*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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# CHECKLIST No. 1

## *NATIONAL TiLV STATUS*

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

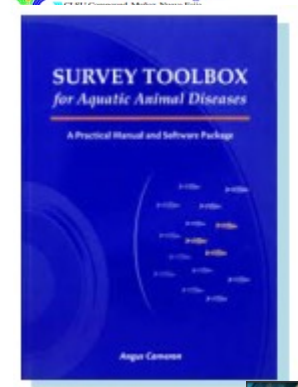
- Histopathology of the liver shows syncytial giant cell or multinucleated giant cells
- Insulated Isothermal PCR shows positive result

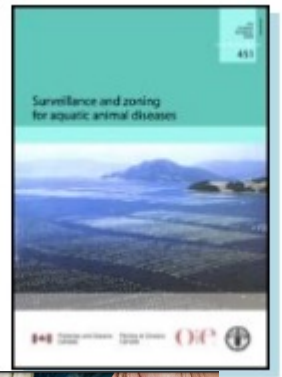
# CHECKLIST No. 6

# *DIAGNOSTIC TESTING*

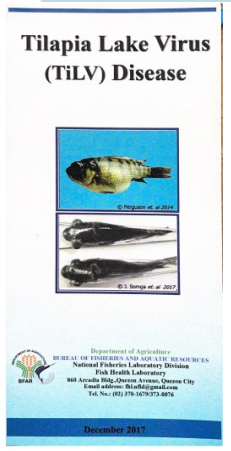
| Diagnostic Level | Technical Requirements |
|---|---|
| I. Observation of animal and environment; Gross Clinical Examination | Farm/ Hatchery Records:<br>- Tilapia profile records<br>- Husbandry practices<br>- Performance Indicators |
| | Guides/ Cards/ Sheets<br>- Fish identification cards<br>- Field key guides<br>- TiLV disease card/ brochure<br>- GIS/ mapping guide<br>- Necropsy procedure guideline<br>- Preservation, Transportation, Sending of samples guides for Level II & III |
| | Sampling equipment & materials<br>- Water Quality Testing parameters (DO, pH, Temp, Salinity, Water Color, Transparency, Other Parameters)<br>- Basic Necropsy Materials<br>- Fixatives<br>- Documentation materials<br>- PPE |

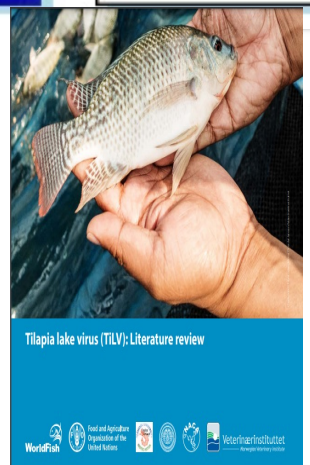


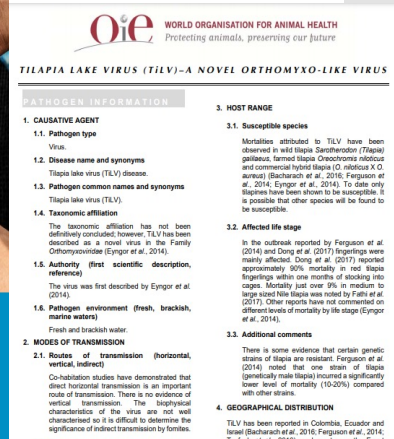


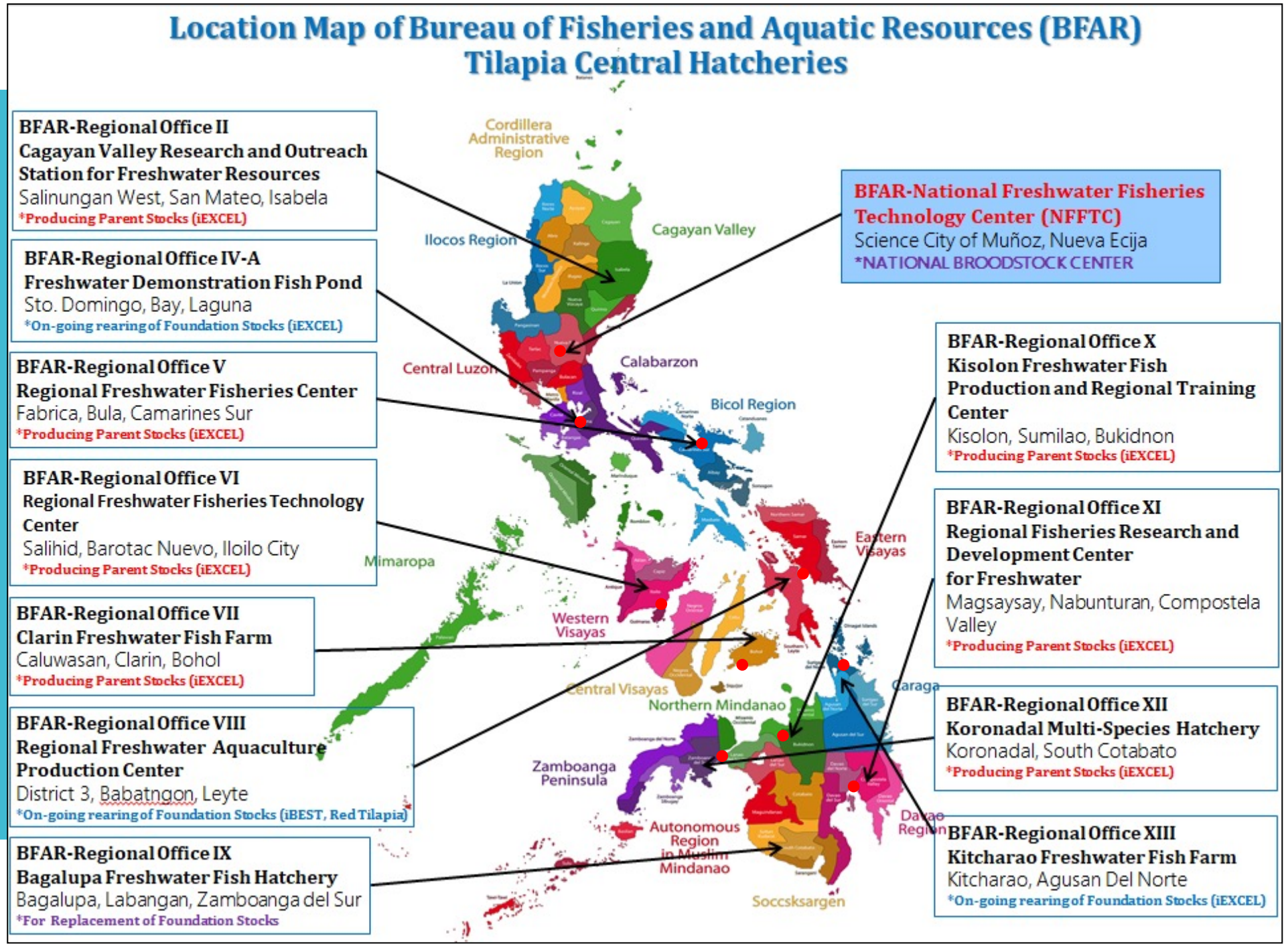




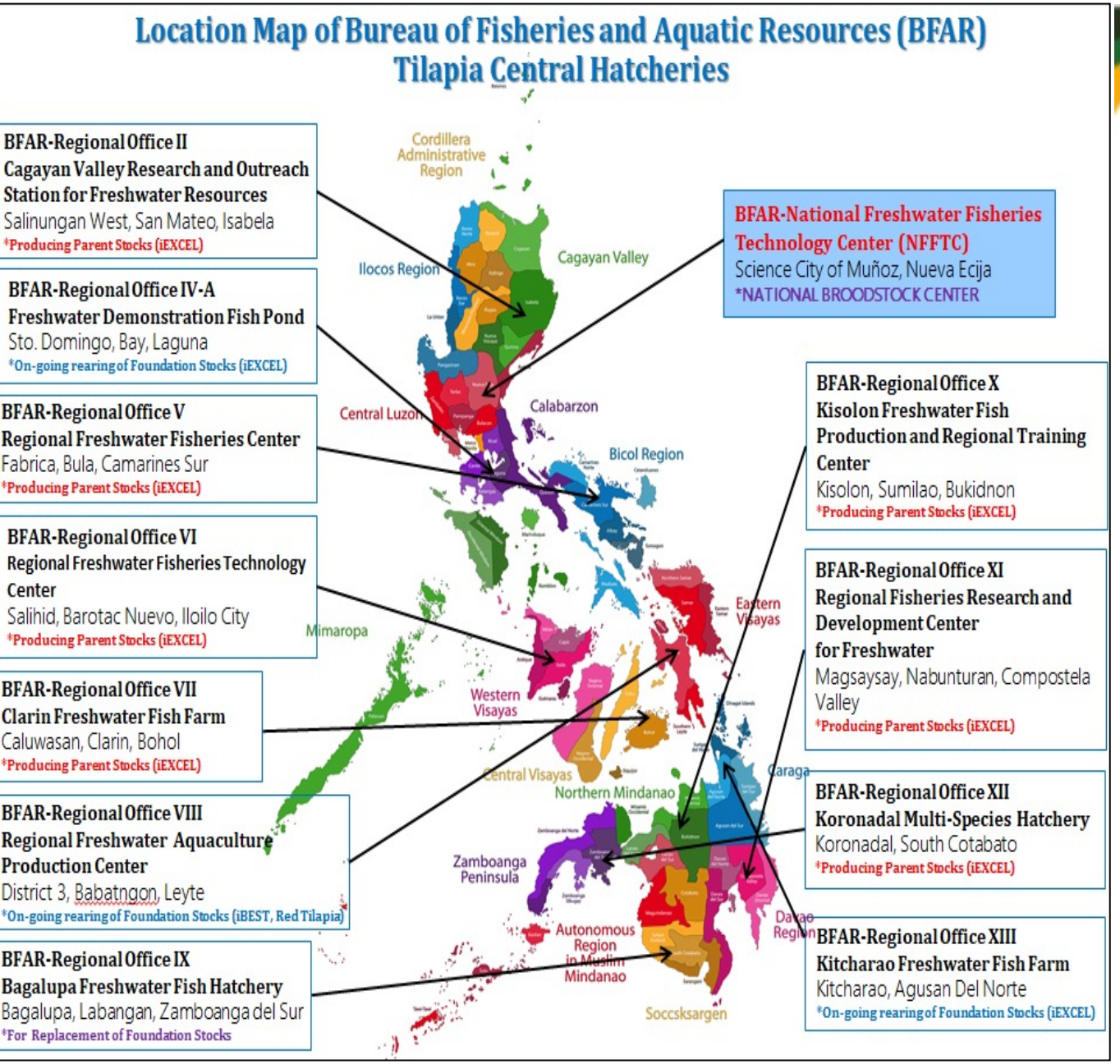
# Location Map of Bureau of Fisheries and Aquatic Resources (BFAR) Tilapia Central Hatcheries




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




# 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):<br>Location: 860 Arcadia Bldg,Quezon Ave., Quezon City, National Capital Region | • Automatic Tissue Processor<br>• Embedding Centre<br>• Microtome<br>• Water Bath<br>• Routine H&E Staining Set<br>• Fumehood<br>• Compound Microscope | Doctor of Fisheries<br>Technologist (1)<br>Veterinarians (6)<br>Biologists (3) |
| | Southeast Asian Fisheries Development Center (SEAFDEC)<br>Location: Tigbauan, 5021 Iloilo, Philippines | • Automatic Tissue Processor/ Tissue Processor<br>• Embedding Centre<br>• Water bath<br>• Microtome<br>• Routine H&E Staining Set<br>• Fumehood<br>• Compound Microscope | Aquatic Animal Health Experts<br>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** | • POCKIT MICROPLUS<br>• POCKIT Portable Nucleic Acid Analyzer<br>• Vortex Mixer<br>• Pipette set<br>• Automatic Nucleic Acid Extraction System<br>• Thermocycler, Thermal Block<br>• Realtime thermocycler<br>• Electrophoresis set<br>• Low temp incubator<br>• Biosafety cabinet/Laminar Flow/ Fumehood<br>• Hotplate<br>• Vortex Mixer<br>• Balance<br>• 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:<br>Location: Arellano Boulevard, Cebu City | • POCKIT MICROPLUS<br>• POCKIT Portable Nucleic Acid Analyzer<br>• Vortex Mixer<br>• Pipette set<br>• Automatic Nucleic Acid Extraction System<br>• Thermocycler, Thermal Block<br>• Realtime thermocycler<br>• Electrophoresis set<br>• Low temp incubator<br>• Biosafety cabinet/Laminar Flow/ Fumehood<br>• Hotplate<br>• Vortex Mixer<br>• Balance<br>• Autoclave | Biologist (2) Veterinarian (1)<br>Fisheries Generalist (1) |
| | Regional Fisheries Laboratory VIII:<br>Location: MRGP Commercial Bldg., Brgy 77, Marasbaras, Tacloban City | | Veterinarian (2)<br>Biologist (1) |
| | Regional Fisheries Laboratory X:<br>Location: BFAR 10 Compound, Julio Pacana St. Macabalan, Cagayan de Oro City | | Biologist (3)<br>Fisheries Technologist (2) |
| | Regional Fisheries Laboratory XI:<br>Location: Ramon Magsaysay Ave., Davao City | | Veterinarian (1) Biologist (1)<br>Chemical Engineering (1) |
| | Regional Fisheries Laboratory XII:<br>Location: D. A. Compond, J. Catolico Ave., Brgy. Lagao, General Santos City | | Chemist (1)<br>Fisheries (2) |
| | Regional Fisheries Laboratory XIII:<br>Location: Sitio Tawilon, Ambago, Butuan City | | Veterinarian (1) Biologist (2)<br>Chemist (1) |
| | Regional Fisheries Laboratory BARMM:<br>Location: MAFAR Compound, OCM Comp., ORC Cotabato City | | Fisheries Technologist (1)<br>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)<br>Location: Tigbauan, 5021 Iloilo, Philippines | • Vortex Mixer<br>• Pipette set<br>• Automatic Nucleic Acid Extraction System<br>• Thermocycler, Thermal Block<br>• Realtime thermocycler<br>• Electrophoresis set<br>• Low temp incubator<br>• Biosafety cabinet/Laminar Flow/ Fumehood<br>• Hotplate<br>• Vortex Mixer<br>• Balance<br>• Autoclave | Aquatic Animal Health Experts<br>Aquatic Animal Health Researchers |
| | Fisheries Biotechnology Center (FBC)<br>Location: BFAR-NFFTC, CLSU Compd, 3120 Science City of Muñoz, Nueva Ecija | | Aquatic Animal Health Experts<br>Aquatic Animal Health Researchers |
| | National Fisheries Reasearch Development Institute (NFRDI)<br>Location: 101 Mother Ignacia Ave., Brgy, Diliman, Quezon City, 1103 Metro Manila | | Aquatic Animal Health Experts<br>Aquatic Animal Health Researchers |
| | ACADEME:<br>- Central Luzon State University<br>- Don Mariano Marcos Memorial State University<br>- University of Sto. Tomas<br>- University of the Philippines-Diliman<br>- University of the Philippines-Los Baños<br>- University of the Philippines-Visayas | | Professors<br>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)<br>Location: Tigbauan, 5021 Iloilo, Philippines | • Laminar air flow hood<br>• $CO_2$ Incubator<br>• Centriuge<br>• Low Temp. Incubator<br>• Liquid Nitrogen container<br>• Flow Cytometry<br>• Inverted Microscope<br>• Dissecting microscope<br>• Water Bath | Aquatic Animal Health Experts<br>Aquatic Animal Health Researchers |
| **III. TEM** | Southeast Asian Fisheries Development Center (SEAFDEC)<br>Location: Tigbauan, 5021 Iloilo, Philippines | Transmission Electron Microscope | Aquatic Animal Health Experts<br>Aquatic Animal Health Researchers |
| | University of the Philippines- Diliman<br>Location: Diliman, Quezon City 1101 Metro Manila, Philippines | Transmission Electron Microscope | Professors<br>Researchers |

# CHECKLIST No.6

## *DIAGNOSTIC TESTING*

a. The method is the recommended method for reasons of availability, utility ad diagnostic specificity and sensitivity
b. The method is a standard method with good diagnostic sensitivity and specificity
c. The method has application in some situations but cost, accuracy or other factors severely limit the application
d. The method is presently not recommended for this purpose

N/A. not applicable

| METHOD | TARGETED SURVEILLANCE | | | PRESUMPTIVE DIAGNOSIS | CONFIRMATORY DIAGNOSIS |
|---|---|---|---|---|---|
| | Fish Fry | Juveniles | Adults | | |
| **Gross signs (I)** | C | C | C | C | D |
| **Histopathology (II)** | B | B | B | B | B |
| **Isolation with Cell-culture (III)** | N/A | N/A | N/A | N/A | N/A |
| **PCR-based assays (III)** | A | A | A | A | A |
| **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

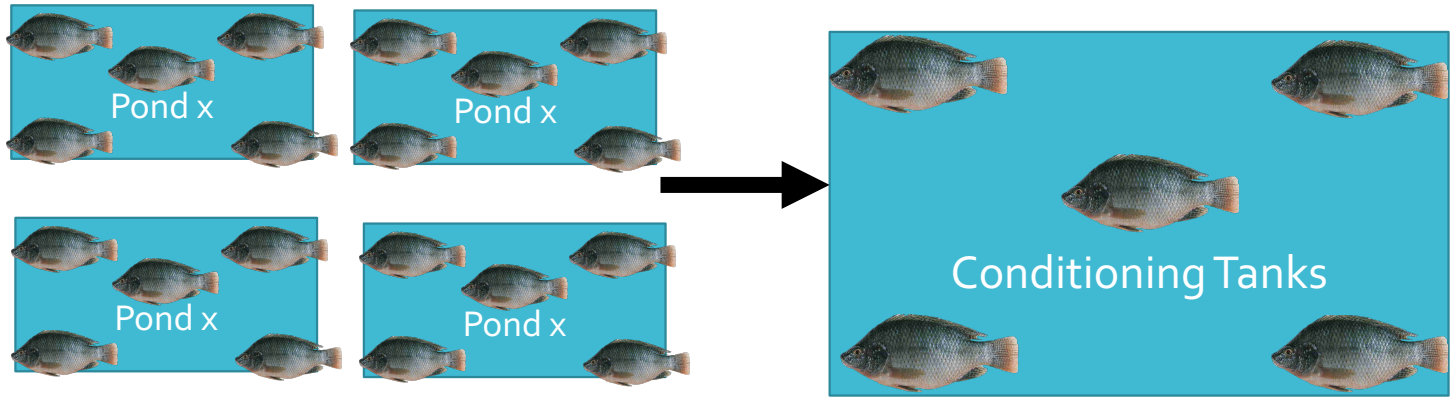
| | |
|---|---|
| Large population | 73 |
| Population = 95 | 42 |

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

CHECKLIST No.

# 8 & 9

## *DATA COLLECTION AND MANAGEMENT*

## *& DATA ANALYSIS*

- **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:

CHECKLIST No.

11

# HUMAN AND FINANCIAL RESOURCES AND LOGISTICS REQUIREMENTS

| 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