The Uganda National Action Plan on Tilapia Lake Virus Disease

Enhancing capacity/risk reduction of emerging Tilapia Lake Virus (TiLV) to African tilapia aquaculture

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Implementing team

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Introduction

In aquaculture sector, tilapias are the second-most important farmed finfish worldwide (next to the cyprinids), with Nile Tilapia (*Oreochromis niloticus*), ranking 6th among the most important cultured species. The world tilapia production (aquaculture and capture in 2015) amounted to 6.4 million tonnes, with an estimated value of USD 9.8 billion, and worldwide trade was valued at USD 1.8 billion. The species are preferred due to their affordability, good source high quality protein and micronutrients and tolerance to high-density aquaculture. The top 10 tilapia producers for 2015 (FAO, 2017b) were: China (1.8 million tonnes), Indonesia (1.1 million tonnes), Egypt (875 thousand tonnes), Bangladesh (324 thousand tonnes), Viet Nam (283 thousand tonnes), the Philippines (261 thousand tonnes), Brazil (219 thousand tonnes), Thailand (177 thousand tonnes), and Colombia (61 thousand tonnes). In Uganda, *O. niloticus* is the main cultured fish species in both ponds and cages throughout the country, contributing approximately 74,900 tonnes to total aquaculture production.

Tilapias are the second-most important commercial fish species (next to Nile Perch) in Uganda's capture fisheries. Four species: *Tilapia Zillii*, *Oreochromis niloticus*, *O. leucusticus*, *O. varibilis* and *O. esculentus* occur in Uganda water bodies, with *Oreochromis niloticus* widely distributed and hence dominates the catches. In 2018, an estimated 59,000 tonnes were produces from Ugandan lakes and rivers. The fisheries sector supports an estimated 1.2million people directly and indirectly along the entire fish value chain.

In 2009, massive mortalities of wild tilapia and farmed occurred in the Sea of Galilee in Israel and were discovered to be associated with a novel Orthomyxo-like virus causing a disease called tilapia lake virus disease (TiLVD) also known as syncytial hepatitis of tilapia-SHT (Ferguson et al. 2014; Eyngor et al. 2014; Bacharach et al. 2016; DelPozo et al. 2017; NACA, 2017; OIE, 2017a). The infection of TiLV was later reported from Colombia (Kembou Tsofack et al. 2017) and Egypt (Fathi et al. 2017) and Thailand (Dong et al. 2017; Surachetpong et al. 2017), Chinese Taipei, India and Malaysia (Amal et al., 2018; Behera et al., 2018; OIE, 2017a,2017b). The outbreaks result in mortalities ranging from 9.2 to 90%, with tilapia fingerlings and juveniles being more vulnerable than larger fish (Ferguson et al. 2014; Fathi et al. 2017; Dong et al. 2017a; Surachetpong et al. 2017). In addition, disease alerts were released including The TiLV disease advisory (NACA, 2017), TiLV disease card (OIE, 2017), TiLV Factsheet (CGIAR, 2017), TiLV special alert (FAO, 2017a). Indeed, some countries (e.g. Israel, Thailand, Malaysia, Chinese Taipei, Philippines and Peru) have notified OIE (OIE 2017b, c, d; OIE, 2018), of the occurrence of TiLV as an emerging disease. The diseased fish is characterized with lethargy, ocular alterations, skin erosions, exophthalmia, abdominal distension, scale protrusion and skin discoloration (darkening) followed by high mortalities. Due to high mortalities associated with the disease, TiLV is threatening the economies of several countries which depend on tilapia for food and nutrition security as well as for poverty reduction and improvement of livelihoods.

In Uganda, Mugimba et al. 2018 reported the presence of TilV in wild and caged tilapia collected from Tanzanian and Ugandan sides of Lake Victoria. Unlike reports from other country, massive mortalities have not been reported Lake Victoria and the samples collected were asymptomatic. It is imperative for the country to ascertain the presence or absence of the pathogen. Besides, owing to the sporadic appearance and the frequent translocation of tilapia fry/fingerlings within and from outside the country, disease status must be known. Notwithstanding, available scientific literature provided information about TiLV in terms of causative agent, modes and risk of transmission, host range, clinical signs and diagnostics, geographic distribution as well as recommended actions and measures to take regarding TiLV. No information, however, is available on survival of pathogen outside host, stability of the agent, levels of mortality at different life stages, suspected aquatic animal carriers/ vectors, and possible treatments.

Objectives of the NAP

Given the importance of farmed and wild-caught tilapia, especially as a widespread source of low-cost protein, TiLV represents a potential threat to food security in Uganda. Therefore, with support from FAO, the ministry together with research and academia have partnered up to develop and implement three strategic priorities to reduce the risk and impacts of introducing TiLV to Uganda on national aquaculture and fisheries production for social and economic development of the country and the communities.

Three key strategies and the associated activities to manage the risk of TiLV include

a. Create awareness and build capacity in TiLV disease
    1. Increase awareness and understanding of TiLV through effective communication, education and training
    2. Build and strengthen infrastructure and human capacity (farmers, fisheries officials and professionals of knowledge) to management disease
    3. Understand and quantify the social and economic impacts associated with TiLV outbreak
b. Establish a surveillance system for TiLV
    1. Generate knowledge and evidence through disease surveillance to guide science-based information for policy makers, farmers and professionals
    2. Develop fish disease management/control programme effective infection prevention and control
c. Develop a response and contingency plan from TiLV
    1. Develop an emergence preparedness and response system
    2. Design a contingency plan for aquatic animal health pathogens for effective infection prevention and control of emergences and re-emergences

a. Create awareness and build capacity in TiLV disease

Prior to conducting surveillance, training sessions will be organized for extension staff (District Fisheries officers, Veterinary Officers and National Advisory Service Providers). In addition, to the training, the extension staff will be enrolled into the passive surveillance system, which will take place throughout the duration of the project. With regard to capacity building of farmers,

tailor-made courses with emphasize to TiLV and general biosecurity will be organized for hatcheries operators and out-growers.

During the training sessions and awareness creation on TiLV, the fishermen, farmers and the public will also be requested to provide information toward accomplishing passive surveillance. For this purpose, the reporting channels and mechanisms will be developed to ensure efficient and smooth flow of information among actors.

b. TiLV Surveillance Programme: Uganda

In response to the confirmation of the occurrence of TiLV disease in Israel, Asia and Scientific report of its possible presence in Uganda, surveillance system for TiLV will be conducted to ascertain the presence in Uganda as well as contribute to understanding the global distribution of TiLV.

1. Purpose of surveillance program

1. To ascertain the presence or absence of TiLV in farmed Tilapia (Oreochromis niloticus) and validate previous results
2. To establish a National Reporting System for aquatic animal health
3. To develop TiLV Disease Control Program

2. Definition of population

TiLV affect mainly Tilapiines both farmed and wild hosts include *Oreochromis niloticus*; *Sarotherodon galilaeus, Tilapia zilli, O. aureus,* and *Tristamellasimonis intermedia* and hybrid tilapias (Eyngor *et al*. 2014; Ferguson *et al*. 2014; Fathi *et al*. 2017; Dong *et al*. 2017a; Surachetpong *et al*. 2017; Behera *et al*. 2018; Koesharyani *et al*. 2018; Mugimba *et al*. 2018; Amal *et al*. 2018). In Uganda, Nile Tilapia, *O. niloticus* is the main cultured tilapia cultured throughout the year, hence the target species for the surveillance. The species is cultured at small-scale (extensive) and commercial (intensive and semi-intensive) levels in ponds (common with small-holder farmers and hatchery operators); cages both at Low-Volume High Density of 150-500 fish/m^3 or High-Volume Low Density of 80-100 fish/m^3 with currently an estimated total of 3000 units on the lake. In addition, the species is also cultivated in tank system with mainly rectangular shaped units with varying sizes depending on production cost, space utilization and management. Therefore, sampling will be conducted throughout the year in ponds and cages at commercial management levels: intensive and semi-intensive.

3. Clustering of disease

Although aquaculture is practices throughout the country, surveillance will be conducted within the shores of Lake Victoria, commonly known as Lake Victoria crescent, a high concentration region of commercial fish farming enterprises (Fig. 1). The water temperature along the L. Victoria crescent ranges between 23 and 30°C, which supports fish farming throughout the year. This water temperature range of 25 -28°C are within the optimum temperature to trigger TiLV disease. The surveillance will also target mainly fingerlings and juveniles life stages, which are more

susceptible (Jansen et al. 2018). Since adults fish become resistance but act as carries, clinically healthy adults will also be including in the sample.

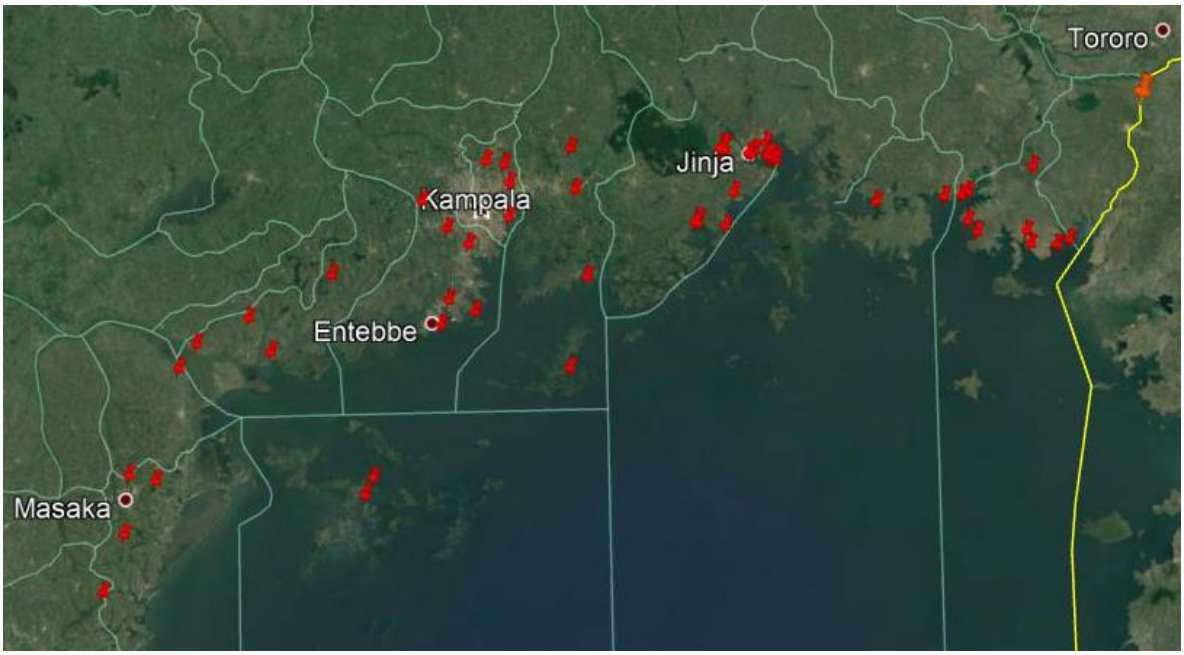

Figure 1: Distribution of commercial fish enterprises within the Lake Victoria Crescent

4. Case definition

This surveillance system will use the following case definitions:

Level	Case definition
Suspicion	A tilapia farming system in which the farmer has observed during the previous and ongoing production cycle - sudden mortalities and /or clinical signs such as skin redness/erosions or eyes protrusion/ruptured/cloudiness or abdomen swollen or scale protrusion/loss attributable to the presence of TiLV (e.g. farmer answer "yes"" to the question whether TiLV occurred for not in the farm of interest)
Confirmation	Upon the collection of 30 moribund or sick fish samples, TiLV is confirmed by a positive test result using PCR and the histopathological signs of TiLV
Validation of previous report	In cases where the levels of case definition (suspected or conformed) highlighted above are absent, asymptomatic fish specimens will also be collected for analyses to validate the previous reports.

5. Sampling frame

The Epidemiological unit in this surveillance will be a tilapia farm. Within a period of 8 months (March 2019 to October 2019), 40 fish farms (Table 1) will be sampled twice with an estimated goal of visiting approximately 3 tilapia farms per week. The farms will be selected using the proportional technique based on production intensity to ensure random distribution of epidemiological unit. The selected farms will be geo-referenced for further follow-ups.

During each sampling event, 30 moribund/sick or clinically healthy tilapia will be collected from ponds/cages at the farm per visit. Physico-chemical water quality especially temperature, dissolved oxygen, pH and conductivity will measured from each pond/cage in-situ. In addition, field questionnaire developed during the Kisumu training workshop will be administered to the farmers/farm managers to collect information of the disease occurrence and severity. Additional field questionnaire focusing understanding the social and economic impacts of TiLV will also be administered to the farmers and other node actors along the farmed tilapia value chain. The diagnostic and surveillance teams will consist of 3 and 4 people respectively from Directorate of Fisheries Resources; The Aquaculture Research and Development Centre of National Fisheries Resources Research Institute and Makerere University.

Culture System	**Eastern**	**Central**	**Total**
Ponds	5	5	10
Commercial	2	8	10
Hatcheries	2	8	10
Cages	5	5	10
Total	14	26	40

6. Methods

Level I – A questionnaire will be applied to the farmers and a designated number of samples will be picked (see table above) and inspected for symptoms of TiLV following a SoP for Level I laboratory tests developed by FAO.

Level II – Histopathology sample preparation will be carried out at the Histopathology lab at the Department of Zoology, Entomology and Fisheries Sciences, Makerere. Confirmed positive sample tissues by PCR screening will be processed and preserved on a microscope slide for expert examination. SoPs for Level II laboratory tests developed by FAO will be applied.

Level III- Screening of TiLV using the TiLV PCR Screening Kit (Pockit-GeneReach) will be done at the Molecular Biology/Genetics laboratory at the Department of Zoology, Entomology and Fisheries Sciences, Makerere followed by RNA extraction and RTPCR for the confirmed positive samples. FAO developed SoPs for Level III will be applied.

7. Data management

Data will be entered, cleaned and stored in Microsoft Office Excel template developed by FAO team. The classical epidemiological approach to quantify risk factors including: 2 by 2 Contingency table; computation of risk factors (exposed vs non-exposed) and computation of relative risk and the Odds Ratio.

8. Validation

The data collected and entered by the data entrants will be validated internally by the both diagnostic and surveillance team and externally by FAO experts and other participants during the second training and data analyses meeting.

9. Quality assurance

All filled data forms will be kept at the focal person both hard and soft copies. The hard copy questionnaires will be entered in excel by data entrant and cross check by the technical persons (diagnostic/surveillance team) for consistency, quality and precision. The SOPs developed by FAO will be followed at all stages.

10. Surveillance in the big picture

The programme fits in the National Policy of Fisheries and Aquaculture: Policy Statement 2.8.1 – "*establish and implement fish health and biosecurity protocols as a security measure to protect biological diversity of fisheries and the life support system*". This is in response to the in significant movement of live fish within the country and the neighbouring countries which poses a danger of spreading diseases in the country, region, and beyond. Further, the surveillance responds to the national effort to Commercialization of Aquaculture through Aquaparks, also underscored in the policy statement 2.9.1: "*invest in the development of aqua parks under a PPP arrangement to increase a competitive, market oriented and profitable, environmentally responsible aquaculture industry*".

11. Communication of Surveillance results to stakeholders

After completion of the field activities, two result sharing and feedback sessions will be organized for stakeholders along aquaculture in Eastern and Central Uganda. During these meetings, plans will be lay-out for developing of biosecurity schemes for different levels of operations.

12. Human and Financial Requirement

The budget estimates to implement successfully the National Action Plan over a period of one year is USD 58,198 (Fifty eight thousand one hundred and ninety eight dollars) as detailed below.

Item	Item Description	USD
Education, awareness and participant recruitment	Training session for surveillance team as well as other ministry personnel, Academia, Research officers from various research organizations for 25 persons	1,830
	Two sensitization and recruitment sessions for 25persons each composed of Extension staff, Fisheries officers and Fish farmers to participate in surveillance programme	3,660
	Hatchery Operators (owners, managers and representatives from the districts) sensitization and recruitment session for 25 persons to participate in surveillance programme	1,830
	Commercial Farmers (owners, managers and representatives from the districts) sensitization and recruitment session for 25 persons to participate in surveillance programme	1,830
Value chain mapping, socio-economic surveys and gender analysis	Identifying aquaculture nodes for different fish production scales for any 25 farms selected - at least 8 commercial; 8 small-medium scale farmers and 4 hatcheries	3,186
	Social, economic and gender analyses along the value chain nodes of at least ten fish farming enterprises	3,186
Field surveillance	Team of 3 persons/trip to make 23 visit to 40 farms, (3farms/week) twice to conduct questionnaire surveys and epidemiological data and sample collection for lab diagnosis and histopathology	13,375
FAO Back-stopping during surveillance	1 officer and 1 driver for total of 8days over a period of 8months/surveillance (DSA+Fuel)	1,197
Small Equipment	TiLV PCR Screening Kit (Pockit-GeneReach) for field rapid screening	2,000
	Pocket PCR screening kit reagents for 1250 samples including asymptomatic specimens to be used to validate the results of Mugimba et al. 2018	3,000
Molecular laboratory analyses	Extraction, RT PCR kit, Other Consumables for 1250 Reactions including asymptomatic specimens to be used to validate the results of Mugimba et al. 2018	10,437
Histopathology Laboratory	Assortment of histopathological Reagents and stains	2,000
Diagnostic Team	Facilitation of molecular technician @$150/month for 8 months	1,200
	Facilitation for Histopathology technician @$150/month for 8 months	1,200
	Facilitation to Data Entrant @$100/m for 8 months	800
Feedback sessions	Organize two feedback sessions of 25 persons each for farmers, policymakers and other aquaculture actors to share surveillance results and lay-out plans for designing biosecurity schemes	3,724
Project cost		**54,391**
Support cost	7% of project cost	3,807
Grand total		**58,198**

Note: Although it was suggested that field surveillance would use only pocket TiLV PCR Screening Kit (Pockit-GeneReach) for field rapid screening, team Uganda strongly believes that a second method (Molecular laboratory analyses) should be used to validate results of Mugimba *et al*. 2018. Hence additional budget for purchase of limited equipment and reagent for this protocol have been incorporated in the above for consideration.

13. Sampling Schedule: Surveillance in ponds, hatcheries and cages

Activities	**Jan 2019**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**	**Jan 2020**
Preparatory work: Stakeholder workshop													
Sensitization: extension staff and farmers													
Pilot test and sampling map													
Training: Diagnostic and surveillance teams													
Field sampling													
Data entry													
Socio-economic & gender analysis													
Lab Analysis													
Culture Period (month)													

c. Response and contingency plan

As future plans will include development of the emergence preparedness and response system in collaboration with all relevant agencies. Further, the team will design contingency plan for aquatic animal health pathogens for effective infection prevention and control of emergences and re-emergences.