



FAO TCP/INT/3707:
*Strengthening biosecurity (policy
and farm level) governance to deal with
Tilapia lake virus*

TiLV Active Surveillance 12-Point Checklist

PHILIPPINES

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NATIONAL TiLV STATUS

CHECKLIST No.

1

CRITERIA	RESPONSE
TiLV Country Status	Country is infected with TiLV
TiLV Surveillance	Passive and Active
TiLV status of Neighboring Countries or Trading Partners	Yes
Sharing of watershed with other Country	No
Data Sources	FAO early warning, OIE/NACA disease card and reports, diagnostic reports, TiLV Profile, On-going Scientific reports, Report of surveillance activity
Country Scenario:	Scenario 1: the country is infected with TiLV

CHECKLIST No.

2

SURVEILLANCE OBJECTIVES

1. To determine the prevalence rate of TiLV among BFAR listed hatcheries in Regions 3 & 4A during the summer months to the onset of rainy season (April-August 2021).
2. To determine the risk factors associated with the occurrence of the disease in an epidemiological unit.

CHECKLIST No.

3

DEFINING POPULATION

CRITERIA	RESPONSE
POPULATION OF INTEREST	Tilapia (<i>Oreochromis</i> spp.)
TARGETED POPULATION	Tilapia (<i>Oreochromis</i> spp.)
POPULATION FOR SAMPLING (Study population)	Tilapia Hatcheries (<i>Oreochromis</i> spp.) in Regions 3 & 4A Strains: Excel, i-BEST, GIFT derivatives
INCLUSION CRITERIA	<u>Swim up Fry</u> : 9-12 days old <u>Fingerlings</u> : 2 weeks size 24 3 weeks size 22 4 weeks size 17 <u>Broodstocks</u> : ≥4 months
EXCLUSION CRITERIA	Grow-out
FARMING SYSTEMS, REGISTRATION, DATA, APPROVAL OF FARMS	System: Modified-Intensive, Semi-Intensive, Hapa-based, Pond-based, Tank-based Registration & Approval: In-placed since 2005
WILD POPULATION	X

CHECKLIST

No.

4

CLUSTERING OF DISEASE

1. Clustering effect of disease is considered and described
 - Temperature: (26-32°C)
 - Time: Summer up to the onset of rainy season (April-August 2021).
2. Clustering effect of disease is accounted in sampling/ survey design and data analysis
 - Hatcheries showing signs of TiLV at the time and area of surveillance activity will be considered

CASE DEFINITION

CHECKLIST

No.

5

Suspected Case: Tilapia Lake Virus Disease is suspected if at least one of the following criteria is met:

Clinical/Field

- Good and/or Poor water quality with temperature ranging from 26-32°C
- Skin discoloration, erosions and redness
- Ocular lesions such as opacities/ alterations and bulging of the eyes
- Abdominal distension and scale protrusion
- Loss of appetite and lethargy
- Abnormal swimming behavior/stop schooling

CHECKLIST

No.

5

CASE DEFINITION

Laboratorial

- Paleness of the gills; liver is watery, green, pale or dark upon necropsy
- Histopathology shows atypical lesions in the liver such as multifocal chronic hepatitis;
reduction of fat-storage cells; karyorrhexis and pyknosis; presence of intracytoplasmic inclusion bodies; reduction of fat-storage cells and foamy cytoplasm
- Histopathology of the brain reveal Perivascular cuffing of lymphocytes in the brain cortex,
congestion and hemorrhage
- Splenic cell degeneration, presence of debris-laden macrophages within splenic ellipsoids,
pyknosis and karyorrhexis and increasing number of melano-macrophage centers (MMC)
are observed in spleen
- In the kidney, aggregation of lymphocytes, pyknosis and karyorrhexis, an increasing number of MMC are seen.

Epidemiological

- High mortality of tilapia species (broodstock and fingerlings) in hatchery farms

CASE DEFINITION

CHECKLIST

No.

5

Confirmed Case: A suspect case of Tilapia Lake Virus Diseases is defined as confirmed case if one of the following criteria is met:

Laboratory

- Histopathology of the liver shows syncytial giant cell or multinucleated giant cells
- Insulated Isothermal PCR shows positive result

CHECKLIST No. 6

DIAGNOSTIC TESTING

Diagnostic Level Technical Requirements

I. Observation of animal and environment; Gross Clinical Examination

Farm/ Hatchery Records:

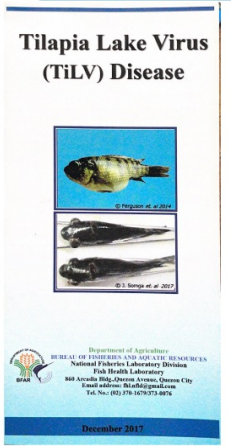
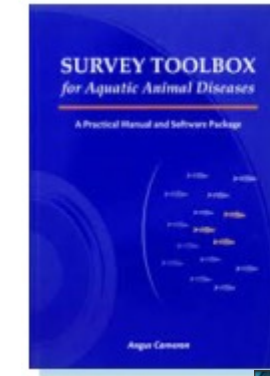
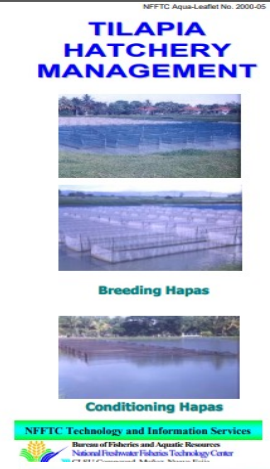
- Tilapia profile records
- Husbandry practices
- Performance Indicators

Guides/ Cards/ Sheets

- Fish identification cards
- Field key guides
- TiLV disease card/ brochure
- GIS/ mapping guide
- Necropsy procedure guideline
- Preservation, Transportation, Sending of samples guides for Level II & III

Sampling equipment & materials

- Water Quality Testing parameters (DO, pH, Temp, Salinity, Water Color, Transparency, Other Parameters)
- Basic Necropsy Materials
- Fixatives
- Documentation materials
- PPE



PATHOGEN INFORMATION	
<p>1. CAUSATIVE AGENT</p> <p>1.1. Pathogen type Virus</p> <p>1.2. Disease name and synonyms Tilapia lake virus (TiLV) disease.</p> <p>1.3. Pathogen common names and synonyms Tilapia lake virus (TiLV)</p> <p>1.4. Taxonomic affiliation The taxonomic affiliation has not been definitively concluded; however, TiLV has been described as a novel virus in the Family Orthomyxoviridae (Eyring et al., 2014).</p> <p>1.5. Authority (first scientific description, reference) The virus was first described by Eyring et al. (2014).</p> <p>1.6. Pathogen environment (fresh, brackish, marine waters) Fresh and brackish water.</p> <p>2. MODES OF TRANSMISSION</p> <p>2.1. Routes of transmission (horizontal, vertical, indirect) Co-habitation studies have demonstrated that direct horizontal transmission is an important route of transmission. There is no evidence of vertical transmission. The biological characteristics of the virus are not well characterized so it is difficult to determine the significance of indirect transmission by fomites.</p>	<p>3. HOST RANGE</p> <p>3.1. Susceptible species Mortalities attributed to TiLV have been observed in wild tilapia <i>Oreochromis niloticus</i> (Tilapia galilaea), farmed tilapia <i>Oreochromis niloticus</i> and commercial hybrid tilapia (<i>O. niloticus</i> X <i>O. aeneus</i>) (Bacharach et al., 2016; Ferguson et al., 2014; Eyring et al., 2014). To date, only tilapia have been shown to be susceptible. It is possible that other species will be found to be susceptible.</p> <p>3.2. Affected life stage In the outbreak reported by Ferguson et al. (2014) and Dong et al. (2017) fingerlings were mostly affected. Dong et al. (2017) reported approximately 90% mortality in red tilapia fingerlings within one month of stocking into cages. Mortality just over 9% in medium to large sized tilapia was noted by Fari et al. (2017). Other reports have not commented on different levels of mortality by life stage (Eyring et al., 2014).</p> <p>3.3. Additional comments There is some evidence that certain genetic strains of tilapia are resistant. Ferguson et al. (2014) noted that one strain of tilapia (genetically male tilapia) incurred a significantly lower level of mortality (10-20%) compared with other strains.</p> <p>4. GEOGRAPHICAL DISTRIBUTION TiLV has been reported in Colombia, Ecuador and Israel (Bacharach et al., 2016; Ferguson et al., 2014, 2016).</p>

Location Map of Bureau of Fisheries and Aquatic Resources (BFAR) Tilapia Central Hatcheries

BFAR-Regional Office II
Cagayan Valley Research and Outreach Station for Freshwater Resources
 Salinungan West, San Mateo, Isabela
 *Producing Parent Stocks (iEXCEL)

BFAR-Regional Office IV-A
Freshwater Demonstration Fish Pond
 Sto. Domingo, Bay, Laguna
 *On-going rearing of Foundation Stocks (iEXCEL)

BFAR-Regional Office V
Regional Freshwater Fisheries Center
 Fabrica, Bula, Camarines Sur
 *Producing Parent Stocks (iEXCEL)

BFAR-Regional Office VI
Regional Freshwater Fisheries Technology Center
 Salihid, Barotac Nuevo, Iloilo City
 *Producing Parent Stocks (iEXCEL)

BFAR-Regional Office VII
Clarín Freshwater Fish Farm
 Caluwasan, Clarín, Bohol
 *Producing Parent Stocks (iEXCEL)

BFAR-Regional Office VIII
Regional Freshwater Aquaculture Production Center
 District 3, Babatngon, Leyte
 *On-going rearing of Foundation Stocks (iBEST, Red Tilapia)

BFAR-Regional Office IX
Bagalupa Freshwater Fish Hatchery
 Bagalupa, Labangan, Zamboanga del Sur
 *For Replacement of Foundation Stocks

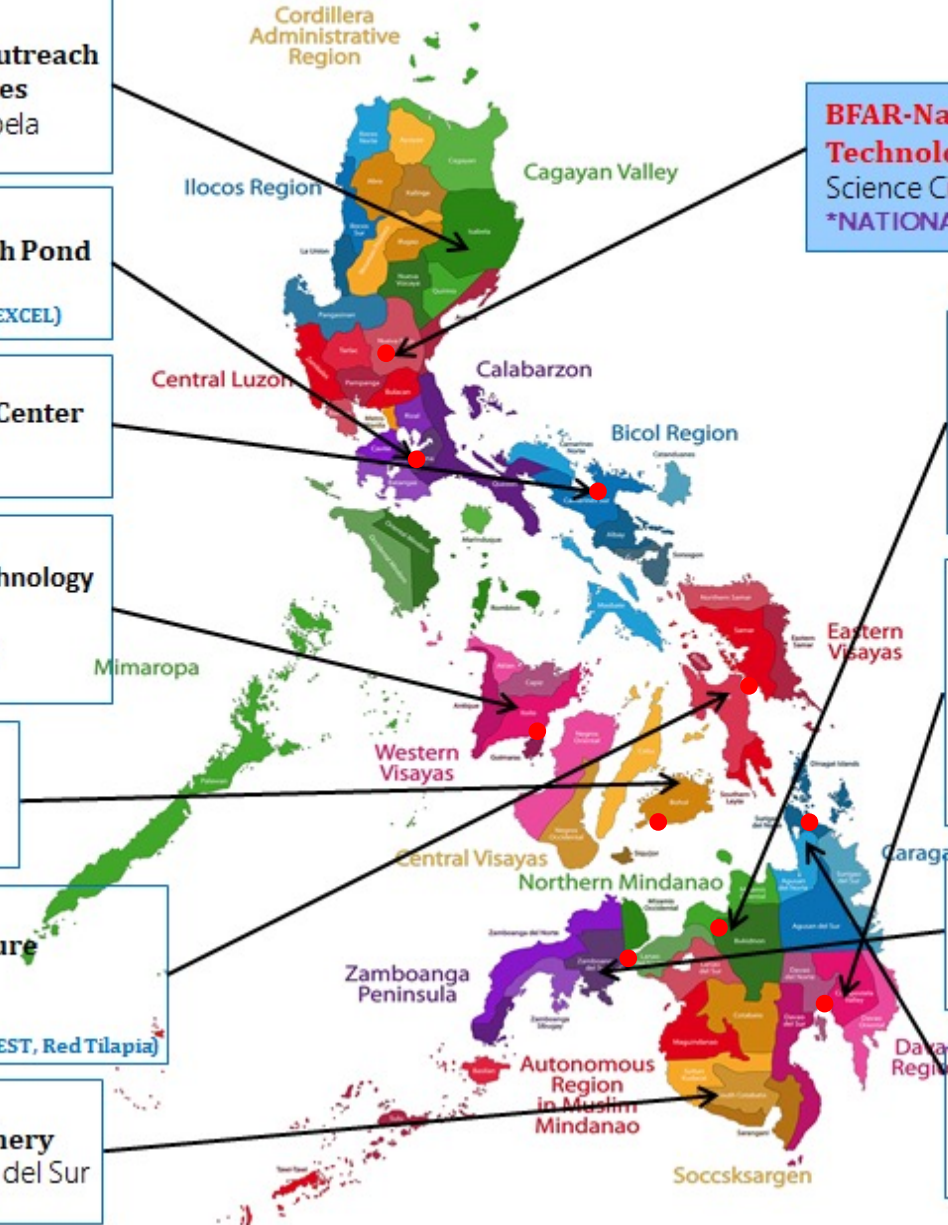
BFAR-National Freshwater Fisheries Technology Center (NFFTC)
 Science City of Muñoz, Nueva Ecija
 *NATIONAL BROODSTOCK CENTER

BFAR-Regional Office X
Kislon Freshwater Fish Production and Regional Training Center
 Kislon, Sumilao, Bukidnon
 *Producing Parent Stocks (iEXCEL)

BFAR-Regional Office XI
Regional Fisheries Research and Development Center for Freshwater
 Magsaysay, Nabunturan, Compostela Valley
 *Producing Parent Stocks (iEXCEL)

BFAR-Regional Office XII
Koronadal Multi-Species Hatchery
 Koronadal, South Cotabato
 *Producing Parent Stocks (iEXCEL)

BFAR-Regional Office XIII
Kitcharao Freshwater Fish Farm
 Kitcharao, Agusan Del Norte
 *On-going rearing of Foundation Stocks (iEXCEL)

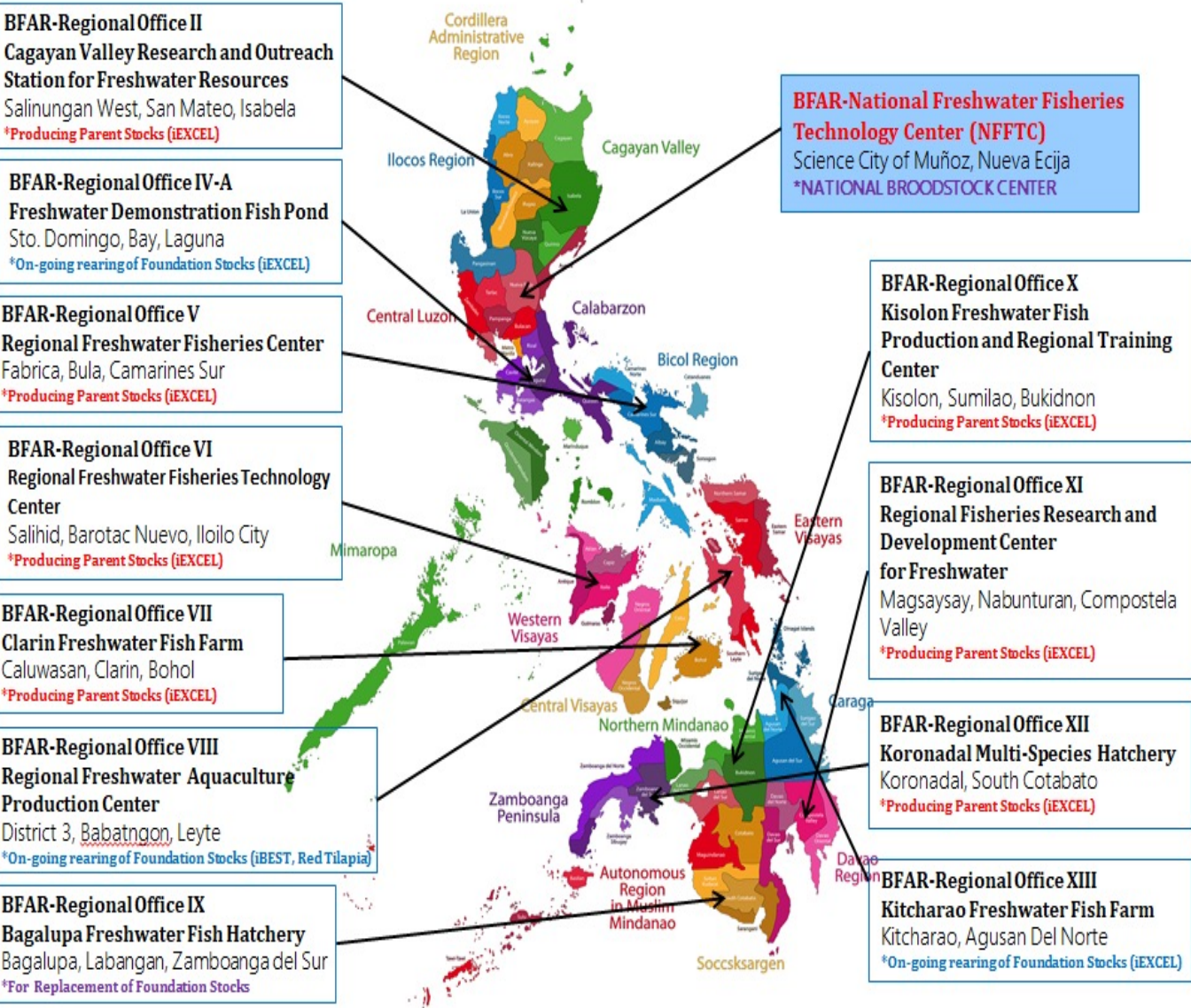


Indicate in the map location of any weather or meteorological station near the tilapia production locations

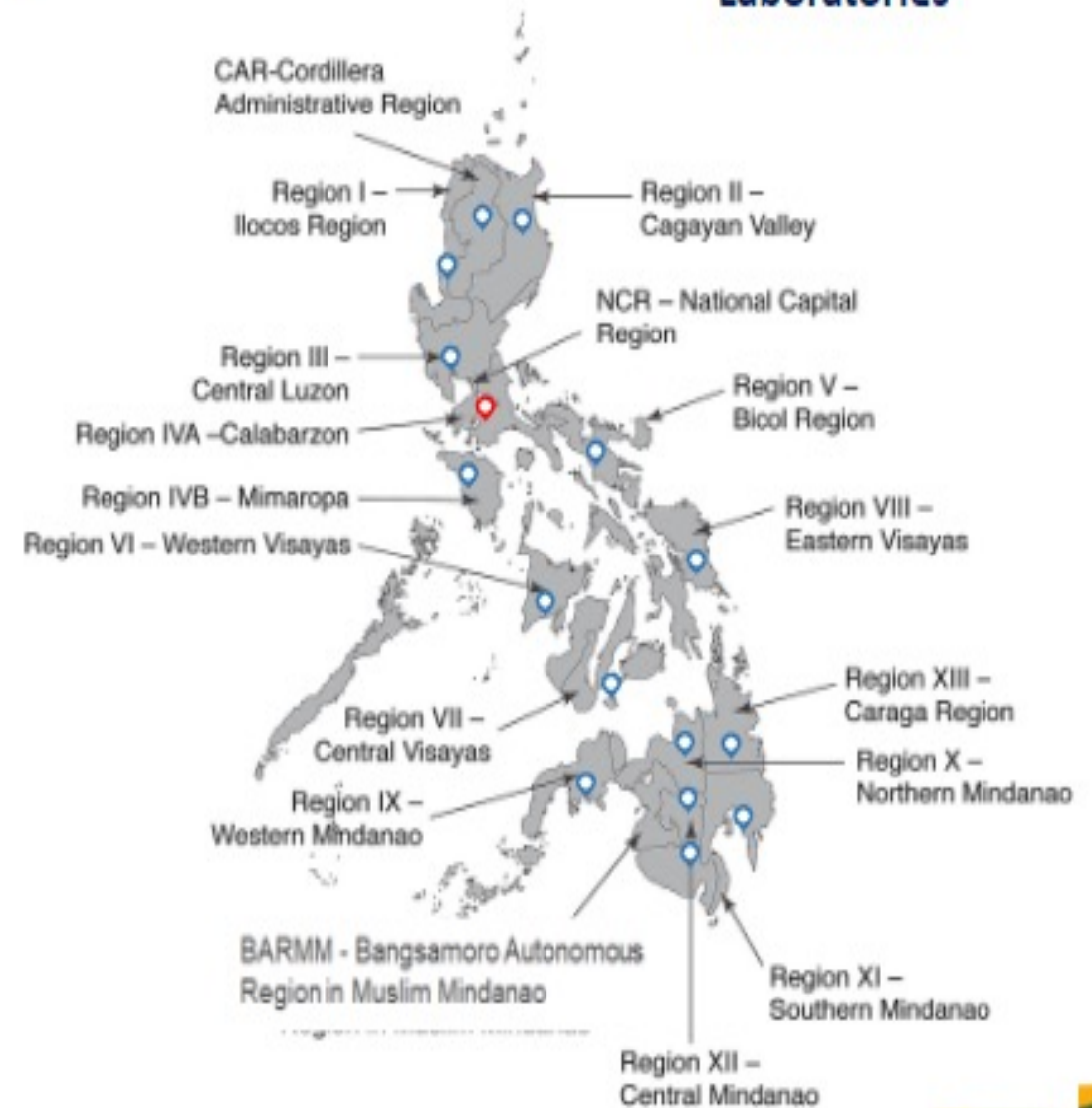
Red dots- meteorological stations

Tilapia hatcheries and BFAR laboratories

Location Map of Bureau of Fisheries and Aquatic Resources (BFAR) Tilapia Central Hatcheries



BFAR Central and Regional Offices and Laboratories



CHECKLIST No. 6

DIAGNOSTIC TESTING

Diagnostic level	No of laboratories national and satellite (regional, provincial, district) and/or national reference laboratory and location	Available equipment for Level II	Available staff expertise (specialization)
II: Histopathology	National Fisheries Laboratory Division (NFLD): Location: 860 Arcadia Bldg, Quezon Ave., Quezon City, National Capital Region	<ul style="list-style-type: none"> • Automatic Tissue Processor • Embedding Centre • Microtome • Water Bath • Routine H&E Staining Set • Fumehood • Compound Microscope 	Doctor of Fisheries Technologist (1) Veterinarians (6) Biologists (3)
	Southeast Asian Fisheries Development Center (SEAFDEC) Location: Tigbauan, 5021 Iloilo, Philippines	<ul style="list-style-type: none"> • Automatic Tissue Processor/ Tissue Processor • Embedding Centre • Water bath • Microtome • Routine H&E Staining Set • Fumehood • Compound Microscope 	Aquatic Animal Health Experts Aquatic Animal Health Researchers

CHECKLIST No. 6

DIAGNOSTIC TESTING

Diagnostic Level	Number of Laboratories	Available equipment for level III	Available staff expertise
III: PCR	National Fisheries Laboratory Division (NFLD): Location: 860 Arcadia Bldg, Quezon Ave., Quezon City, National Capital Region	<ul style="list-style-type: none"> • POCKIT MICROPLUS • POCKIT Portable Nucleic Acid Analyzer • Vortex Mixer • Pipette set • Automatic Nucleic Acid Extraction System • Thermocycler, Thermal Block • Realtime thermocycler • Electrophoresis set • Low temp incubator • Biosafety cabinet/Laminar Flow/ Fumehood • Hotplate • Vortex Mixer • Balance • Autoclave 	Doctor of Fisheries Technologist (1) Veterinarians (6) Biologists (3)
	Regional Fisheries Laboratory III: Location: Maalaga st., Diosdado Macapagal Government Center, Maimpis, CSFP, Pampanga		MsC in Aquaculture (1) Veterinarian (1) Chemist (1) Fisheries Technologist (1)
	Regional Fisheries Laboratory IV-A: Location: Purok 3, Brgy. Bambang, Los Banos, Laguna		Fisheries Technologist (1) Veterinarian (1) Chemist (2) Chemical Technician (2) Biologist (2)
	Regional Fisheries Laboratory I: Location: AB Fernandez West, Dagupan City, Pangasinan		MsC in Aquaculture (2) Chemist (1) Veterinarian (1) Fisheries Technologist (1)
	Regional Fisheries Laboratory II: Location: Government Center, Carig Sur, Tuguegarao, City, Cagayan		Veterinarian (2) Fisheries Technologist (2)
	Regional Fisheries Laboratory V: Location: RFFC Compound, Fabrica, Bula, Camarines Sur		Aquacultural technologist (1) Biologist (1)
	Regional Fisheries Laboratory VI: Location: Muelle Loney St., Iloilo City		Chemist (3)

CHECKLIST No. 6

DIAGNOSTIC TESTING

Diagnostic Level	Number of Laboratories	Available equipment for level III	Available staff expertise
III: PCR	Regional Fisheries Laboratory VII: Location: Arellano Boulevard, Cebu City	<ul style="list-style-type: none"> • POKKIT MICROPLUS • POKKIT Portable Nucleic Acid Analyzer 	Biologist (2) Veterinarian (1) Fisheries Generalist (1)
	Regional Fisheries Laboratory VIII: Location: MRGP Commercial Bldg., Brgy 77, Marasbaras, Tacloban City	<ul style="list-style-type: none"> • Vortex Mixer • Pipette set 	Veterinarian (2) Biologist (1)
	Regional Fisheries Laboratory X: Location: BFAR 10 Compound, Julio Pacana St. Macabalan, Cagayan de Oro City	<ul style="list-style-type: none"> • Automatic Nucleic Acid Extraction System • Thermocycler, Thermal Block 	Biologist (3) Fisheries Technologist (2)
	Regional Fisheries Laboratory XI: Location: Ramon Magsaysay Ave., Davao City	<ul style="list-style-type: none"> • Realtime thermocycler • Electrophoresis set 	Veterinarian (1) Biologist (1) Chemical Engineering (1)
	Regional Fisheries Laboratory XII: Location: D. A. Compond, J. Catolico Ave., Brgy. Lagao, General Santos City	<ul style="list-style-type: none"> • Low temp incubator • Biosafety cabinet/Laminar Flow/ Fumehood 	Chemist (1) Fisheries (2)
	Regional Fisheries Laboratory XIII: Location: Sitio Tawilon, Ambago, Butuan City	<ul style="list-style-type: none"> • Hotplate • Vortex Mixer 	Veterinarian (1) Biologist (2) Chemist (1)
	Regional Fisheries Laboratory BARMM: Location: MAFAR Compound, OCM Comp., ORC Cotabato City	<ul style="list-style-type: none"> • Balance • Autoclave 	Fisheries Technologist (1) Research Assistant (2)

CHECKLIST No. 6

DIAGNOSTIC TESTING

Diagnostic Level	Number of Laboratories	Available equipment for level III	Available staff expertise
III: PCR	Southeast Asian Fisheries Development Center (SEAFDEC) Location: Tigbauan, 5021 Iloilo, Philippines	<ul style="list-style-type: none"> • Vortex Mixer • Pipette set • Automatic Nucleic Acid Extraction System • Thermocycler, Thermal Block • Realtime thermocycler • Electrophoresis set • Low temp incubator • Biosafety cabinet/Laminar Flow/ Fumehood • Hotplate • Vortex Mixer • Balance • Autoclave 	Aquatic Animal Health Experts Aquatic Animal Health Researchers
	Fisheries Biotechnology Center (FBC) Location: BFAR-NFFTC, CLSU Compd, 3120 Science City of Muñoz, Nueva Ecija		Aquatic Animal Health Experts Aquatic Animal Health Researchers
	National Fisheries Research Development Institute (NFRDI) Location: 101 Mother Ignacia Ave., Brgy, Diliman, Quezon City, 1103 Metro Manila		Aquatic Animal Health Experts Aquatic Animal Health Researchers
	ACADEME: <ul style="list-style-type: none"> - Central Luzon State University - Don Mariano Marcos Memorial State University - University of Sto. Tomas - University of the Philippines-Diliman - University of the Philippines-Los Baños - University of the Philippines-Visayas 		Professors Researchers

CHECKLIST No. 6

DIAGNOSTIC TESTING

Diagnostic Level	Number of Laboratories	Available equipment for level III	Available staff expertise
III. Cell Culture	Southeast Asian Fisheries Development Center (SEAFDEC) Location: Tigbauan, 5021 Iloilo, Philippines	<ul style="list-style-type: none">• Laminar air flow hood• CO₂ Incubator• Centriuge• Low Temp. Incubator• Liquid Nitrogen container• Flow Cytometry• Inverted Microscope• Dissecting microscope• Water Bath	Aquatic Animal Health Experts Aquatic Animal Health Researchers
III. TEM	Southeast Asian Fisheries Development Center (SEAFDEC) Location: Tigbauan, 5021 Iloilo, Philippines	Transmission Electron Microscope	Aquatic Animal Health Experts Aquatic Animal Health Researchers
	University of the Philippines- Diliman Location: Diliman, Quezon City 1101 Metro Manila, Philippines	Transmission Electron Microscope	Professors Researchers

CHECKLIST No. 6

DIAGNOSTIC TESTING

	METHOD	TARGETED SURVEILLANCE			PRESUMPTIVE DIAGNOSIS	CONFIRMATORY DIAGNOSIS
		Fish Fry	Juveniles	Adults		
a. The method is the recommended method for reasons of availability, utility and diagnostic specificity and sensitivity	Gross signs (I)	C	C	C	C	D
b. The method is a standard method with good diagnostic sensitivity and specificity	Histopathology (II)	B	B	B	B	B
c. The method has application in some situations but cost, accuracy or other factors severely limit the application	Isolation with Cell-culture (III)	N/A	N/A	N/A	N/A	N/A
d. The method is presently not recommended for this purpose	PCR-based assays (III)	A	A	A	A	A
N/A. not applicable	In situ hybridization (III)	N/A	N/A	N/A	N/A	N/A
	Antibody-based assays	N/A	N/A	N/A	N/A	N/A
	TEM (III)	N/A	N/A	N/A	N/A	N/A

STUDY DESIGN AND SAMPLING

CHECKLIST No. 7

STUDY DESIGN DECIDED (Prevalence Study)

- Cross Sectional
- Epidemiological Unit: Hatchery
- Two-Stage Random Sampling:
 - **1st stage sampling: 42** hatcheries out of 95 total hatcheries
 - Region 3= 29 hatcheries out of 66
 - Region 4A= 13 hatcheries out of 29

Sample size to estimate a simple proportion (apparent prevalence)

Analysed: Thu Apr 08, 2021 @ 14:52 UTC

Inputs

Estimated Proportion	0.05
Desired precision of estimate	0.05
Confidence level	0.95
Population size	95

Results

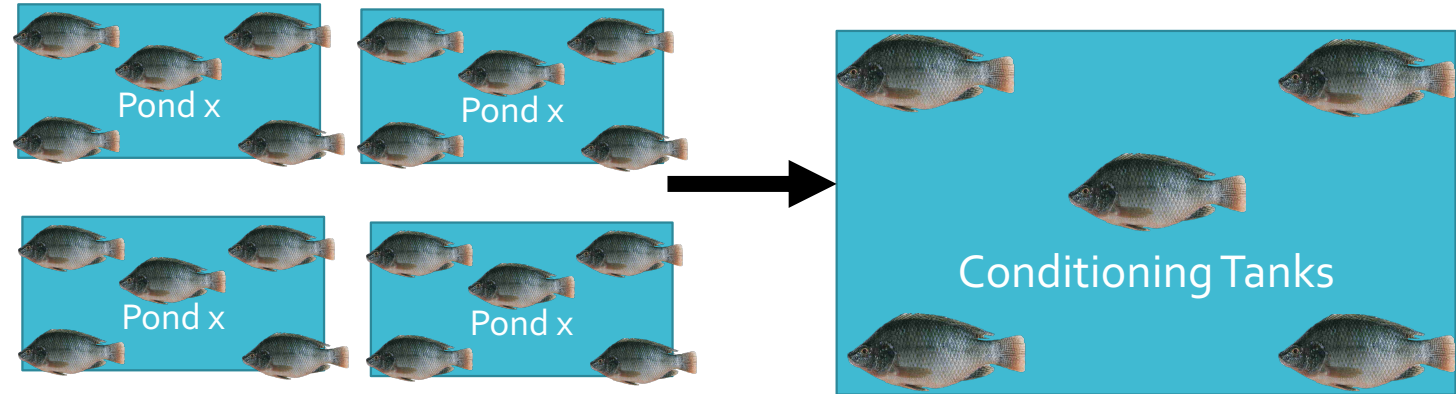
Sample size required for specified inputs

Large population	73
Population = 95	42

CHECKLIST No. 7

STUDY DESIGN AND SAMPLING

- **2nd Stage Sampling:** Combination of Apparently Healthy and Sick Population in the conditioning tanks



- **Design prevalence: 5%** based on 100% specificity and 95% sensitivity.
- **Number of fingerlings to be sampled:**

Design prevalence	Sensitivity (%)	Specificity (%)	Sample size	Maximum number of false positive if the population is free
5	95	100	62	0

- **Field Sampling:** To access breeding ponds for environment (H₂O quality) and animal observation and testing before going to conditioning tanks.
- **Laboratory Testing (Histopathology):** Samples with TiLV signs
- **Laboratory Testing (iiPCR):** Pooled

DATA COLLECTION AND MANAGEMENT

& DATA ANALYSIS

CHECKLIST No.

8

&

9

- **Forms:**

1. Waiver/ Letter/ MOA
2. TiLV Disease Surveillance Form
3. Necropsy Form
4. Request for Laboratory Analysis (RLA) Form
5. Test Results

- **Data analysis and Database:**

- 1) A data analysis team will be created to analyze all the information collected and collated during the surveillance. They will also be tasked to manage the database for the surveillance program
- 2) All documents will be uploaded in a cloud (One Drive/Google), back up hard drive and compiled hard copies of the Project (e.g. Project proposal, Memorandum, etc.)
- 3) A mapping system (E.g. QGIS) will be used to accurately point the location of the hatcheries in the region

VALIDATION AND QUALITY ASSURANCE

CHECKLIST No.

10

- Quality Assurance Checklist
 - Field Checklist
 - Environment (H₂O quality)
 - Animal behavior (Broodstock and fingerlings in ponds and in conditioning tanks)
 - Farm Records
 - Other records
 - Laboratory Checklist
 - Use of Controls
 - Turn Around Time of test results
 - Data Analysis
 - Crosschecking of data

CHECKLIST No.

11

HUMAN AND FINANCIAL RESOURCES AND LOGISTICS REQUIREMENTS

- Personnel Involved in the Surveillance
 - Field Teams
 - Laboratory Teams
 - Data Analysis Teams
 - Audit Teams – Plan, Do, Check, Act (PDCA) / Cross-checking of processed data
- Budgetary Requirements
 - Co-sharing of resources (BFAR-FAO)
 - Overtime & hazard pay
 - Hiring of vehicle vs. Gov't vehicle
 - Travel Allowance
- Meeting with the hatcheries
- COVID-19 Restrictions
 - Swabbing of the team
 - Vaccination
- Timeline of Activities:

HUMAN AND FINANCIAL RESOURCES AND LOGISTICS REQUIREMENTS

CHECKLIST No. 11

Output	Activity	J	F	M	A	M	J	J	A	S	O	N	D
		2021											
PHASE 1 (Preparation)	Proposal from FAO		X										
	Invitation of Participants for the On-line Virtual Course for TiLV Active Surveillance			X									
	Creation of Core Group for TiLV Active Surveillance			X									
	Online course for TiLV Active SURveillance				X								
	Project Proposal/ Concept Note for the TiLV Active Surveillance				X								
	Procurement of supplies for the implementation of the Project				X	X							
	Planning/Training for the surveillance				X	X							
PHASE 2 Field and laboratory Sampling	Region 3 Sampling					X	X	X					
	Region 4a Sampling						X	X	X				
PHASE 3 Data Analysis	Creation of database					X	X						
	Analysis of data				X	X	X	X	X	X			

CHECKLIST No.

12

SURVEILLANCE IN THE BIGGER PICTURE

- Surveillance as an essential component of aquatic animal health/aquatic biosecurity strategies, disease management and control plans .
 - Promotion on Good Aquaculture Practices (GAqP)
 - Identify information on emerging diseases
 - Basis for Biosecurity , Health Certification and Traceability
 - Gather other problems for the formulation of policies and guidelines
 - Sustainable and good quality tilapia in relation to food security
- One Health
 - Information in the use of antimicrobials
 - Information on Antimicrobial Resistance (AMR)
 - GAqP