FAO/ASTF project: GCP/RAF/510/MUL:

Enhancing capacity/risk reduction of emerging Tilapia Lake Virus (TiLV) to Africa tilapia aquaculture: Intensive Training Course on TiLV

In cooperation with Kenya Fisheries Service (KeFS) and Kenya Marine Research Institute (KMFRI

KENYA NATIONAL ACTION PLAN ON Tilapia Lake Virus (TiLV)

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1. INTRODUCTION

Food and agriculture are key to achieving the entire set of Strategic Development Goals (SDGs), and many SDGs are directly relevant to fisheries and aquaculture, in particular SDG 14 whose objective is to Conserve and sustainably use the oceans, seas and marine resources for sustainable development. Tilapias are a good fish for warm water aquaculture. They are an important global food source due to their omnivorous diet, tolerance for high-density aquaculture, and relative disease resistance. They are easily spawned, use a wide variety of natural foods as well as artificial feeds, tolerate poor water quality, and grow rapidly at warm temperatures. These attributes, along with relatively low input costs, have made tilapia the most widely cultured freshwater fish in tropical and subtropical countries.

Nile tilapia (*Oreochromis niloticus*) is the foremost cultured species (FAO 2005). In 2014, the global tilapia production was valued at 4.5 million tons and is expected to surge to 7.3 million tons by 2030 (FAO 2014). Worldwide harvest of farmed tilapia has now surpassed 800,000 metric tons, and tilapias are second only to carps as the most widely farmed freshwater fish in the world. Specifically, Egypt represents over 90% of the commercial Arab aquaculture production and currently ranks second to China with regard to global tilapia output (FAO 2005). Tilapia and Nile perch are the top most traded fish in terms of value and are the most popular with customers.

Freshwater aquaculture development in Kenya in the new millennium is remarkable making Kenya one of the fast growing major producers in Sub-Saharan Africa. From the annual production of about 1 000 tonnes in 2001–2006, the harvest of farmed fish leaped to over 4 000 tonnes in 2007–2009. In a nationwide fish farming mass campaign launched by government in 2009, the total area of fish ponds was increased from 220 ha to 468 ha by building 7 760 new fish ponds. Together with the improved seed supply and supports covering other aspects, it lead to a hike in farmed fish production reaching 23 501 tonnes in 2013, more than four times of the production in 2009. Main species farmed is Nile tilapia (75 percent), followed by African catfish, common carp and rainbow trout (Fig. 1).

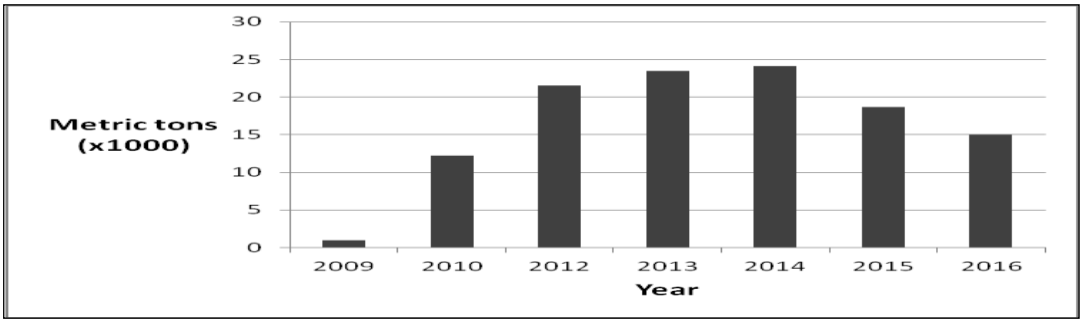

Figure 1; Kenya aquaculture production trends between 2009 and 2016, main species harvested from ponds and cages is Nile tilapia. (State Department for Fisheries and The Blue Economy)

Since 2009, tilapia aquaculture has been threatened by mass die-offs in farmed fish in Israel and Ecuador. The tilapia lake virus (TiLV) has a 10-segment, negative-sense RNA genome. The largest segment, segment 1, contains an open reading frame with weak sequence homology to the influenza C virus PB1 subunit. The other nine segments showed no homology to other viruses but have con- served, complementary sequences at their 5= and 3= termini, consistent with the genome organization found in other orthomyxo-viruses. In situ hybridization indicates TiLV replication and transcription at sites of pathology in the liver and central nervous system of tilapia with disease.

2. TILAPIA AQUACULTURE IN KENYA

Cichlids of the genus tilapia endemic to Kenya are distributed throughout the country's fresh water bodies (Fig. 2), which include lakes, rivers and man-made lakes. The table 1 below shows the distribution of known tilapine species in Kenya.

Table 1: Distribution of wild tilapia stocks in Kenya

Cichlidae Species	Distribution
Oreochromis hunter	Lake Chala
Alcolapia graham	Lake Nakuru, Lake Elementaita, Lake Magadi
Alcolapia alcalicus	Ewaso ngiro
Oreochromis jipe	Lake Jipe,
Oreochromis esculentus	Lake Jipe, Lake Kanyaboli, Lake Victoria
Oreochromis leucostictus	Lake Victoria, Lake Kanyaboli, Lake Naivasha
Oreochromis niloticus	Lake Victoria
Oreochromis niloticus baringoensis	Lake Baringo
Oreochromis niloticus sugutae	River Suguta
Oreochromis niloticus vulcani	Lake Turkana
Oreochromis spirulus spirulus	Lower Tana, North Ewaso Ngiro, Galana-Sabaki River, Lake Kamnarok
Oreochromis spirulus niger	Athi River, Upper Tana
Oreochromis spirulus percivalis	Hot springs of Ewaso Ngiro
Oreochromis variabilis	Lake Victoria Basin
Pseudocrenilabrus multicolor victoriae	Lake Victoria, Lake Kanyaboli, Upper Athi and Tana Rivers
Salotherodon galilaeus galilaeus	Lake Turkana drainage
Tilapia rendalli	Lake Victoria, Pangani drainage, Tana River
Tilapia zillii	Lake Turkana, Lake Victoria system, Lake Naivasha, Tana River

(Common freshwater Fishes of Kenya, 2013: Nyingi W. D.)

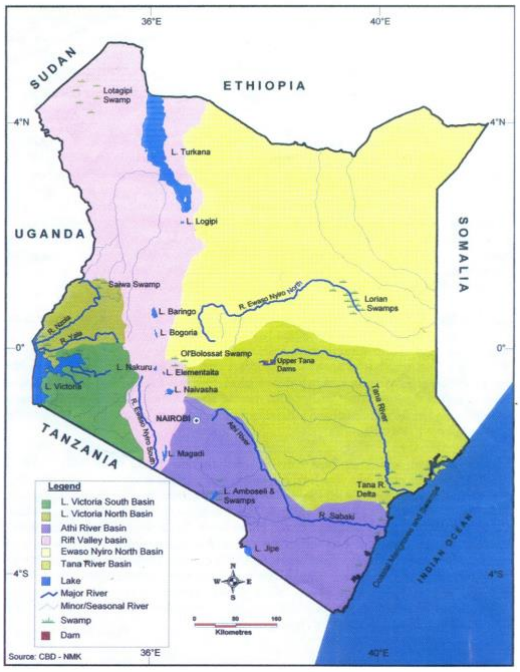

Figure 2: Map of Kenya Showing distribution of Water bodies, They include Marine (Indian Ocean) and Inland Waters; Lake Victoria, Lake Turkana, and Major Rivers.

3. SPECIES OF FRESH WARM WATER FISHES FARMED IN KENYA

- Nile tilapia (*Oreochromis niloticus*)
- African catfish (*Clarias gariepinus*)
- Common carp (*Cyprinus carpio*)

Commercial aquaculture producers are mainly focused on three production systems:

1. Cage fish production – Food fish/Grow-outs
2. Pond fish either liner or earthen ponds – Food fish/Grow-outs
3. Hatchery - seed production

The three practices are at either semi or intensive levels of production and as carrying capacities rise, monitoring water quality gain importance because fish becomes more susceptible to diseases.

4. AQUACULTURE PRODUCTION IN KENYA

Most farmers who were farming fish at a subsistence level are now categorized as small-scale commercial fish farmers earning as much as KSh 450,000 (i.e. US$ 6,000) per acre of water surface annually and some now focused on value addition and export markets. Therefore, it is more likely that in the coming years, aquaculture will contribute significantly to food security and foreign exchange earnings in Kenya (FAO, 2006).

Farmed fish production is dominated by Nile tilapia (*Oreochromis niloticus*), which makes about 90% of the production. Majority of aquaculture farms are found in areas with moderate to high rainfall i.e. Nyanza, Western, Central Rift Valley, Eastern and the Coastal Region. The climatic conditions in these regions allow production to be done throughout the year. The production is done under semi intensive pond systems and cages. However intensive production in tanks and raceways is on the rise. Over 90% of the cages are found in Lake Victoria. Majority of fish farms and hatcheries are concentrated in Western Kenya (around the Lake Victoria basin) and Central and Eastern Kenya (Tana River basin).

Figure 3: Earthen pond and Lined ponds in aqua farms in Kenya

Under intensive practices, farmers use raceways, various forms of tanks made of concrete, PVC, Fibre glass and even iron. Figure 3 below show examples of concrete raceways, concrete tanks and wooden tanks used in fish production in Kenya.

Figure 4: Concrete Trout raceways

Figure 5: Concrete and wooden indoor fish tanks

Figure 6: PVC and glass tanks used in an indoor hatchery in Kenya

Cage aquaculture is increasingly becoming important especially in Lake Victoria. Majority of the cages are made of polyethylene netting material supported by a steel frame. The cages are fitted with a steel lid, plastic barrels as floaters, locally made sinkers and anchors. Majority of the cages are fabricated locally on site.

Figure 7a: Completed cage ready for installation

Figure 7b:Installed cages in the lake

As at the end of 2016 there were about 65,000 fish ponds (including tanks and raceways) covering about 2,500 Ha and 1,663 cages with a total volume of about 12,850m^3. These production units

are owned by about 67,000 fish producers. Majority of the fish ponds are operated by small scale, semi intensive farmers with 1-4 ponds measuring between 200m^2 to 300m^2.

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5. EMERGENCE OF TILAPIA LAKE VIRUS (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.

5.1 Diagnostic methods for TiLV

Clinical signs of TiLV infection include: skin erosion, haemorrhage at the base of fins and opercula, scale protrusion, abdominal swelling, skin darkening, gill pallor, and ocular alterations. Infected fish also exhibit abnormal behaviour like lethargy, loss of appetite, swimming at the surface, and loss of balance.

Syncytial hepatitis is the most common histopathology feature found in TiLV outbreaks. In 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. 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. For 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.

5.2 Suspect TiLV case

A fish population showing at least 20% mortality for 3 consecutive days with or without the following clinical signs: skin erosion, haemorrhage at the base of fins and opercula, scale protrusion, abdominal swelling, skin darkening, gill pallor, and ocular alterations. Infected fish also exhibit abnormal behaviour like lethargy, loss of appetite, swimming at the surface, and loss of balance.

5.3 Suspect TiLV Location

This refers to location in where one or more suspect TiLV fish have been found.

5.4 Confirmed case

A fish showing the above mentioned clinical signs with positive results from histopathology (e.g. syncytial hepatitis in liver cells) and RT-PCR and/or qRT-PCR

6. THE NATIONAL ACTION PLAN ON TiLV

6.1 Aims/Purpose of surveillance program

Kenya's TiLV Surveillance scenario is that there are no reported cases and no known surveillance activities have been done and therefore Kenya is at Risk.

For this reason, a National Action Plan (NAP) on TiLV shall be established, documented and implemented by the Competent Authority.

The objective of this NAP is to investigate the presence or absence of TiLV in Kenya so as to acquire knowledge and build capacities on TiLV pathology, diagnostics, surveillance and emergency preparedness.

6.2 Specific Objectives are to:

- To investigate presence or absence of TiLV in farmed and wild tilapia
- To develop mechanisms for securing early detection of TiLV in the country
- Adopt effective farm level TiLV bio-security governance to reduce the negative impacts of the disease.
- Enhance capacity building and training among stakeholders and participating institutions in dealing with TiLV to reduce risk
- Identify possible risk factors for spreading TiLV in order to develop a TiLV surveillance mechanism in Kenya
- Maintain freedom of TiLV status in the country though continuous surveillance
- To ensure tilapia sector profitability through production of healthy tilapia

6.3 Expected outcomes will be:

i) Effective bio-security governance through well-planned TiLV national action plans to reduce the negative impacts of the disease.
ii) Enhanced information and knowledge on good and effective aquaculture bio-security and Aquatic Animal Health (AAH) management practices shared among participating institutions and fish farmers
iii) Shield/protect Tilapia aquaculture industry from the negative effects of TiLV
iv) Increase profitability to producers through increased healthy tilapia sold and consumed

This Plan of Action is based on the following strategic lines of action:

a) Promote strong and accurate investigative mechanism or surveillance to ascertain the health status of a given population aimed at early detection and control systems

b) Develop strategic disease diagnostic mechanisms for those tilapia showing signs of health deterioration

c) Strengthen laboratory capacity to support diagnosis, surveillance, and maintain freedom of healthy animals to ensure they are not carrying subclinical infections.

7. DEFINITION OF POPULATION:

This refers to fish species susceptible to TiLV. In Kenya's case, this will be farmed and wild Nile Tilapia (*Oreochromis niloticus*) at all developmental stages during their growth in:

i) Cages
ii) Ponds
iii) Hatcheries

Tilapia Lake Virus (TiLV) hotspots in Kenya are as shown in figures 6 and 7 and include:

i) Cages in Lake Victoria where the following zones will be selected for the study: Kadimu bay zone I, Naya Zone II, Matara Zone III, Winam Zone IV

ii) Tilapia Hatcheries: These have been zoned into Western region (as Region I) and Central region (as Region II)

iii) Tilapia Fish Farms: This will involve pond fish farms in Western region (as Region I) and Central region (as Region II)

iv) Wild Tilapia in Lake Victoria: Four sampling stations have been selected as follows - Kadimu bay zone I, Naya Zone II, Matara Zone III, Winam Zone IV

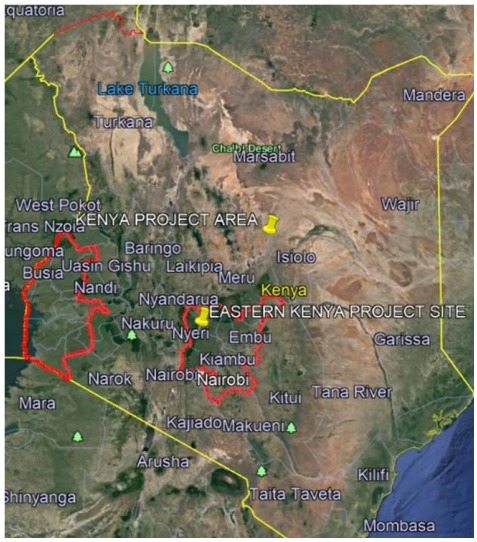

Figure 8: TiLV Sampling zones for Ponds and Hatcheries

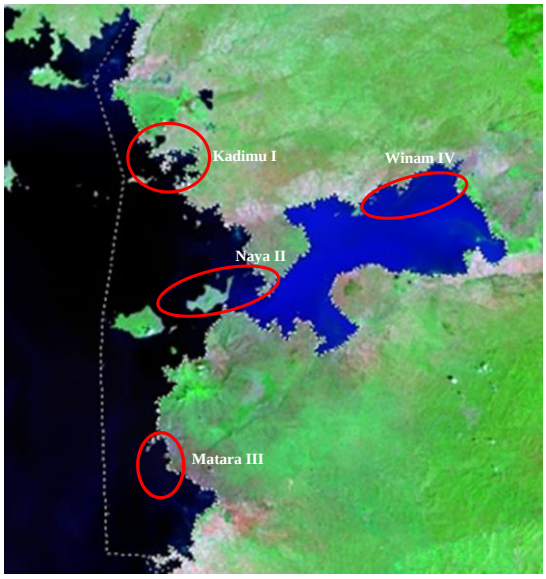

Figure 9: TiLV Sampling zones for cages and wild tilapia in L. Victoria

8. SAMPLING

In the case of wild tilapia populations, non-random spatial sampling will be used while in the case of farmed tilapia populations, random sampling from a list of known fish farms will be used.

Samples in the case of farmed tilapia will be taken from 40 sampling units as summarized in table 2.

Table 2: Distribution of farms/zones and numbers to be sampled

		Western (Region I)	Central (Region II)
System/ Operation	**Percentage (%)**	**No. Of farms**	**No. of farms**
Cages	50	20	0
Ponds Farms	30	4	8
Hatcheries	20	4	4
Total	100	28	12

Cages in Lake Victoria will be selected from Kadimu bay zone I, Naya Zone II, Matara Zone III, and Winam Zone IV (Figure 9)

Wild Tilapia in Lake Victoria: 4 sampling stations have been selected as follows - Kadimu bay zone I, Naya Zone II, Matara Zone III, Winam Zone IV (Figure9)

Aside from sampling for TiLV, basic water quality parameters will be recorded that include temperature, DO, pH, Nitrates, Ammonia and Phosphates

8.1 Sampling plan

The frequency of sampling and sampling matrices and parameters that will guide the sampling plan in regions I and II is shown in Table 3.

The following shall apply:

i. Samples shall be taken at farm level, on fish at all stages of farming
ii. Samples shall be accompanied by sample submission forms (i.e. Documentary Evidence).

Procedure for disease screening shall be adhered to and screening of specific fish disease shall be done in accordance with the Animal diseases Act CAP 364. TiLV Validation will be done by the Competent Authority (CA) and assisted by experts in aquatic animals' health. The CA will also select the laboratory to carry out the various tests.

Table 3: Sampling plan:

Zones	**Parameter**	**Sampling frequency**	**Target species**	**Sampling matrix**	No of farms to be sampled per week
Western (Region I)	TiLV	Twice annually	*Oreochromis niloticus*	Clinical signs etc Organs of interest	4
Central (Region II)	TiLV	Twice annually	*Oreochromis niloticus*	Clinical signs etc Organs of interest	4

8.2 Sampling materials:

Target organs for this surveillance will be the liver for both histopathology and RT-PCR and spleen for histopathology.

8.3 Sample collection:

Samples will be collected from tilapia both from the wild and culture facilities with and without clinical signs at the specific time of sampling. The frequency of sampling for TiLV monitoring will be twice per year during the months of March to April and November to December when the water temperatures will be between 22°C and 32°C.

Duplicate samples of fish shall be sampled from randomly selected Cages/Farms/Lake/Hatcheries. The assumption here is that the samples drawn shall be representative of the tilapia populations. The samples will be handled aseptically and preserved appropriately until tested for TiLV.

9. CLUSTERING OF THE DISEASE

TiLV occurs mostly at water temperatures with a range of 24 - 25°C. Although TiLV has not been reported in Kenya, we would like to investigate the presence or absence of TiLV in all stages of growth of Tilapia both in culture and capture situations. In Kenya, tilapia production is done mostly in water whose temperatures range between 22-32°C throughout the year.

The ideal water quality parameters for optimum growth and survival of the target fish species are shown in Tables 4 and 5.

Table 4: Water Quality Parameters for Hatcheries

Type of Hatchery	**Parameters**					
	pH	**Temperature (◦C)**	**Dissolved oxygen (ppm)**	**Nitrites (ppm)**	**Nitrates (ppm)**	**Salinity (ppt)**
Fresh water prawns	7.0-8.5	28-30	>5.0	<0.1	<2.0	-
Marine shrimps	7.0	28	>5.0	-	<0.1	35
Finfish	6.5-8.5	28-30	>4.0	-	<0.1	-

Table 5: Water Quality ranges for Fresh Water Cultured Fish

Parameters	Cold water fish	Warm water fish (e.g. Nile Tilapia)
Temperature (°C)	14 – 17	22 – 32
Salinity (ppt)	0 – 25	0 – 25
Alkalinity/hardness (as CaCO3) (mg/l)	40 – 70	40 – 70
Ph	6.5 – 8.5	6.5 – 8.5
Dissolved oxygen (mg/l)	≥ 5	≥ 3
Phosphate (mg/l)	10 – 100	10 – 100
Unionized ammonia (NH3) (mg/l)	< 0.5	< 0.5

10. CASE DEFINITION

For the purpose of this study, the case is defined as farmed and wild Nile Tilapia (*Oreochromis niloticus*) at all developmental stages during their growth.

11. DIAGNOSTIC TESTING

11.1 List of Laboratories:

Two national reference laboratories will be involved in the sampling and testing of the surveillance samples:

1. KeFS laboratory at Kisumu – Histopathology and preparations of samples for PCR confirmatory tests

2. KMFRI laboratory at Kisumu – Preparations for samples for histopathology and subsequent tests

11.2 Laboratory tests to be conducted:

The following tests will be performed:

i. Observation of clinical signs and gross pathology: (red spots or small to large ulcerative lesions on the body)
ii. Microscopic pathology: pathological observations in sections of liver and the spleen.
iii. Isolation of pathogen and PCR or semi nested RT-PCR if kit is availed
iv. Validation test – send samples to the reference labs for confirmation

Table 6: TiLV Monitoring Plan

Parameter	Matrix Analyzed	Frequency	Requirement
TiLV	Liver and Spleen	Twice per year	Shall be Absent

During implementation of the sampling plan, the following shall apply:

- Samples shall be taken at farm level, on fish at all stages of farming including live fish imports.
- Samples shall be accompanied by sample submission forms (i.e. Documentary Evidence).
- Procedure for disease screening shall be adhered to and Screening of specific fish disease shall be done in accordance with the Animal diseases Act CAP 364.

The procedure for screening will include;

a) Selection of diagnostic criteria for identifying infected or progeny at risk and development of a response plan.
b) Tracking of sampled stock including progeny until screening results are received and reviewed.

11.3 Diagnostic methods for TiLV

Gross Pathology or checking of Clinical Signs for TiLV infection will include: skin erosion, haemorrhage at the base of fins and opercula, scale protrusion, abdominal swelling, skin darkening, gill pallor, and ocular alterations. Infected fish also exhibit abnormal behaviour like lethargy, loss of appetite, swimming at the surface, and loss of balance.

Histopathology will involve checking for histopathology feature found in TiLV outbreaks. These are syncytial hepatitis, massive cellular necrosis with pyknotic and karyolitic nuclei; eosinophilic cytoplasmic inclusion bodies in the liver cells. Confirmatory Tests will involve molecular detection method, RT-PCR, RT-qPCR. For Pond-site detection, a portable TiLV RT-PCR detection kit will be used. Prepared samples will be shipped to a competent laboratory for confirmation tests.

12. STUDY DESIGN AND ANALYSIS METHODOLOGY

To ensure success of this survey, target participants in the selected sampling regions will be invited to enrol through invitation letters. Those tilapia farms who will respond in affirmation will form the epidemiological units for the purpose of this survey.

The unit of sampling will be a mix of 30 tilapia fish with or without clinical signs of infection from ponds, hatcheries and cages at the selected farms.

The total number of enrolled and participating tilapia farms will be 40 which will be sampled (visited) twice during the sampling period which will begin on March 2019. A total of eighty (80) field visits will be done. The first round of visits will be made during the months of March and April 2019 and the second round of visits during the months of November and December 2019. It is estimated that 3 tilapia farms will be sampled per week during the sampling activities

12.1 Survey Design

Wild fish population: The surveillance is designed to provide 95% confidence of detecting the presence of TiLV, if present in 1% of the fish population or more using a diagnostic test with 95% sensitivity (Se) and 100% specificity (Sp).

The value of 10% (design prevalence) is selected to be practical and to reflect the current knowledge of TiLV in Kenya.

Farmed fish population: The surveillance is designed to provide 95% confidence of detecting the presence of TiLV in farms if TiLV occurs in 5% of the farms or more and if TiLV occurs in 2% of the farmed fish population or more within a farm using a diagnostic test with 95% sensitivity (Se) and 100% specificity (Sp).

13. DATA ANALYSIS METHODS

The surveillance will use a sample size of at least 10% of the fish population

13.1 Data flow Management

Data collection will be done using separate questionnaires for wild and farmed fish depending on population sampled.

After sampling, clinical signs of TiLV will be observed and two (2) copies of the laboratory form will be completed and one (1) copy will be sent together with fish samples to a diagnostic laboratory.

All data including laboratory results will be entered into an excel data sheet.
Data traceability will be secured (farm or population data with laboratory results) and correct data recording in the field will be ensured.

14. VALIDATION

This step will be done throughout the whole process from the design until the actual implementation. The surveillance design and implementation plan will be reviewed by Kenya CA in The State Department for Fisheries and The Blue Economy with the assistance of relevant project proponents.

15. QUALITY ASSURANCE

There will be administrative and procedural activities which will to be used in order to avoid problems and where problems or mistakes occur, corrective measures will be introduced. These will guarantee quality implementation of the surveillance plan.

These activities include:

a) Establishing a National surveillance team (NST)
b) Training and education of NST on TiLV pathogen biology, pathology, diagnostics and surveillance; data collection and a questionnaire described and explained clearly and common understanding achieved;
c) Diagnostic laboratory accredited in line with ISO 17025, if possible; trained field and laboratory personnel;
d) A clear standard operating procedures (SOPs) developed and used during implementation,
e) Aseptic technique procedures for minimizing contamination from potential areas of sample collection developed and made clear to the sampling teams;
f) Sampling teams closely supervised; and a pilot survey will be conducted as a sampling exercise.

16. BIO-SECURITY PLANS IN FARMS/CAGES

Every effort will be made to make sure that every aquaculture facility shall have an effective bio-security program incorporating;

i) Disease prevention,
ii) Disease monitoring,
iii) Management of disease outbreaks,
iv) Cleaning and disinfection between production cycles, and
v) General security precautions.

17. MANAGEMENT COMMITMENT

Surveillance and diagnostic team:
A Total of 8 officers will be involved in this project for the entire sampling and data analyses period.

Table 7: Institution Capacity and staff that will be involved are as follows:

Institution	Number of officers	Remarks
KeFS	4 officers: i) Stanley Tonui (QA) ii) Henry Mbugua (Aqua) iii) Stephen Gichimu (Lab Tech) iv) Peter H. Okelo (Lab Tech)	
KMFRI	2 officers: i) Venny Mziri (Researcher) ii) Ephaim Odada (Lab Tech)	One social economist will be needed and will be selected from KMFRI – Kisumu station
UNIVERSITY	2 University researchers: i) Prof. Charles Ngugi ii) Prof. Julius Manyala	

NB: 5 Ship crews will be used when the KMFRI boat will be on hire to sample cage and wild fish on Lake Victoria only.

Due to limitations on the number of officers that can be involved in the activity, all the officers indicated in Table 7 above will participate in collection and diagnosis of the samples in both regions I and II.

18. ANNEX

I. TILV NECROPSY DATA SHEET

Form No. I: Farmed fish

Part: A

Particulars of aquaculture farm/facility:		**Particulars of sample collector:**	
Name:	…….	Name:	…….
Address:	…….	Address:	…….
	…….		…….
Telephone:	…….	Telephone:	…….
	…….		…….
E-mail:	…….	E-mail:	…….
County:	…….	Institution:	…….
Sub-county:	…….	Title:	…….
Location:	…….	Signature	…….
GPS	…….		

Part B

Sample particulars *(One location per submission form)*

Aquaculture farm/facility's Code …… Sample(s) Reference No./ID(s): .....

Species sampled: ..... Life stage sampled: .....

Sampling point (s) ..... Date of sampling ……

Temperature required during transport: …… Specimen type: Spleen: ☐

Liver: ☐

Others: .....

Necropsy Data Sheet

Date: ……

Case No.: ……

Name: ……

Phone: ……

Address: ……

History

Species affected ……

Specie in the system ……

Average size ……

Age of affected fish ……

No. of fish in the system ……

Estimate mortality (%)……

When mortality started/ended ……

Any new introduction? If yes, when and what……

Behavioural changes ……

Appearance of fish ……

Appetite ……

Others ……

Environmental parameters

DO …… mg/L
To …… mg/L
pH …… mg/L
Ammonia (total ammonia nitrogen)…… mg/L
Hardness…… mg/L

Nitrite…… mg/L
Nitrate…… mg/L
Salinity…… ppt
Chloride…… mg/L

Physical Examination

Behaviour ……
Appearance ……
Skin ……
Gills ……
Photo/video taken……Yes_____ No_____

Necropsy

Visceral cavity ……
Liver ……
Gall ladder……
Stomach ……
Intestine ……
Spleen ……
Kidney ……
Heart ……
Brain ……
Others……
Photo/video taken……Yes_____ No_____

Part C

B. Sample Delivery *(One location per submission form)*

Samples delivered by*:* ***Name:*** ……
Contacts: ……
Title: ……
Signature……

Reasons for Sampling: ……
……

Specimen delivered as:

Fixed:	☐	Describe: …… ……
Frozen:	☐	Temp:……
Live:	☐	Describe: …… ……

Parameters to be tested:

Spleen:	Histopathology	☐	RT-PCR,	☐	RT-qPCR	☐
Liver:	Histopathology	☐	RT-PCR	☐	RT-qPCR	☐

Original form goes with the sample to the Laboratory.
Duplicate form to be kept in the Farm's/Establishment's file

Form No. II: Wild fish

Part: A

Particulars of sampling locality:		**Particulars of sample collector:**	
Zone:	……	Name:	……
County:	……	Address:	……
Sub-county:	……		……
Location:	……	Telephone:	……
GPS location	……		……
BMU	……	E-mail:	……
Address:	……	Institution:	……
	……	Title:	……
Telephone:	……	Signature	……
E-mail:	……		

Part B

Sample particulars *(One location per submission form)*

Sampling location Code ……

Species sampled: ……

Sampling point (s) ……

Temperature required during transport: ……

Sample(s) Reference No./ID(s): ……

Life stage sampled: ……

Date of sampling ……

Specimen type: Spleen: ☐ Liver: ☐ Others: ……

Necropsy Data Sheet

Date: ……

Case No.: ……

Name: ……

Phone: ……

Address: ……

History

Species affected ……

Specie in the system ……

Average size ……

Age of affected fish ……

No. of fish in the system ……

Estimate mortality (%)……

When mortality started/ended ……

Any new introduction? If yes, when and what……

Behavioural changes ……

Appearance of fish ……

Appetite ……

Others ……

Environmental parameters

DO …… mg/L
To …… mg/L
pH …… mg/L
Ammonia (total ammonia nitrogen)…… mg/L
Hardness…… mg/L

Nitrite…… mg/L
Nitrate…… mg/L
Salinity…… ppt
Chloride…… mg/L

Physical Examination

Behaviour ……
Appearance ……
Skin ……
Gills ……
Photo/video taken……Yes_____ No_____

Necropsy

Visceral cavity ……
Liver ……
Gall ladder……
Stomach ……
Intestine ……
Spleen ……
Kidney ……
Heart ……
Brain ……
Others……
Photo/video taken……Yes_____ No_____

Part C

B. Sample Delivery *(One location per submission form)*

Samples delivered by*:* ***Name:*** ……
Contacts: ……
Title: ……
Signature……

Reasons for Sampling: ……
……

Specimen delivered as:
Fixed: ☐ Describe: ……
……
Frozen: ☐ Temp:……
Live: ☐ Describe: ……
……

Parameters to be tested:
Spleen: Histopathology ☐ RT-PCR, ☐ RT-qPCR ☐
Liver: Histopathology ☐ RT-PCR ☐ RT-qPCR ☐

Original form goes with the sample to the Laboratory.
Duplicate form to be kept in the relevant BMU file

ANNEX

II. DATA COLLECTION QUESTIONNAIRES

Questionnaires for collection of TiLV surveillance data form cage, pond, hatchery and wild fish population.

The purpose of these survey questionnaires is to collect specific data on wild and farmed fish populations relevant to the diagnosis of TiLV disease affecting susceptible fish. The results of laboratory analysis of field samples, together with analysis of complementary data collected by these questionnaires, will allow a science-based evaluation of the available data and information through a corresponding epidemiological study format (cross sectional). This will provide – comprehensive information on absence/presence of TiLV, frequency and distribution as well as an understanding of potential risk factors and their degree of association. Epidemiological data from this study will support the formulation of appropriate risk management measures to prevent and control spread of infection and to mitigate the impact of this disease.

(i) Questionnaire for collection of TiLV surveillance data from wild population

Name of sample collector		**Date**		**Data ID**	
County		**Sub County**			
Location		**Other**			
Location (GPS coordinates or narrow geographical location) Provide separate locations if inspections are not at the same place	Longitude:				
	Latitude:				
Describe water body	• Stream • River • Lake/Reservoir • Swamp • Flood plain • Other (specify)				
Flow/water level	• Drought • Average • Floor				
Water pH					
temperature					
Turbidity	• Clear • Muddy • Green • Other (Specify)				
Submerged vegetation present at sampling site	• Yes • No				
TiLV lesion seen on site	• Yes • No				
Other disease seen on site (if yes, describe)					
Total number of fish sampled at location?					
Sampling methods	• Dip net • Cast net • Gill net • Fisherman's catch • Live fish market • Other				
Number of fish inspected (per species)					
Number of fish (per specie) with suspected TiLV lesions					
Other observations					

(ii) Questionnaire for collection of TiLV surveillance data from farmed fish

Name of the interviewer		**Date**		**Data ID**			
County		**Sub County**					
Location		**Village**					
Farm ID		**Farm Owner**		**Gender**	• M • F	**Age (years)**	
Farm Contact Info							
Location (GPS coordinates or narrow geographical location) Provide separate locations if all ponds are not at the same location		Latitude: Longitude:					
What is the total area of cultured farm/ponds (ha)?							
What is the source of water used for the ponds/cages?	• Fresh water • River • Lake/reservoir • Brackish water (salinity less than 2 ppt) • Surface water • Other (Specify)						
What type of water management is used?	• Open (frequent change of water during the culture period) • Semi closed (water changed few times during the culture period) • Closed (no change of water during the culture period) • Recycled (water changed using recycled water)						
Water temperature							
Water pH							
Pond substrate	• Clayish • Muddy • Sandy • Concrete • Synthetic lining • Other (specify						
Do you use aerators in pond?	• Yes • No	If Yes what type					
Number of ponds/cages (overall)							
Are any of ponds/cages adjacent to or intermingled with ponds of other farms?						• Yes • No	
Are there other nearby farms (within 10km radius) using the same water source?						• Yes • No	
Type of culture	• Small scale • Intensive						

Fish species cultured	
What type of feed is used in the farm?	• Commercial fee • Self-made fee • Fertilized ponds/natural food
Sources(s) of fish for restocking (during previous year)	• Purchased from outside hatchery • Own hatchery • Wild-caught
Control of health of restocking – tested/certified by supplier to be free from diseases	• Yes • No
Treatment of ponds before restocking	• Pond is left dry for more than one week before restocking • Pond is left dry for less than one week before restocking • Top soils scraped out of pond when dry • Lime is applied: O Dolomite / O Agricultural lime / O Hydrated lime • Fertilizer is applied: O Chicken manure / O Chemical fertilizer (specify)
Applied bio-security measures	• Pond/farm has fencing • Farm uses sun-drying to disinfect nets • Pond/farm has disinfectant foot bath • Other
TiLV lesion seen on site	• Yes • No
Other disease seen on site (if yes, describe)	
Total number of fish on farm	
Number of fish sampled on site?	
Sampling methods	• Dip net • Cash net • Seine net • Harvested catch • Other
Species inspected on farm if other than the cultured species	
Number of fish (per species) on farm with suspected TiLV lesions	
Other observations	

19. KENYA NAP WORK PLAN AND ACTIVITY TIMELINES

Annual Kenya NAP work plan and activity timelines

Activities	Jan 2019	Feb	Mar	Apr	May	June	Jul	Aug	Sept	Oct	Nov	Dec	Jan 2020	Feb
Preparatory work														
Training/ Sensitisation														
Pilot test and sampling map														
Field sampling														
Data entry														
Data analysis														
Report writing and reporting														
Water temperature for TiLV (28-30 C)														
Culture Period		1		2	3	4	5	6	7	8	9			

20. BUDGET

Budget requirement for data collection and analysis for 8 months

Cages, Hatcheries, Ponds		
Activity	**Description**	**Cost in US$**
Sensitisation and meetings	2 meetings for 40 farmers	10,000
Data collection/Social economic survey/Sample collection and laboratory tests	80 samples (8 officers on data collection and 2 Lab technicians)	64,000
Equipments and Reagents		40,000
Total		**114,000**
Wild Tilapia in L. Victoria		
Activity	**Description**	**Cost in US$**
Hire of Boat (Lake Victoria)	4 excursions	40,000
Data collection/Social economic survey/Sample collection and laboratory tests	20 samples (3 officers from Kisumu and 5 ship crew)	20,000
Equipment and Reagents		6,000
Total		**66,000**
Grand Total in US Dollars		**180,000**

21. AQUACULTURE FACILITIES FOR POSSIBLE SAMPLING

WESTERN REGION: Region I (4 grow out farms and 4 hatcheries)

Homa Bay	Migori	Kisii	Siaya	Kisumu	Vihiga	Kakamega
HATCHERIES						
Jewlet Fish Farm Enos Were 0722958594 emacwere@yahoo.com		Kisii Fish Multiplication Centre	Yala Fish farm	LBDA fish farm	Bidii Fish Farm	Labedcash Marine Enterprises Limited Laban Mwanzo Malava 0722 565 686
Raphael Owaka Upper Nyakach, Homabay County						Safe Farm International Winfred Makokha Navakholo 0716 052 180
						Khwisero Fish Farm Salim Owiti Khwisero 0708 678 660
GROWOUT FARMS						
				RIAT Fish farm		Prof. Philip Museve Kutima Malava 0720 467 120
						Bukura Atc Fish Farm Joseph Chiteri Lurambi 0702 066 215 Grow Out Ponds
						Ingotse Fish Farm Navakholo Grow Out Ponds
						Lea Luchivya Malava 0722 827 087

CENTRAL REGION: Region II (From this we will get 8 grow out farms and 4 Hatcheries)

Kirinyaga	Machakos	Kiambu	Muranga	Meru/Tharaka Nithi	Embu	Nyeri
HATCHERIES						
National Aquaculture Development Centre	Kamuthanga Fish Farm Tonny Ndetto 0722522169			Desert Fish Farm		
Mwea Aqua Fish Farm Eutycus Githinji 0726 165 127 Eutycusgithinji8@gmail.com				East Africa highland Fish Farm This farm is in Tharaka Nithi County		
GROWOUT FARMS						
Green algae Fish Farm William Kiama 0722 899 904 afridozers@yahoo.com	PeterBragaza Fish Farm 0727000026	Kungu Fish Farm 0728930726	Makindi fish farm		Good Shephard Fish Farm Sr. Margaret Wangeci 0726446493 wangecirgs@gmail.com	
Ornamental fish farm, Nyangati James Mugo 0712 031 294		Gitonga Fish Farm 0710852773	Stanley Munyori 0720827990		Nyati Fish Farm Mbogo Mkulima 0725110030	Karatina University Fish Farm James Mbundi 0735 370 058
Hill top fish farm Nancy Kariuki 0722 785 542		Mwihaki Fish Farm 0717456816	Edward Muhia 0723163596		EEEPO Fish Farm Jacob 0723409834	Francis Wahome Gichoki 0728809547
			Sammy Mugo 0702753548			Nikson Ngumo Maina 0722690492
						Charles Muriithi Githaiga 0720809727

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