

FAO/ASTF Project: GCP/RAF/510/MUL:
**Enhancing capacity/risk reduction of emerging Tilapia Lake Virus (TiLV) to
African tilapia aquaculture: Intensive Training Course on TiLV**

4-13 December 2018. Kisumu, Kenya
in cooperation with Kenya Marine Fisheries Research Institute (KMFRI) and Kenya Fisheries Service (KeFS)

Session 13:
TiLV disease strategy manual

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Food and Agriculture
Organization of the
United Nations

Contingency plan goal

- To ensure **preparedness** (not a reaction) for rapid responses to TiLVD outbreaks, it should be developed **before** the occurrence of outbreaks

Objectives

- **Protect tilapia health**
- **Minimize economic losses (fish production and socio-economy)**
- **Minimize negative impacts to the environment.**

Contingency Plan

- **To consider the worst scenarios (such as the negative impacts on trading) and develop intervention strategies for it**
- **It needs to have a risk assessment analysis that serves as a guide to determine the context**
- **Required elements: **TiLVD strategy manual**, policies, SOPs, financial and technical resources**
- **Identify gaps and weak areas through simulation exercises**
- **Establishing systems and procedures for TiLVD control**

Components of a contingency plan

Technical plans

Disease strategy manuals (one for each high priority disease)

General procedures manuals (SOPs)

Enterprise manuals

Job descriptions (needed skilled, responsibilities)

Support plans

Financial (individual farmer, fish farming industry, government)

Resource (equipment, diagnostic reagents, vaccines, etc)

Legislation and other agencies

Operational capability

Management manuals (coordination, arrangements)

Diagnostic resources

Field personnel

Training resources

Awareness and education

Response exercises (simulation)

See: <http://www.fao.org/docrep/009/a0090e/A0090E10.htm>



<http://www.agriculture.gov.au/animal/aquatic/aquavetplan/>

TiLVD strategy manual

Chapter 1: Introduction

produced 6 million tons of tilapia in 2016, US\$9.8 billion in trades

Chapter 2: The nature of TiLVD

Chapter 3: Diagnostics of infection

Chapter 4: Prevention and treatment

Chapter 5: Epidemiology

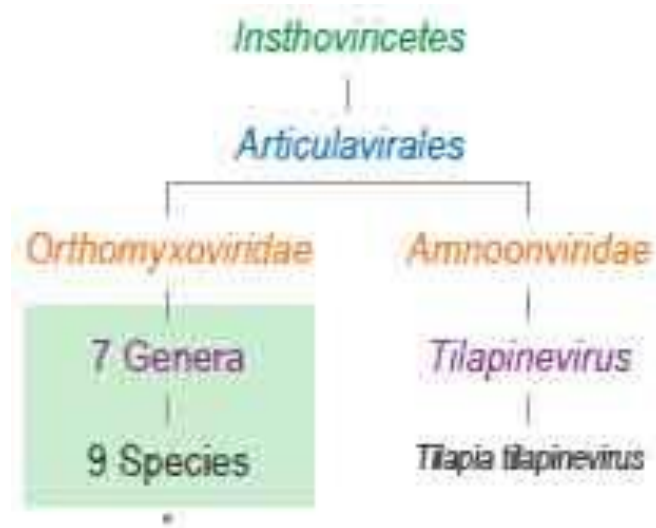
Chapter 6: Principles of control and eradication

Chapter 7: Policy and rationale



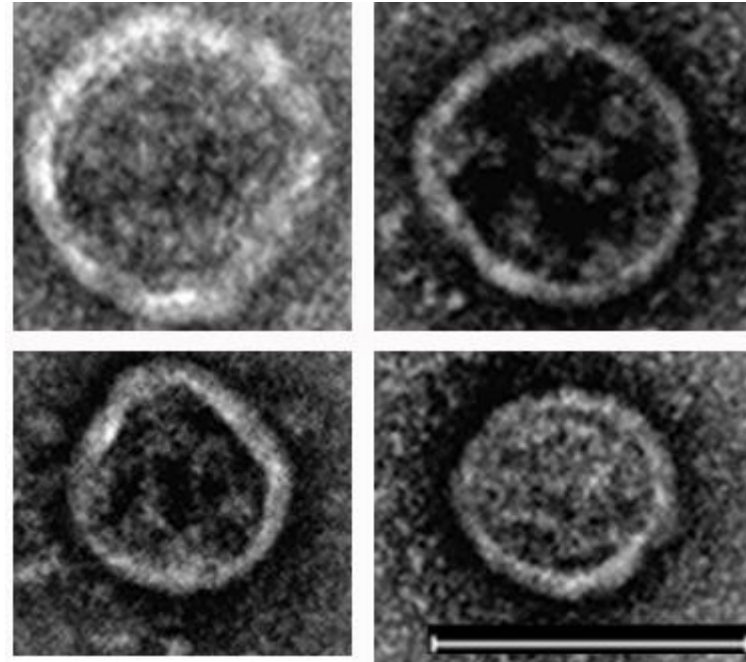
Chapter 2. The nature of TiLVD

2.1 Etiology: TiLV



2.2 Susceptible species

2.3 Global distribution



Transmission electron micrographs (TEM) of purified TiLV showing round-shaped virions. scale bar=0.1 μm.

Chapter 3: Diagnostics of infection

3.1 Gross signs

3.2 Laboratory methods

a) Sample submission:

For demonstrating TiLV-free: 149 fish
(at a 2% prevalence)

For follow-up surveillance:

59 fish (5%), or 29 fish (10%)

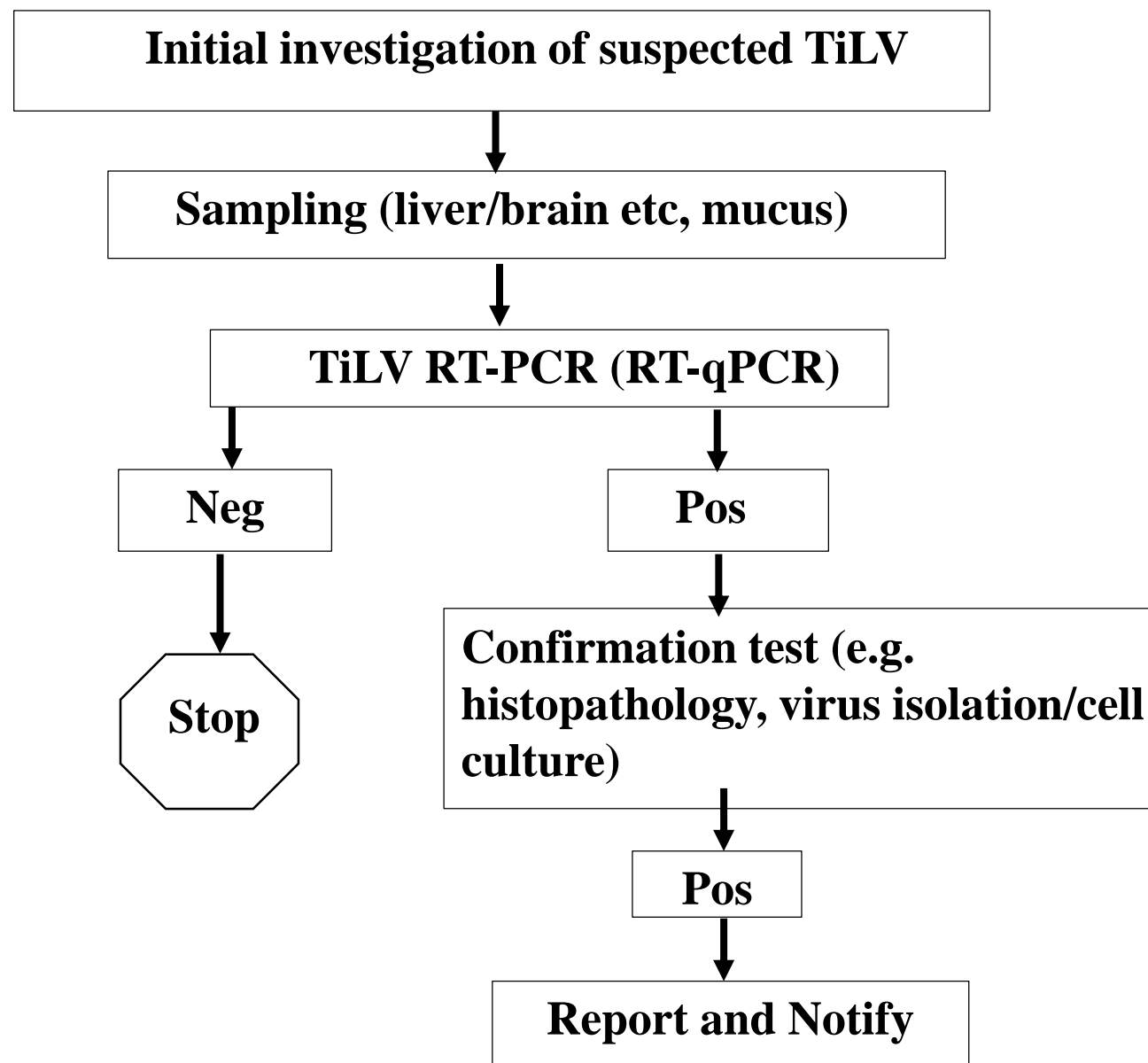
From the **clinically diseased** fish, **10 (or 30)** fish should be sufficient for each epidemiological unit

3. Diagnosis of infection with TiLV

3.1 Gross signs:



- (A) diseased red tilapia showed hemorrhage;
- (B) diseased Nile tilapia showed skin erosion, hemorrhage on various parts of body, abdominal swelling, and swelling of the eyeball (exophthalmos);
- (C) diseased wild tilapia (*Sarotherodon galilaeus*) showed shrinkage of the eye and loss of ocular functioning (phthisis bulbi).



Diagnostic flowchart

Suspect and confirmation of TiLV infection

- Suspect case

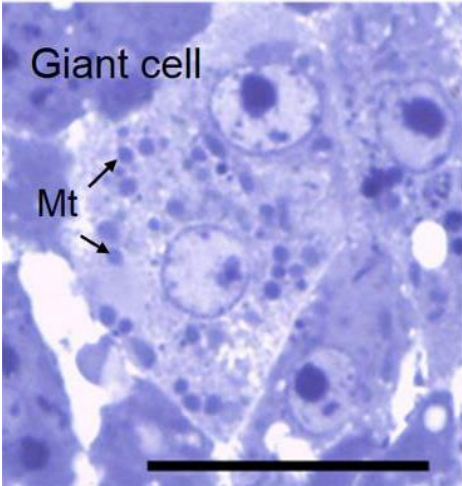
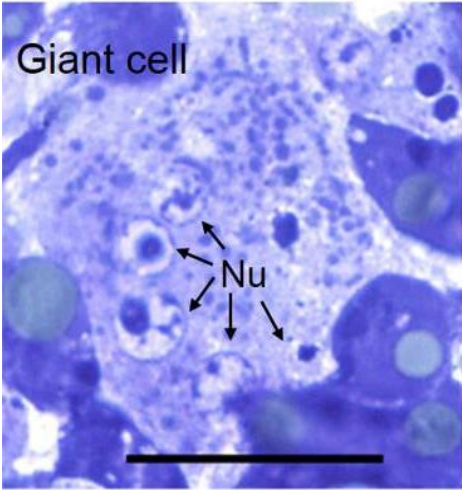
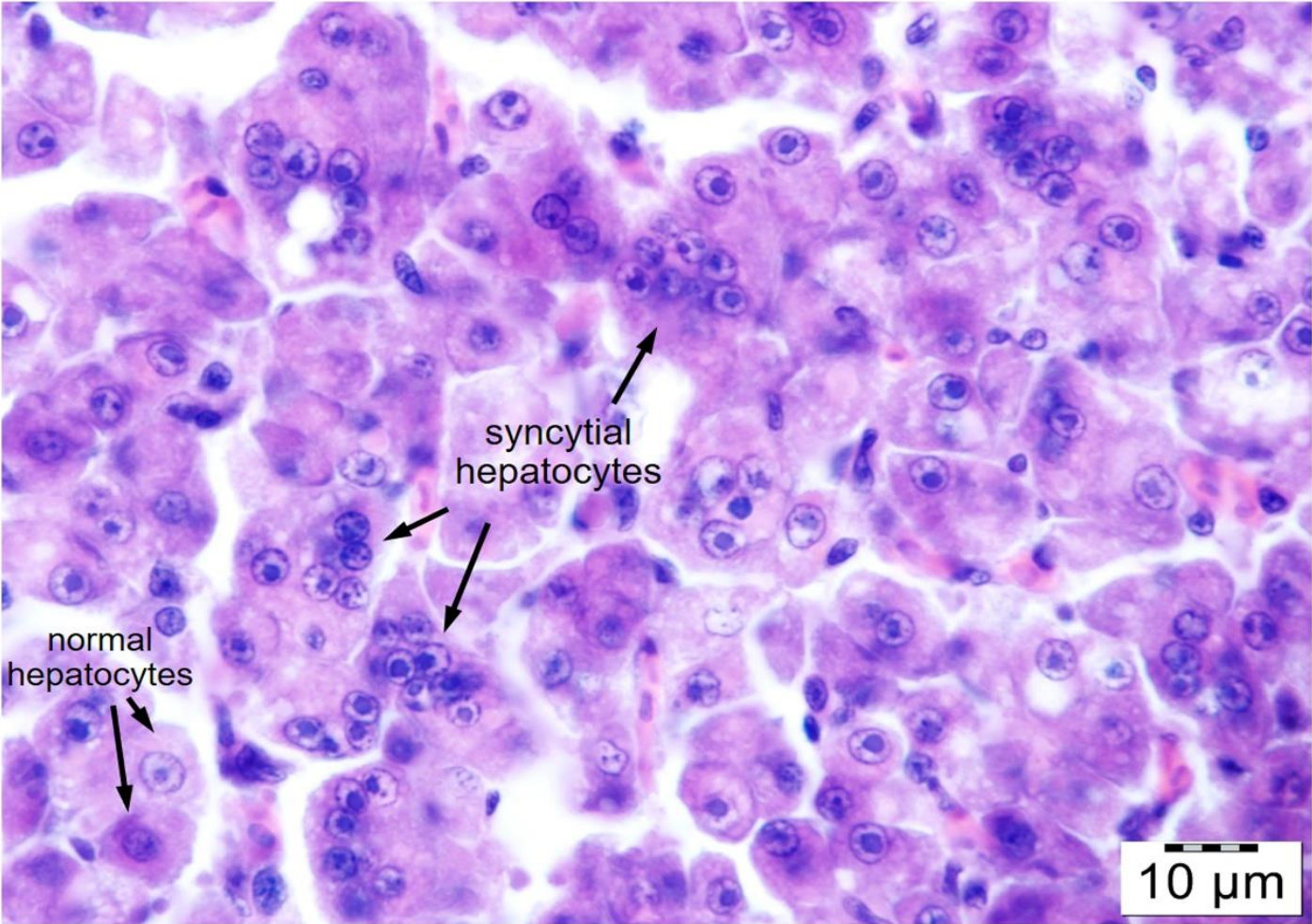
Infection of TiLV is **suspected** if at least one of the following criteria is met:

- (1) mortality and clinical signs consistent with TiLV disease
- (2) histopathology consistent with disease
- (3) detection of TiLV by RT-PCR (or RT-qPCR).

- Infection of TiLV is considered to be **confirmed** if **two or more** of the following criteria are met:
- histopathology consistent with disease
- detection of a TiLV by RT-PCR and amplicons' sequence analysis
- TiLV are isolated from infected fish, followed by performing the cell-culture or laboratory infection in conjunction with the diagnostic methods for TiLV.

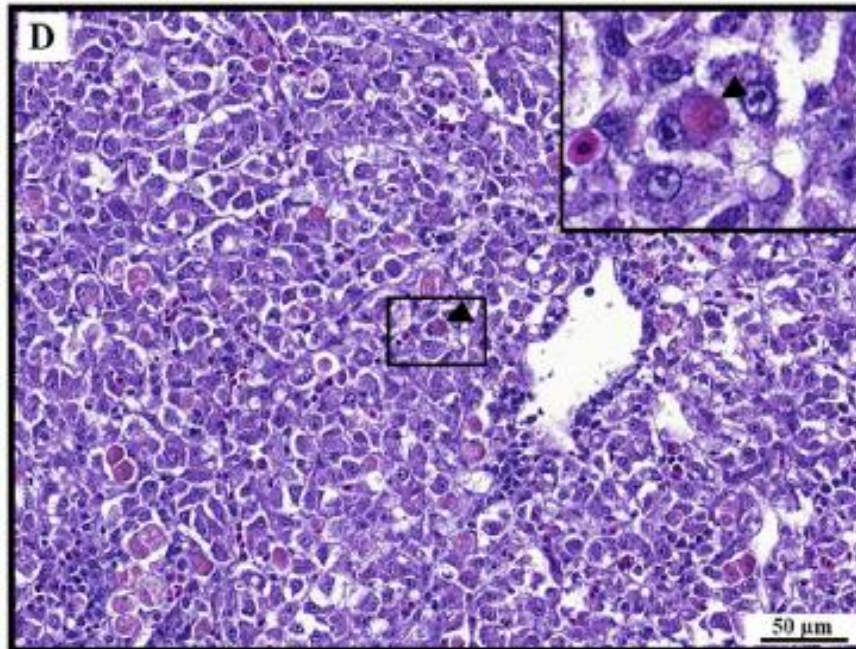
3.2 Laboratory methods

a) histopathology

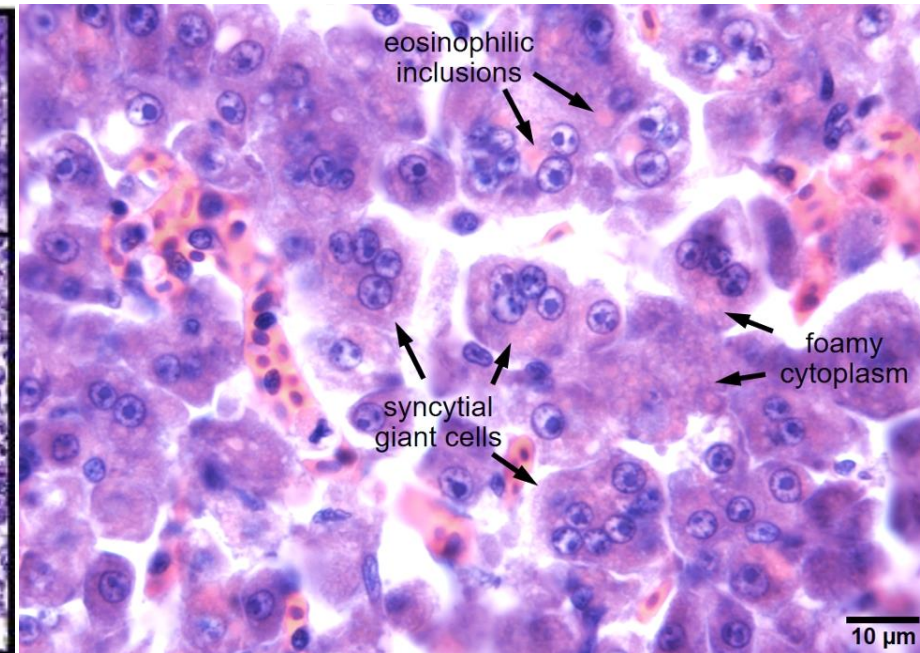


Other histopathological changes

Liver



Intracytoplasmic inclusion bodies
Tattiyapong et al. 2017



Syncytial giant cells, intracytoplasmic
inclusion bodies, foamy cytoplasm
(HT Dong)

b) Molecular techniques

i) TiLV specific RT-PCR (RT-qPCR)

- nested RT-PCR
- RT-qPCR
- POCKIT Micro (42 min)



ii) In situ hybridization

c) Virus isolation and cell culture

d) Laboratory infection

Chapter 4: Prevention and treatment

4.1 Vaccination:

TiLV vaccine is not available. Vaccines are pathogen-specific and relatively expensive, TiLV vaccines are under development in Israel and Thailand.

The use of vaccines needs to be approved by Competent Authority.

4.2. Immunostimulants

- Alternative to vaccines
- Cost effectiveness
- Enhance the innate and adaptive immunity
- Broad spectrum activity
- Natural products, ecological friendly
- Protect younger fish their immunity are not fully developed

4.3 Probiotics

- enhance the physiological and immunological responses of fish
- improve water quality
- Protect from the pathogens
- Reduce the use of chemicals and antibiotics

Successful probiotic agents used in fish aquaculture mostly belong to the genera *Bacillus*, *Lactobacillus*, *Enterococcus*, *Carnobacterium*, *Saccharomyces*.

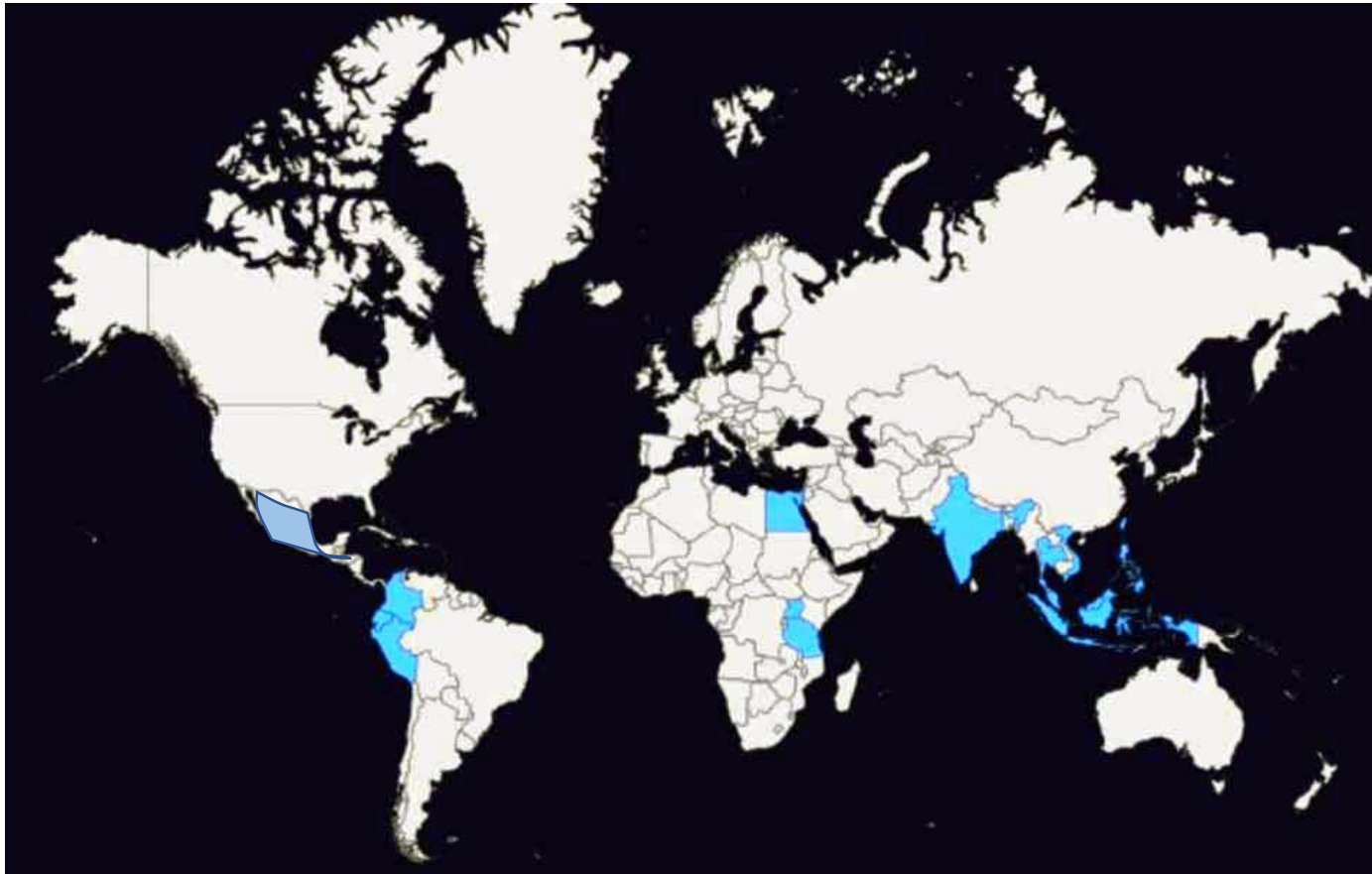
4.4 Biosecurity

a set of preventive measures designed to:

- reduce the risk of introducing pathogens into fish farms,
- reduce the viral loads in the farm and prevent viral transmission within the farm, and
- avoid spreading viruses to neighboring facilities and the environment.

Chapter 5. Epidemiology

5.1 Geographic distribution



TiLV was reported in 15 countries, most recently in Vietnam and Mexico during 2018

To clarify non-confirmatory reports/publications:

Senapin et al. (2010) False rumours of disease outbreaks caused by infectious myonecrosis virus (IMNV) in the whiteleg shrimp in Asia. **Journal of negative results in Biomedicine (Journal is discontinued)**

Other journals, such as **BMC Research Note**

5.2 Persistence in the environment: no data

As ISAV, which can survive up to 18 days in seawater at 28°C. ISAV were stable in the pH range 5 – 9.

- pH 4: the virus was completely inactivated after 30 min
- pH 11: a 90 % reduction in infectivity was observed after 30 min
- 56°C: completely inactivated the virus in 30 min
- Chlorine (100 mg/mL): inactivate the ISAV in 15 min

5.3 Mode of transmission:

a) Vertical transmission: by RT-PCR, TiLV were detected in the fertilized eggs and yolk-sac larvae, suggesting maternal transmission may be involved.

Hatchery	Year	Species	Fish stage	RT-PCR/sequencing
H1	2012	Red tilapia	Yolk-sac larvae	+
H2	2013	Red tilapia	Fertilized eggs	+
		Nile tilapia	Fertilized eggs	+

b) Horizontal transmission: via cohabitation (healthy fish became infected after 10 days), contaminated water (viral shedding) or contaminated sediments.

5.4 Vectors and reservoir hosts

5.5 Factors influencing disease transmission and expression: handling, temperature (summer mortality), stocking density, water quality

5.6 Impact of the disease: Tilapia is the second most important fish after carps cultured worldwide, with annual production surpassed 6 million tons in 2016. Diseases could cost the global tilapia aquaculture industry billions of dollars.

Chapter 6. Principles of control and eradication

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6.1 Methods to prevent spread and to eliminate TiLV

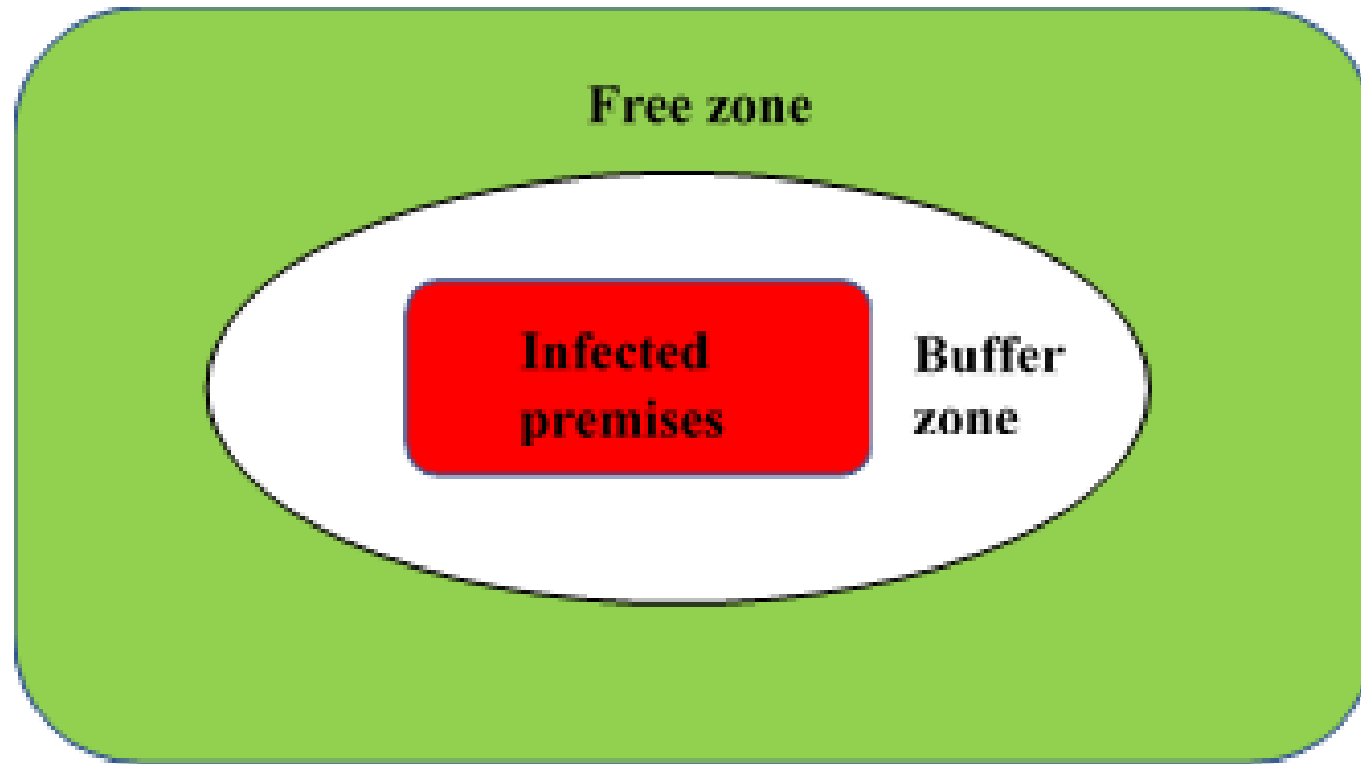
6.1.1 Quarantine and movement controls

Establish appropriate zone and compartment designations

- (1) Infected premises or area - the premises (e.g. farm) or area where the infection is present, and the immediate vicinity
- (2) Buffer zone —an area adjacent to infected premises or area
- (3) Free area—non-infected area

Control area: infected area and buffer zone.

Ideally, Should have the disease prevention plan prior to building a tilapia farm, or before the introduction of fish/eggs



Designation of zone, area, and premise in the TiLV outbreak response.

Movement controls from the infected areas should include:

- bans on the movement of **live tilapia** from the infected premises into TiLV-free areas
- restrictions on releasing **fish and pondwater** from the infected area into aquatic environments
- restrictions the use and movement of equipment and vehicle between farms within the infected area
- Restrictions on harvest and then transporting fish in the infected area to off-site processing plants
- Bans on discharging of processing plant effluent within the infected area
- Control of disposal of infected fish (**formic acid**)

6.1.2 Tracing

-to investigate if the TiLV infection has spread to other areas

-to identify the sources of the TiLVD

- **live tilapia**—for example, broodstock, fry, fingerlings
- **fresh fish**—uncooked fish intended for consumption
- **effluent and waste products**—from processing plants and farms
- **farm water**—discharge into or nearby coastal or inland waters
- **vehicles**—potentially contaminated transport vehicles, feed trucks, cars and boats
- **materials**—nets, buckets and other and farm instruments

6.1.3 Surveillance

- (a) to detect the presence of TiLV
- (b) to determine its prevalence in populations
- (c) used for maintaining, and certification of, farms freedom from TiLV

-Diagnosis: histology, RT-PCR (RT-qPCR), cell-culture

-Rapid on-site diagnostic assays: e.g. hand-held POCKIT™

6.1.4 The use of TiLV-free fish

6.1.5 The use of immunostimulants

6.1.6 The use of probiotics

6.1.7 Disinfection of fish and fish products

6.1.8 Destruction of infected fish

6.1.9 Disposal of hosts

6.1.10 Decontamination

6.1.11 Vector control

6.1.12 Environmental considerations: the presence of wild hosts

Avoid the presence of unwanted animals (such as fish-eating birds) through the use of screens, as they can be a source for fish pathogens.



piscivorous birds,
such as herons,
hammerhead stock



Avoid to feed
piscivorous birds
the viscera around
the fish pond (lake)
site

6.1.13 Public Awareness

- (a) To support the response strategy by coordinating government agencies and stakeholders to provide unified messages to the local, national and international audiences,
- (b) To address the concerns relating to food safety, public health, the environment,
- (c) To address issues related to regional commerce, continuity of business, and international trade,
- (d) To disseminate information to consumers and producers through workshops, agency technical reports, industry bulletins, social media.

6.2. Control, containment, and eradication options

6.2.1 Eradication

- (a) Prohibiting imports of potentially infected fish,
- (b) Screening imports of live or frozen commodity fish as well as other susceptible species,
- (c) Destruction and safe disposal of all fish at infected farms,
- (d) Disinfection of pond and reservoir water
- (e) Decontamination of pond bottom, tanks, equipment, supplies and facility surfaces through drying, application of appropriate chemical agents.

6.2.2. Containment and zoning

- (a) Establishing infected and free zones.
- (b) Prohibiting movement of infected tilapia, un-cooked fish products, or any contaminated materials into TiLV-free areas.
- (c) Establishment of well monitored buffer zones where spread of TiLV can be detected before TiLV-free zones are affected.

6.2.3. Control and mitigation of disease

- (a) Implementing more rigorous methods of eliminating potential vectors
- (b) Control the movements of live fish from the infected premises
- (c) **Reduce the stress factors**

6.3 Trade and industry considerations

6.3.1 Domestic markets

Place restrictions on transporting or marketing some products between infected and disease-free areas.

6.3.2 Export markets

In countries where TiLV is exotic, import countries may require imports to be certified free of TiLV and **reject tilapia batches that test positive for TiLV**

7. Policy and Rationale

7.1 Overall Policy

Implement national aquatic animal health strategies (NAAHS).

7.2 TiLV-specific objectives

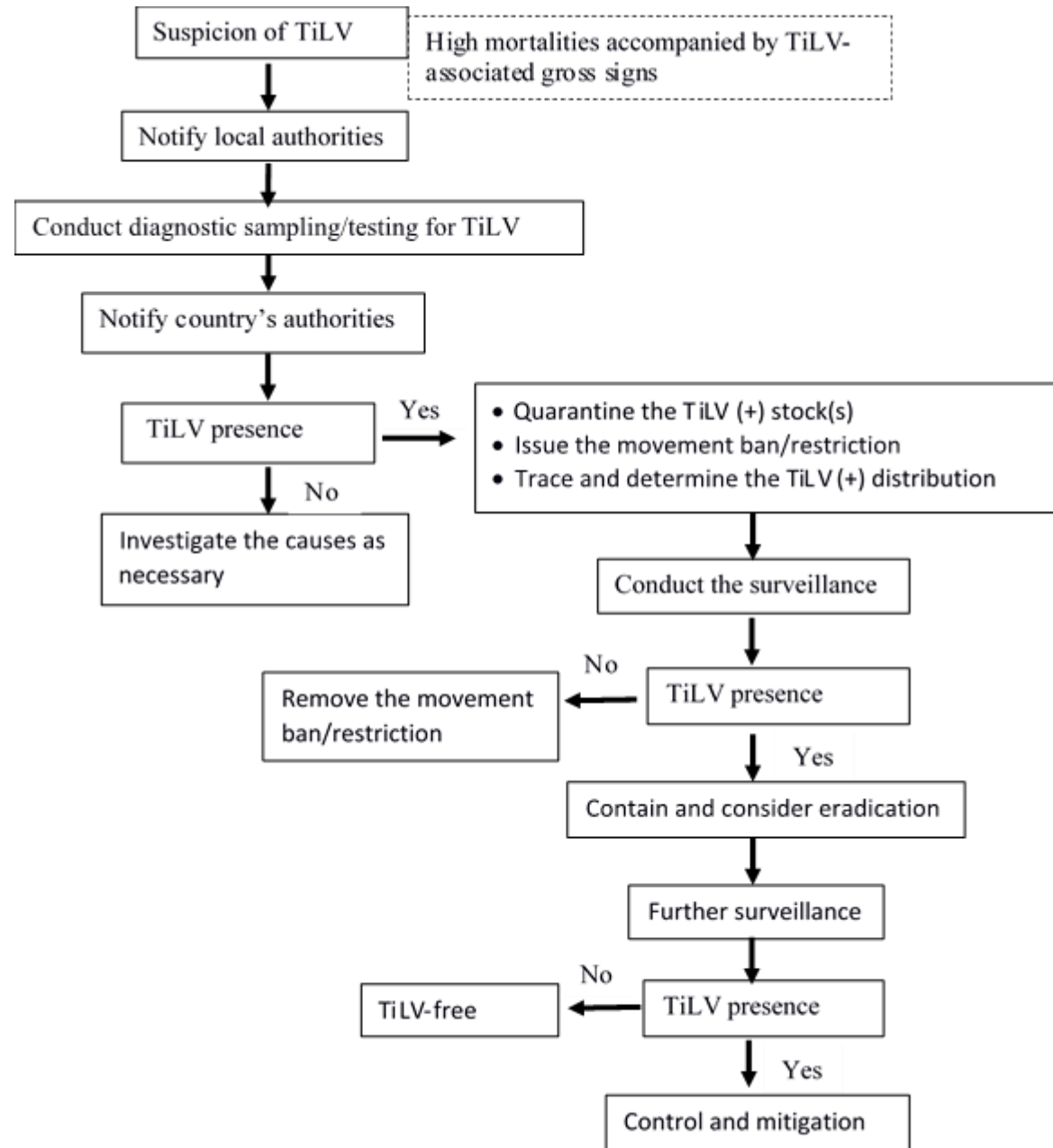
- (1) to eliminate TiLV from the country if possible
- (2) to prevent re-emergence of TiLVD
- (3) to prevent the spread of the disease to farmed or wild populations
- (4) to minimize the impact of the disease on commercial production
- (5) to prevent loss of domestic and international markets for locally farmed tilapia
- (6) to ensure that stakeholders and the public are informed of the issues involved in preventing the introduction or spread of TiLV through improper importation or movement of fish products

7.3 Problem definition

An outbreak of TiLV can result in severe economic losses due to high mortality. Without a proper response to its emergence, **this disease can be rapidly spread**, and the virus could become **established in the farmed and wild populations**.

Initial efforts should be to quickly identify the TiLV through **diagnostic procedures** and then to immediately institute procedures to **eradicate or to control the disease**.

7.4 Overview of response options



7.5 Strategies for eradication and control

7.5.1 Eradication from production facilities

- (a) Diagnosis and surveillance
- (b) Disposal of diseased stock and disinfection**
- (c) Fallowing**
- (d) Restocking with TiLV-free stocks**
- (e) TiLV-free declaration**

TiLV has been eliminated

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- based on a series of surveys of farmed and wild populations of susceptible species over at least a two-year period
- 2 years of basic biosecurity
- There should be 2 surveys per year to be conducted 3-4 more months apart,
- provide a greater than 95% confidence with a prevalence of 2 % or lower,
- tilapia to be sampled are preferred to display any gross signs,
- with the involvement of the competent authority of the country.

7.5.2 Containment and movement control

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- (a) Restrictions on movement of fish products
- (b) Restrictions on water discharge
- (c) Prevention of spread by wildlife
- (d) Surveillance

7.5.3 Management and mitigation

- (a) Manage facilities restored to TiLV-free status:
stock healthy fish and high levels of biosecurity
- (b) Manage farms showing low levels of TiLV infection:
reduce stress factors, the use of best management practices

7.6 Social and economic effects

- (a) Farmers may fall into debt
- (b) Devastating to the communities depending entirely on incomes from fish farming

7.7 Funding for contingency planning and compensation

- (a) Adequate funding by government
- (b) Fish farmers association

Barriers to contingency planning

- **Neglect early warning**
- **Lack of commitment**
- **Lack of support from government in policies and actions**
- **Lack of financial support and resources**

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Thank you for your attention

Hakuna Matata



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