Food and Agriculture Organization of the United Nations

FAO/China Intensive Training Course on Tilapia Lake Virus (TiLV)

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TRAINING COURSE SESSION MODULES
SESSION 2
What is currently known about TiLV

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Session 2

What is currently known about TiLV

Introduction

Emergence of TiLV. Tilapia lake virus (TiLV) is a recently described virus affecting wild and farmed tilapines, infection with TiLV has caused extremely variable mortalities (ranged from 0 to 90%) and may cause significant production losses. The virus was first recognized in Israel in 2011 and was assumed to be linked to previously unexplained outbreaks in Israel and Ecuador in 2009; at present, it has been reported on three continents (Asia, Africa and South America) and the number of countries where the agent has been detected is likely to increase rapidly as a result of increased awareness, surveillance and availability of diagnostic methods.

TiLV biology. TiLV genome has 10 segments of negative-sense RNA. Under the transmission electron microscope, the virions are enveloped, spherical-shaped, with diameters of 70-110 nm. Its morphology and size are similar to members of the family *Orthomyxoviridae*, and was described as an orthmyxo-like virus, but its genomic sequence showed little homology to those of orthmyxoviruses. TiLV has recently been placed as a new species, *Tilapia tilapinevirus*, under a new genus *Tilapinevirus*, under an unassigned family.

Gross pathology. The gross signs of TiLVD include: skin erosion, hemorrhage at the base of fins and opercula, scale protrusion, abdominal swelling, skin darkening, gill pallor, and ocular alterations. Infected fish also exhibit abnormal behavior like lethargy, loss of appetite, swimming at the surface, and loss of balance.

Histopathology. Currently available information suggests syncytial hepatitis to be the most common histopathology feature found in TiLV outbreaks, which is a reason that TiLVD was also named as syncytial hepatitis of tilapia (SHT). In the TiLV-infected tilapia, massive cellular necrosis with pyknotic and karyolitic nuclei are found in the hepatocytes; eosinophilic cytoplasmic inclusion bodies are also present in the liver cells.

There appears to be some geographical variations in the histopathology features associated with this disease. Observed lesions in affected fish in Israel include congestion of internal organs (kidney and brain), foci of gliosis and perivascular cuffing in the brain cortex and ocular lesions. In infected fish from Egypt, histopathology findings included gliosis, encephalitis and mild perivascular cuffing in the brain, multifocal chronic hepatitis, and multifocal interstitial haemorrhage in the kidney. The cases in Ecuador and Colombia showed hepatocyte necrosis and syncytial cell formation, necrosis of gastric glands and diffuse congestion in multiple tissues. The lesions observed from Thailand samples include aggregation of lymphocytes and perivascular cuffing in brain tissue.

Individual variation was also found in tilapia sampled from a single farm in Thailand. While syncytial hepatitis and foamy cytoplasm were observed in the liver of the majority of the fish analyzed, all infected fish showed severe pancreatic necrosis and some occasionally exhibited the presence of cytoplasmic inclusions in the hepatocytes. Severe infiltration of lymphocytes was

observed in some areas of the kidney tubules and brain where syncytial cells were located in the center of areas of inflammation.

Virus isolation and Koch's postulate. The presence of TiLV was determined by isolating the viruses from the suspect fish and inoculate in continuous cell lines, such as E-11 (derived from snakehead fish, *Ophicephalus striatus*), or primary cell lines. After incubation for approximately 10-14 days at 25^{o}C, cytopathic effect (CPE) was observed, and virions purified were injected intraperitoneally into healthy tilapia, their gross signs and histopathological lesions were identical to those observed in the original clinical specimens. TiLV were then re-isolated from these experimentally infected fish and shown to be replicating in the cell culture, thus completing Koch's postulate for demonstration of a viral etiology.

Preparation of reference TiLV stocks. For preparing the viral stock, the infected cell culture can be centrifuged, the TiLV in the supernatant can be filtered through a 0.22 µm membrane, then dispensed into sterile vials and stored at -80^{o}C, liquid N_2 or lyophilization for a long-term storage.

Diagnostic methods. Several molecular detection methods, including RT-PCR, RT-qPCR and *in situ* hybridization protocols have been described for the specific and sensitive detection of TiLV. A semi-nested RT-PCR targeting the TiLV genomic segment 3 was developed and shown to have a sensitivity of 7.5 copies of viral sequence. This method is listed in the OIE disease card. Recently, a SYBR green-based reverse transcription quantitative PCR (RT-qPCR) method targeting the same segment 3 RNA was developed for detection of TiLV with a reported sensitivity of 2 copies. Commercial TiLV RT-PCR and RT-qPCR kits are also available, some of these kits also target segment 3.

Pond-site detection. A commercial TiLV RT-PCR detection assay, based on insulated isothermal polymerase chain reaction (iiPCR, use the TaqMan-based qPCR principle), is available for on-site diagnosis. The hand-held device is called POCKIT™ Micro (manufactured by GeneReach Biotechnology Corp.). The assay can be completed within 45 min and has advantages of rapid, inexpensive, sensitive, and easy to maintain and operate for non-specialists.

***In situ* hybridization (ISH)**. ISH is a technique to determine and localize target nuclei acids in fixed tissue sections. Over the years, this method has been improved and greatly increased its sensitivity; it is possible to detect viruses in the cells that no visible signs of infection by histological evaluation. ISH has been applied to reveal the tissue tropism for TiLV. The use of the digoxigenin-labeled probe in conjugation with alkaline phosphatase detection is simple and practical, the tissue sections after color development can be stored for long periods.

Laboratory practical training. For the diagnosis of TiLV, we will have hands-on practice in histopathological evaluation with the infected tissue sections. From fish tissues sampled from a local tilapia farm, we will extract RNA, followed by both RT-PCR and RT-qPCR analyses. The extracted RNA will also be analyzed for the presence of TiLV by a pond-site diagnostic devise. For ISH, we will have a video for demonstrating the procedure using digoxigenin-labeled probe followed by color development with a NBT/BCIP chemistry, and examination of hybridized sections under the microscope.

Learning objectives

To allow workshop participants to

- update on emerging, re-emerging and new diseases of tilapia;
- gain knowledge on the discovery of TiLV, the pathology and its molecular characteristics;
- update on the currently available scientific knowledge related to TiLV;
- learn the diagnostic methods, including gross pathology and molecular methods, for detecting infection with TiLV;
- learn how to isolate TiLV from infected fish, propagate viruses using cell cultures and prepare the reference viral stocks.

Learning outcomes

At the end of the course module, participants are able to:

- understand diagnostic procedures for TiLV, including clinical signs, histopathology, TiLV RT-PCR and RT-qPCR, and *in situ* hybridization using a digoxigenin-labeled probe;
- apply these diagnostic methods in their home laboratories and determine the TiLVD status in farmed and wild populations of tilapia;
- understand the concept of Koch's postulate through TiLV isolation and inoculation in cell culture;
- learn methods for preparation and storage of infectious viruses as laboratory reference stocks.

Module duration

Day 1 (6/18): 13:00-17:00
Day 2 (6/19): 08:30-17:00
Day 5 (6/22): 13:00-17:00

Lectures

- Emerging, re-emerging and new diseases of tilapia (1 hr, HTD)
- TiLV, the discovery of a new pathogen (1 hr, MDJ)
- TiLV-biology, epidemiology and economics (1 hr 30 m, MDJ)
- TiLV pathology (45 min, MDJ,)
- TiLV diagnostics (1 hr 30 min, WS)
- Necropsy, Histology, RT-PCR (1 hr, HTD)
- TiLV isolation and Koch's postulate (1 hr, WS)
- TiLV *in situ* hybridization (1 hr 45 min, HTD&KT)

Working group activity

- Interactive session on pathology and diagnostics
- Perform tissue preparation for histology and RT-PCR analyses
- Examine the histopathological changes in tissue sections prepared from TiLV-infected tilapia
- Perform RNA extraction from tilapia tissues
- Carry out TiLV RT-PCR and RT-qPCR analyses
- Carry out TiLV RT-qPCR with a pond-side detection kit

Background documents

- ppt presentations
- video presentation
- handouts of laboratory procedures (ISH, RNA extraction, RT-PCR, RT-qPCR)

Key references

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