pathogens with heavy protozoan infestation (*Trichodina* sp), multi bacterial infection (*Aeromonas hydrophilla, Photobacterium damsela, Staphylococus sciuri, Vibrio vulnificus*) and TiLV. High presence of augmented melanomacrophages centers (MMCs), were seen in mostly at spleen and liver while eosinophilic intracytoplasmic inclusion body eosinophilic were observed mostly at liver. Several major risk factors were identified and among them were water quality, multiple pathogen infections, source of fry and fish movements.

Control measures taken during and after outbreak are implemented on case by case basis. Both reported cases have been solved as no mortalities were reported after two months for the wild black tilapia, while for the farmed red tilapia, no mortalities was observed after implementation of break cycle. At national level, under National Fish Health Strategy for Malaysia (NFHSM), a few measures have been taken. Among them are a) notification to affected countries on Malaysia requirement on screening for TiLV before importation, b) importation of fry is monitored closely at the entry point by Biosecurity Division, and c) monitoring on TiLV status to be included in national surveillance program by Biosecurity Division.

Acknowledgements.
The authors would like to thank Biosecurity Division and Aquaculture Division from Department of Fisheries Malaysia for their assistance during sampling. We are grateful for the NaFisH's Epidemiology staffs and laboratory assistants for their assistance during the handling of the reported cases.

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Tilapia Health Management with Focus on Status of and National Action Plan on TiLV in Myanmar

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Tilapia Aquaculture in Myanmar

Myanmar has a long coastline and large river system with huge networks of their tributaries those are rich in fisheries and aquaculture resources. According to the FAO fishery and statistic (2013), Myanmar is the tenth biggest aquaculture producer in the world in 2013. It also has one of the highest global annual consumption rates of fishery products, 34 kg per capita, second only to rice in the diet and 3 times beef consumption. Employment in production, processing and sales of fisheries and aquaculture products is reported to be 2,562,230 of Myanmar's 50,000,000 populations. Therefore, aquaculture sector plays an important role in economy development.

In aquaculture production, more than 95 percents comes from freshwater aquaculture. Freshwater fishes that are cultured in Myanmar are mostly carp and catfish species such as Rohu (*Labeo rohita*), Catla (*Catla catla*), Mrigal (*Cirrhinus mrigala*), Common Carp (*Cyprinus carpio*), Grass carp (*Ctenopharyngodon idella*), Big head carp (*Aristichthys nobilis*), Silver carp (Hypophthalmichthys molitrix) and Striped catfish (*Pangasius hypopthalmus*). Recent decade, people are interested to culture Tilapia (*Oreochromis* spp) due to short culture period and market demand.

However, it was not popular among Myanmar people almost two decades ago. Tilapia *Oreochromis mossambicus* had been imported from Thailand by Department of Fisheries, Myanmar in 1953. *Oreochromis niloticus* and *O. aureus* were introduced in 1976 by Peoples' Pearl and Fisheries Corporation, Myanmar from Israel. Due to low market demand compared to carp species, farmers were not paying attention on Tilapia farming. In 2002, mono-sex Tilapia from Thiland had been introduced and hatchery was operated in Dha Pei, Ayeyarwaddy Division. Total production of 20 – 40 million fingerlings per month was produced. Cyclone Nargis hit the area in 2007 and all hatcheries were shutdown. During the time, tilapia was spread to the farms as well as to natural rivers.

Nowadays, tilapia has been produced in many polyculture ponds with rohu, common carp, catla and catfish. In Myanmar, carp has been cultured in earthen pond extensively. The sizes of the ponds vary from four to thirty hectares with an average water depth of 1.5 meters. Some mega farms are over 200 hectares. Tilapia spawn without control in many polyculture ponds with other carps. The farmers said Tilapia is a bonus when they harvest carp in the farm. Recently, Tilapia production from polyculture farm has been reached 45000 metric tons in the year 2013 (Fig. 1). A total of 1640 metric tons are exported to international market such as Singapore and Central Arabic.

Tilapia has more market demand than previous time due to increased tourism, economic expansion and very high local market demand. Grilled Tilapia becomes very popular street food among the young people. Farmers are interested to culture Tilapia instead of carp species since they need species diversification to more marketable fish instead of freshwater carps.

Farmers like to change species but they do not obtain assistance for quality seed supply and technology. To help develop small-scale farming of tilapia, for both domestic consumption and international market, WorldFish has provided quality seed of 3200 Genetically Improved Farmed Tilapia (GIFT) fry to the Department of Fisheries (DoF) in Myanmar in 2016. Now, the Myanmar Government, with technical support from WorldFish, is raising the new GIFT seed into brood, breed the brood and produce seed. In 2016, USIAD's Sustainable Seafood Industry Development project donated 3000 mono-sex tilapia fry imported from Thailand to farmers from rural area who lost huge amount of fish in seasonal flood. US Soybean Export Council (USSEC) has been established Tilapia mono-sex demo farms in Kayan, Yangon Division in 2017 and is giving the seminar on mono-sex tilapia hatchery in different regions of Myanmar. However, more partnership efforts for research, education, training and extension or technology transfer are still needed through private-public partnership in order to get rapid growth.

Tilapia Health Management

There are two laboratories in Myanmar, Laboratory of Aquatic Bioscience, Yangon University and Fish Health Section, Department of Fisheries, those can check diseases of aquatic organisms using molecular technologies. Information or fish samples related to mass mortality of Tilapia has not been reported yet to the laboratories.

However, the demand of Tilapia seeds are very strong among the farmers since they do not get seeds supply from local hatchery. Disease problems will be emerged in the balance between supply and demand. There is only one quarantine check post at the Yangon international airport for imported livestock to Myanmar. We have 26 border gates along the border line with neighboring countries. The policy towards import and movement of live aquatic animals in border line has not been implemented yet. Imported seeds from infected area will be threatening for biosecurity. Besides, we have poor extension services including lack of technology transfer to rural areas. Existing experiences from NACA, FAO and USAID support in the Asian region are requested to support development of a responsible Tilapia aquaculture sector in Myanmar.

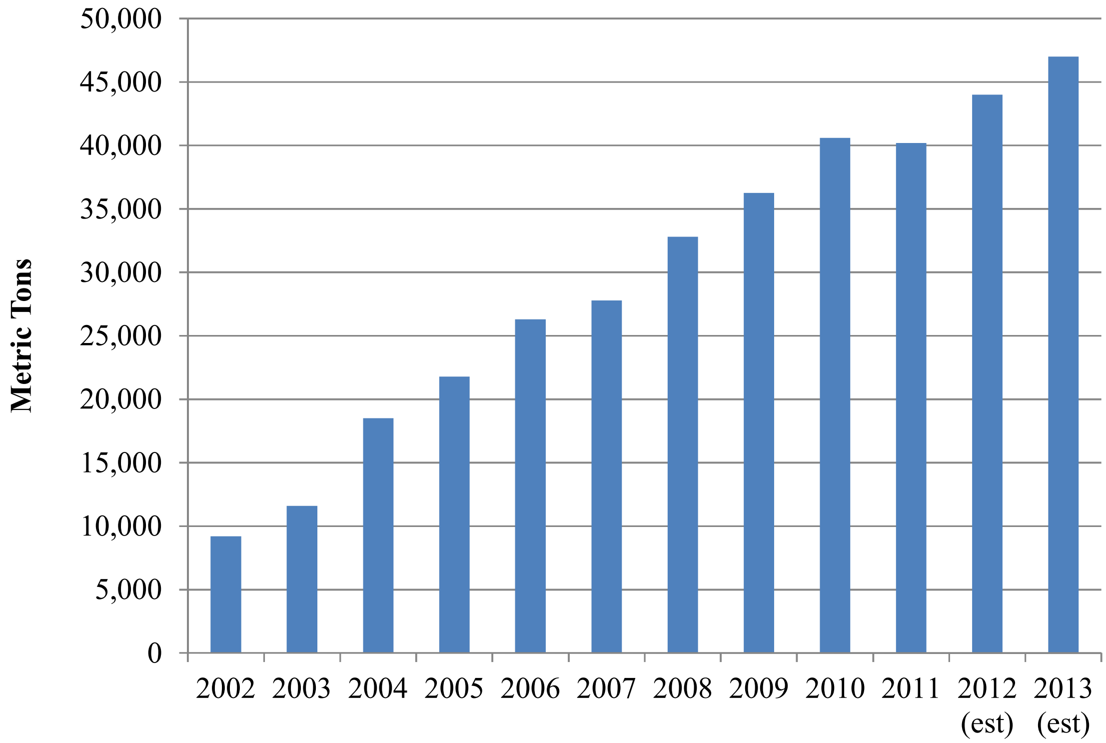

Figure 1. Tilapia production in Myanmar.

Tilapia Health Management with Focus on Status of and National Action Plan on TiLV in the Philippines

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Overview of Tilapia Aquaculture in the Philippines
In Philippine fisheries, the aquaculture sector contributes 50% of the total fisheries production. In 2015, production from aquaculture totaled to 2,348,161 MT while municipal and commercial fisheries production were 1,216,527 MT and 1,084,625 MT, respectively. Among the major aquaculture species, tilapia is the second most important farmed fish next to milkfish. In 2015, tilapia production reached 261,210.45 tons valued at P35.143 Billion. Table 1 shows the major aquaculture species and production. Table 2 indicates tilapia production data per region (Philippine Fisheries Profile, 2015)

Table 1. Major aquaculture species and production data (2015).

Species	**Quantity(MT)**	**% Production**
Seaweeds	1,566,362	66.71
Milkfish	384,425	16.37
Tilapia	261,210	11.12
Shrimps/Prawns	52,124	2.22
Others	84,040	3.58
Total	2,348,161	100.00

Tilapia is produced mainly for local consumption, and sold either fresh chilled or live in wet markets and as frozen fillet packs in some supermarkets. The farmers can harvest them after four to six months of growing period from the fingerling stage. The intensification of the culture system is a challenge to maintain the optimum condition of the aquatic environment for sustainable production. The health of the fish is compromised in cases where conditions become undesirable due to poor culture environment brought about by over stocking, poor water quality and poor husbandry practices that even hardy fish such as tilapia cannot tolerate. Disease may occur as a result of stressful conditions that lower the resistance of the fish and environmental conditions that are favorable for the proliferation of pathogens and which enhance its virulence.

Table 2. Regional tilapia production for 2015

Region	Production (MT)
CAR	2,692.06
I	14,093.36
II	9,505.37
III	124,033.64
IV	81,510.92
IVB	298.31
V	8,894.89
VI	1,660.62
VII	192.36
XVIII	753.04
VIII	278.50
IX	1,189.42
X	1,511.46
XI	2,588.68
XII	5,707.26
CARAGA	219.40
ARMM	6,081.12
TOTAL	261,210.41

Tilapia Health Management

The Philippines has developed the Philippine National Standard on Code of Good Aquaculture Practice (GAqP). It includes elements on food safety, animal health and welfare, environmental integrity and socio-economic aspects. After its approval, BFAR initiated trainings for the fish health officers, production staff, Local Government Units, and farmers on the GAqP standard and the compliance requirements of the standard. The manual of operation for the implementation of the GAqP was also developed through the assistance of the EU-TRTA project, wherein a checklist for the farm audit is included as a guide for the farm inspectors.

Status of TiLV and national action plan

In May 2017, the Network of Aquaculture Centers in Asia Pacific (NACA) and the World Organization for Animal Health (OIE) released a Disease Advisory and Disease Card on Tilapia Lake Virus, respectively. The Food and Agriculture Organization (FAO) also released a Special Alert on TiLV (FAO GIEWS dated 26 May 2017). As a precautionary measure, BFAR temporarily suspended the processing of import permit for tilapia importation. BFAR through a memorandum dated 04 May 2017 also disseminated TiLV disease advisory to all its regional offices and centers including instruction to monitor, coordinate, and report any tilapia mortality events. All BFAR fish health officers, extension officers and quarantine officers are also directed to strictly implement monitoring on health status of tilapia culture and immediate reporting of any tilapia disease outbreak events in their respective areas.

To develop diagnostic capability, BFAR Fish Health Laboratory conducted a training on TiLV detection test using insulated isothermal PCR (iiPCR) on June 27-30, 2017. The training was jointly conducted with GeneReach, Chinese Taipei and participated by analysts of BFAR Central and Regional Fish Health Laboratories, SEAFDEC-AQD and Fisheries Biotechnology Center. The Fish Health Laboratory is in coordination with the Fisheries Biotechnology Center for further research study regarding TiLV and its detection method.

Surveillance is being conducted to determine TiLV presence and distribution in the country. Samplings at registered hatcheries are conducted and information education campaign for awareness of farmers were done simultaneously with surveillance activities. The farmers are also adviced on the threat posed by TiLV and reporting of unexplained mortalities to BFAR extension officers is encouraged.

BFAR also issued Fisheries Office Order requiring Health Certificate for TiLV for the in-country transboundary movement of tilapia intended for aquaculture purposes.

Tilapia Health Management in Thailand: Status of and National Action Plan on Tilapia Lake Virus

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Tilapia lake virus (TiLV) was firstly recorded in Israel in 2009. The disease was initially reported in Thailand in 2017 that tilapia farms experienced extensive losses since October 2015 (Surachetpong *et al*., 2017). The affected fish samples tested by RT-PCR were positive for TiLV (Orthomyxo-like virus), however most of the cases investigated were co-infected with bacteria and/or external parasites. The disease surveillance action by Department of Fisheries also found TiLV in tilapia farms in Chachoengsao, Prachin Buri, Pathum Thani, Kamphaeng Phet, Kalasin and Chiang Rai provinces.

It is noteworthy that TiLV has been detected in cultured tilapia in Thailand. However, the overall production has not been significantly reduced. Tilapia production statistics in Thailand from 2012 to 2016 (Table 1) shows that the tilapia production slightly decreased every year as the culture area is reduced. Considering the yield per area, tilapia production in 2015 and 2016 decreased by 1.64 and 1.98 %, respectively compared to previous years. The decrease can be attributed to climate change and drought in many areas from late 2015 to mid 2016, which resulted in insufficient water resources for fish cultivation during the dry season. Consequently, the farmers have practiced early harvest as a result of limited water supply in culture ponds. In addition, the 2016 rainy season in Thailand was delayed which made many

Table 1. Culture area and production of tilapia in Thailand in 2012-2016.

Year	Culture area (hectares)			Production (ton)			Production (ton)/Area (hectare)		
		decrease	% decrease		decrease	% decrease		decrease	% decrease
2012	72,243.20			203,095			2.81		
2013	70,516.80	1,726.40	2.39	197,644	5,451	2.68	2.80	0.01	0.30
2014	68,126.24	2,390.56	3.39	189,948	7,696	3.89	2.79	0.01	0.52
2015	67,038.56	1,087.68	1.60	183,856	6,092	3.21	2.74	0.05	1.64
2016	66,315.84	722.72	1.08	178,265	5,591	3.04	2.69	0.05	1.98

Source: Office of Agricultural Economics farmers to halt their fish farming activities. Subsequently, when rainy season came, it has brought rainstorms in the North, the North East and the Central parts, while the South was flooded in late 2016.

Though Tilapia lake virus disease (TiLVD) is an emerging disease and not listed by OIE, Thailand Department of Fisheries (DOF), the competent authority for aquatic animal affairs, paid close attention to this disease and put in place the action plans for prevention and control of TiLV as follows:

1. Disease diagnosis preparedness: DOF has TiLV testing laboratories both for isolation of the virus in cell culture and detection by PCR techniques.
2. Surveillance and monitoring program: DOF has taken action on both active and passive TiLV surveillance and monitoring program in exporting farms and non-exporting farms:
   2.1 Exporting farms: all registered tilapia exporting farms participated in the TiLV surveillance program for demonstrating the absence of disease in accordance with OIE Aquatic Code. Tilapia samples must be taken from each farm twice a year at minimum intervals of 3 months at 5% prevalence. In addition, the registered exporting farms must have biosecurity measures in place.
   2.2 Non-exporting farms: hatchery and farm prevalence survey for TiLV is being conducted by DOF.
3. Farm standard:
   3.1 Exporting farms must be registered and comply with "aquaculture establishment for export standard". This standard requires
      - Biosecurity measures in place;
      - Farm must be inspected at least twice yearly;
      - Fish samples must be taken from farm at least 2 times per year for disease surveillance according to OIE Aquatic Code.

   3.2 Non-exporting farms are encouraged to register and comply with "good aquaculture practice (GAP) standard".
4. Aquatic animal health certificate for export: tilapia to be exported should come from registered farm establishment and which has participated in TiLV surveillance program to demonstrate freedom from the disease. These farms can also apply for "freedom from TiLV" health certificate. For newly participating farm in TiLV surveillance program, the DOF will negotiate with the competent authority of importing country to use alternative measures to certify the "freedom from TiLV" such as pre-export testing for the disease.
5. National listed diseases: TiLVD will be added in the national list of diseases for aquatic animals for the purpose of disease prevention and control under Animal Epidemic Act. When the disease or suspicion or un-explained mass mortality occurred, the fish owners have to report to the competent authority for disease investigation and diagnosis.

6. Communication: DOF invited fish farmers to attend the meeting for sharing information on TiLV as well as finding some resolutions.
7. International reporting: DOF has reported the status of TiLV to NACA/OIE.
8. Research on TiLV especially in field of epidemiology such as identifying the potential factors associated with TiLVD are underway.

Tilapia Health Management and Active Surveillance for TiLV in Vietnam

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Tilapia production in Vietnam is mainly in the northern provinces with around 85 ha culture area and 7,647 cages. Total tilapia culture area is about 109 ha and over 10,700 cages. Tilapia are mainly farmed together with other freshwater species. The export of tilapia is expected to increase as per information from Vietnam Association of Seafood Exporters and Producers (VASEP). It is expected that export will generate revenues of US$45 million in 2017 which is around 32% higher than 2015. Recently, the Ministry of Agriculture and Rural Development of Vietnam has issued Decision No. 1639/QD-BNN dated 6 May 2016 on approval of planning for tilapia production to 2020 with a view in 2030. The aim of this Decision is to achieve total yield of tilapia to around 300,000 MT by 2020, of which 50 to 60 percent should meet export criteria.

The common diseases of farmed tilapia in Vietnam include bacterial (Aeromonads and Streptococcosis) and parasitic infestation. For Tilapia lake virus (TiLV), surveillance has been initiated by collecting samples (142 in total) in 10 provinces (Quang Ninh, Hai Phong, Hanoi, Yen Bai, Phu Tho, Thai Nguyen, Hung Yen, Thai Binh, Bac Ninh and Tien Giang) for testing, but no positive samples was detected. The government has taken measures to strengthen quarantine and border control for imported tilapia, and to quarantine tilapia seeds movement between provinces. The Competent Authorities also advise and guide tilapia farmers to follow some on-farm preventive measures including: proper stocking density; improved biosecurity conditions at farms; treat bacterial diseases of tilapia with antibiotics prudently and responsibly.

Recently, the government of Vietnam has organized a training on TiLV diagnosis for diagnostic laboratory and epidemiology staff of the Department of Animal Health (DAH; Regional Animal Health Offices 1 to 7 and NCVD) and Sub-DAH in Quang Ninh and Hai Phong provinces. The trainors are Dr. Saenchan Senapin and Dr. Ha Thanh Dong from Thailand. During the training, a diagnostic protocol for TiLV has been developed which will be applied among DAH laboratory system, and preventive measures were proposed to prevent TiLV introduction in Veitnam.

Surveillance programme was also planned to be carried out for detection of TiLV in farmed tilapia in Norther Vietnam (Quang Ninh, Hai Phong, Bac Ninh, Ha Noi, Thai Nguyen, Thai Binh, Hai Duong provinces). Duration of surveillance will be two months (October-

November 2017) with twice a month sampling (target number of samples: 140). TiLV detection will be by semi-nested RT PCR which will be done by the DAH.

The following activities were also proposed:

- Epidemiological/observation study to identify roles of each of the reported disease in causing losses of tilapia and the risk factors involved for disease outbreak;
- Indepth analysis of each of the pathogen involved in the disease or of any new pathogen that has not been identified;
- Study on treatment of infected tilapia using antibiotics and any other alternative methods;
- Technology for improvement of biosecurity measures and its application;
- Improved technology for breeding of tilapia;
- Any measure to improve productivity of tilapia and disease control and prevention.

PANEL DISCUSSION

A panel of experts representing national, regional and international organisations has facilitated the discussion on the prevention and management of TiLV in the region, with the participation of all the country representatives. The panelists were as follows:

- Dr. Hong Liu (AQSIQ, PR China)
- Prof. Jianguo He (SYSU, PR China)
- Dr. Yan Liang (NFTEC, PR China)
- Prof. Hong Yang (FFRC, PR China)
- Dr. Stian Jonsen (OIE, France)
- Dr. Shimaa Ali (WorldFish, Egypt)
- Dr. Rolando Pakingking, Jr. (SEAFDEC AQD, Philippines)
- Dr. Eduardo Leaño (NACA, Thailand)

Included in the discussion were important issues on TiLV including the need for steps to be undertaken for prevention and control of TiLV in the region including research and improved diagnostics. The panelists also emphasized the need to develop a regional strategy and legislative framework for prevention and control of TiLV, and the importance of capacity building and awareness programmes in different countries. It was also suggested that TiLV be listed in OIE to encourage surveillance and reporting, but the need for contribution from member countries (which will be the ones to submit request for listing) and team work were highlighted. The economic impact of TiLV was also suggested as one of the priorities for further study, as well as certification and accreditation of diagnostic laboratories for TiLV and the importance of biosecurity and management for prevention of TiLV.

For disease surveillance and diagnosis, a standard diagnostic test is still needed. There is also a need to set up two working groups to deal with TiLV investigations, one for national surveillance and another for the development of standard operating procedures (SOP) for biosecurity. Understanding the genetic variations of the virus, carriers (sub-clinically infected fish) and susceptibility of other fish species to the virus are also important. Similarly, research studies should focus on validation of diagnostic tests (study of coinfection of virus and bacteria; survival of virus in frozen sample; abundance of virus in mussel tissue; relationship between host and pathogen; phylogenetic analysis of different strains; susceptibility of marine ornamental fishes). It is also necessary to know whether the virus is already present in the natural aquatic environment, and to identify the root cause of the disease. As the clinical signs appear to be non-specific which causes confusion, it might be worthwhile to pay attention to several areas inclduing unbalanced "complete" feed which might cause nutrient deficiency, and environmental parameters that might trigger disease outbreak and proliferation of the virus.

Other important questions raised include the following:

- Is TiLV already present in the region or is it introduced through introduction of broodstock or fingerlings?
- How harmful is TiLV and under what conditions that the virus becomes harmful?

- Is TilV is a new pathogen?
- Is tilapia the only host of TilV?
- How to detect the virus in the early stage of the disease before it cause heavy mortality?

The course of the discussion were focused on several issues including diagnostics, reporting, epidemiology, disease prevention, and research gaps. These are summarized below:

Diagnosis

- Prepare/improve the Disease Card which can be used for diagnosis.
- How to reduce false positive results (molecular diagnostics).
- It is important to correlate gross pathology with internal pathology, electron microscopy and cell culture studies.
- Since clinical signs are non-specific, there might be a need to detect mixed or secondary infections:
    1) pop eyes and sunken eyes might suggest the presence of mixed infection;
    2) co-infection with *Streptococcus*, the most common bacterial disease in tilapia farming;
    3) in mixed infection, how to determine which one is the primary causative agent?
- SOP and standardization of confirmatory diagnosis;
- It is suggested that PCR should not be used for 1st time report in a country
- Histopathology needs to be done on infected organs. Koch's postulates must be fulfilled.
- Areas to consider for disease diagnosis:
    1) check lesions in internal organs
    2) physiology parameters such as blood chemistry
    3) use of electron microscopy to observe the virus
    4) check fo presence of parasites
    5) use of host cell line(s) for isolation, cytopathology changes, purification of the virus, propagation of the virus for use in animal challenge in case of co-infection
    6) development of immunoassays especially hemoagglutination assay which is more simple and fast.
- At present, diagnosis is done based on PCR, cell culture and histopathology. It is suggested that NACA to propose the formation of a working group with support from relevant institutes and individuals, for developing recommendations on proper diagnotic method.
- Other recommendations:
    1) standardized PCR protocol: routine vs real-time PCR. Probably real-time PCR plus sequencing can be used as confirmation for reporting to OIE and NACA.
    2) in situ hybridization

3) cell culture, establishment of cell lines
4) establishment of reference laboratories
5) challenge study in ornamental fish and pathogenicity study in different fish species. Observe clinical sign in laboratory challenge study in comparison with field infection.
6) phylogenetic sequence: which segment to be used? sequence 1 for pyogenesis and sequence 3 for detection.

Epidemiology

- There is a strong need to get an epidemiologist involved in the overall investigation of the disease. Dr. Jansen (Norwegian Veterinary Institute, OIE collaborating centre on Epidemiology and Risk Assessment of Aquatic Animal Diseases) can be contacted in this regard.
- There is a need to look into other potential hosts and carriers of the virus besides tilapia.
- How about vertical transmission? This will have impact on farm biosecurity.
- Susceptibility of the fish at different life stages.
- Environmental factors such as water temperature, ammonia, nitrite content, water exchange, etc.; these might have roles in triggering disease outbreaks and/or proliferation of the virus.
- presence and involvement of other microbes (pathogenic/nonpathogenic) in triggering or enhancing the TiLV infection.

Disease Prevention

- Is TiLV present in broodstock?
- Development of biosecurity measures at regional, national and farm levels.
- Vaccine development can be considered. However, there is a need to pay attentiaon to the nature of TiLV (as an Orthomyxovirus which is similar to bird flu virus) as they can easily mutate and "escape" from host immunity, the same as what have been obseved in Avian influenza vaccination. Moreover, there might be different strains of the virus, thus a developed vaccine might work in some areas but not in other.
- Effective disinfectants.
- SOP for quarantine of incoming stocks.
- Control and management of water quality and feeding.
- Establishment of SPF stock; as the mortality ranges from 10% to 90%, it is likely that there is a variation in tilapia breed susceptibility.

Reporting (and impact on the farming industry)

- It is important to share information and to raise awareness, as it is not good to wait until the disease is becomes out of control. The report of AHPND by NACA with support from member countries is a good example.
- It is important to report early and timely, as it can help in the prevention of further spread of the disease.

- OIE encourages everyone to find a way to encourage notification but not causing panic. So far, only Thailand and Chinese Taipei have officially reported the presence of TiLV to either OIE or NACA, and another two is in the process or preparing the report (India and Malaysia). TiLV has not been listed in OIE because of lack of specificity and sensitivity in diagnosis. If further supporting documents and comments are submitted, the earliest for TiLV being listed will be May 2019.
- The main question is "do we have the right knowledge in diagnosis and validation on the test?"
- OIE encourages member country to investigate morbidity and mortality, and submit samples to Gene Bank for information sharing.
- When recommendation is made for listing of TiLV, the farmers and the industry should be kept in mind, as listing the disease can impose trade barrier; i.e. how can we help the farmers (disease diagnosis, disinfection and biosecurity protocols, etc.) and how they will be impacted by the reporting system. They are the ones who live day-in and day-out with the disease situation at farm level. There is also a need for their buy-in for disease reporting and implementation of preventive measures.
- There is a need to report the disease causative agent, no matter with or without clinical signs; as some fish might be sub-clinically infected.
- On-line questionnaires shall be used to encourage reporting on clinical signs, economic losses and for sample tracking.

Research Gaps

- Information on surveillance, disease prevalence, reliable mortality data, development of online questionnaires
- Epidemiology of the disease.
- Phylogenetic sequence of the virus.
- Factors affecting viability of TiLV: water condition, UV, chemical agents. This will help in formulating a sound biosecurity strategy.
- What is the economic losses caused by TiLV and how to estimate it?

Way Forward

Some of the important activities for regional management of TiLV are information sharing, collaboration and capacity building. The China-ASEAN Center for Joint Research and Promotion of Marine Aquaculture Technology (managed by Prof. He of Sun Yat-sen University) can provide financial support for training, which can be done through colloaboration with and coordination of NACA (or other relevant institutes in the region). The formation of a Working Group is necessary to determine the scope of capacity building activities as well as roles and responsibility of the team members. Sun Yat-sen University is willing to provide both human resource and financial support.

FAO and NFTEC will also organize a one-week TiLV training course to be held in China in 2018. Tentative agenda of the training include: 1) Tilapia and aquaculture country presentation; 2) What are currently known (diagnosis, field findings, water quality, PCR techniques, etc); 3) TiLV active surveillance; 4) Focus on emergency response. All expenses of experts and the selected participants will be covered by FAO and China-ASEAN Center for Joint Research and Promotion of Marine Aquaculture Technology.

It is also recommended that a formal online discussion group be created for sharing of relevant results, publications, emerging issues and other relevant information on TiLV. The issues identified above on diagnosis, epidemiology, preventive measures, reporting and research gaps should be the priority areas for furture activities related to the manangement, prevention and control of TiLV in the region.

ANNEXES

Annex 1. Provisional Agenda

Emergency Regional Consultation for Prevention and Management of Tilapia Lake Virus (TiLV) in the Asia-Pacific
27-28 September 2017
Sun Yat-Sen Kaifeng Hotel, Guangzhou, China

Time	Topic	Speaker
27 September (Wednesday)		
8:30-9:00	Registration	
9:00-9:30	Opening Session	
	• Welcome Remarks	**Mr. Youheng Sun** (Deputy Director, NFTEC, China)
	• Message	**Dr. Eduardo Leano** (NACA)
	• Self Introduction	All participants
9:30-10:15	***Tilapia Aquaculture in the Asia-Pacific Region: Status and Trends***	**Dr. Derun Yuan** (NACA
10:15-10:35	Coffee Group Photo	
10:35-11:25	***Important Diseases of Cultured Tilapia***	**Prof. Jianguo He** (Sun Yat Sen University)
11:25-12:10	***Overview of TiLV***	**Dr. Mona Dverdal Jansen** (NVI) and **Dr. Chadag V. Mohan** (WorldFish)
12:10-12:30	Open Forum/Discussion	
12:30-13:30	Lunch	
13:30-14:00	***Update on TiLV Research in Thailand and Potential Strategies for Control***	**Dr. Dong Ha** (King Mongkut University of Technology)
14:00-14:30	***Virus characterization, Clinical presentation and Pathology of TiLV***	**Dr. Win Surachetpong** (Kasetsart University)
14:30-15:00	***The Role of Trade in Spread of Transboundary Aquatic Animal Diseases***	**Dr. Eduardo Leano** (NACA)
15:00-15:30	Open Forum/Discussion	
15:30-16:00	Coffee	
16:00-16:30	***Import Risk Assessment: Role in Prevention of Transboundary Aquatic Animal Diseases***	**Dr.Hong Liu** (AQSIQ)
16:30-17:00	***Biosecurity: Role in Aquatic Animal Diseases Prevention and Control***	**Dr. Jie Huang** (YSFRI)
17:00-17:30	Open Forum/Discussion	
18:30-	Welcome Dinner	
28 September (Thursday)		

9:00-12:30	***Country presentations:*** ***Tilapia Health Management with Focus on Status of and National Action Plan on TiLV***	
9:00-9:15	• China	**Dr. Qing Li**
9:15-9:30	• Egypt	**Dr. Shimaa Elsayed Mohamed Ali**
9:30-9:45	• India	**Dr. Pavata Pradhan**
9:45-10:00	• Indonesia	**Dr. Ratna Amalia Kurniasih**
10:00-10:15	• Malaysia	**Dr. Azila Binti Abdullah and Mr. Gerald Misol, Jr.**
10:00-10:30	Open Forum/Discussion	
10:30-11:00	Coffee	
11:00-11:15	• Myanmar	**Dr. Kay Lwin Tun**
11:15-11:30	• Philippines	**Dr. Sonia Somga**
11:30-11:45	• Thailand	**Ms. Jaree Polchana**
11:45-12:00	• Vietnam	**Dr. Phạm Hồng Quân**
12:00-12:30	Open Forum/Discussion	
12:30-14:00	Lunch	
14:00-16:00	***Panel Discussion:*** • Formulation of regional plan for prevention and control of TiLV • Research priority • Surveillance and Reporting • Listing in OIE • Way Forward	Panelists: **Dr. Hong Liu** (AQSIQ) **Prof. Jianguo He** (SYSU) **Dr. Yan Liang** (NFTEC) **Prof. Hong Yang** (FFRC) **Dr. Shimaa Elsayed Mohamed Ali** (WorldFish) **Dr. Stian Johnsen** (OIE) **Dr. Rolando Pakingking, Jr**. (SEAFDEC AQD) **Dr. Eduardo Leaño** (NACA)
16:00-16:30	Coffee	
16:30-17:30	Panel Discussion Continued	Panelists
17:30-18:00	Closing	**Dr. Jianguo He** (SYSU)

Annex 2: List of Participants

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