Food and Agriculture Organization of the United Nations

SUSTAINABLE DEVELOPMENT GOALS

VIRTUAL COURSE

26 March to 15 April 2021

Design of an Active Surveillance for Tilapia Lake Virus (TILV) Disease and Its Implementation

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

Food and Agriculture Organization of the United Nations

SUSTAINABLE DEVELOPMENT GOALS

CHECKLIST 6

05 April 2021

Field and laboratory preparation checklists and forms

Kathy Tang-Nelson

ktangnelson@gmail.com

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

Outline

- To achieve optimal conditions for tilapia culture and the need for accurate record-keeping on water quality, fish health, and farm operation
- To identify gross signs of TiLVD-affected tilapia
- To conduct fish necropsy and examine for internal lesions of TiLVD-affected tilapia
- To sample TiLV-infected fish tissues (liver, spleen, brain, heart, kidney, gills and muscle) for diagnostic analyses
- To design a sample submission form for diagnosing TiLVD
- To develop checklists of supplies and equipment needed for TiLV diagnosis

The optimal temperature and water quality for Nile tilapia.

Growth condition	Optimum	Range
Temperature (°C)	27-30 (warm water)	12-38
Dissolved oxygen (mg/L)	> 5	
Salinity (ppt)	5-10	<25
pH	6-9	5-10
Ammonia (NH_3) (mg/L)	< 0.1	
Nitrate (NO_3^-) (mg/L)	Keep < 27	n/a
Alkalinity (mg $CaCO_3$/L)	Keep <300	

Poor water quality can result suppression in immunity, increase risk for disease and mortality

Checklist for record-keeping of fish hatchery/farm

Information recorded	Yes	No	Comments
A. Feeding activities			
Date, time, tank/pond#			
Feed source			
B. Water quality management			
Date, time			
Salinity			
Algae blooms			
Dissolved O_2, temperature, pH, NH_3, No_2^-			
Water exchange			
Pipe flushing			
Filter back flushing			
C. Growth condition			
Weight, length, condition factor			
D. mortalities			
Date, time			
Gross signs			
Sampling for diagnosis			
E. Disinfection			
Date, chemical's name, concentration, treatment duration			
F. Human activities			
Date, name of visitor(s)			
G. Others (e.g. source and transfer of stock)			

(A) diseased red tilapia showed **hemorrhage** (black arrows)

(B) diseased Nile tilapia showed **skin erosion**, **hemorrhage** on various parts of body, loss of scales, **abdominal swelling**, and swelling of the eyeball (**exophthalmos**)

(C) diseased wild tilapia (*Sarotherodon galilaeus*) showed **shrinkage of the eye** and loss of ocular functioning.

Supplies and data sheet for gross signs examination

Lab work	Equipment, supplies, data sheets	
External gross signs examination (Level I)	☐data sheet recording any abnormalities in behavior, body, skin, gills, eyes	☐disposable lab coats and gloves ☐camera ☐Compound microsope ☐microscope slides, coverslips ☐scalpel, forceps, scissors

External examination data sheet

1. behavior (normal, gasping, flashing, crowding in the water inlet/outlet, lethargic, swim erratically, etc).

_____

2. body appearance (normal, swelling, color change, etc)

_____

3. skin (normal, hemorrhagic, loss of scales, erosion, etc.)

_____

4. gills (normal, pale, tissue losses, etc)

_____

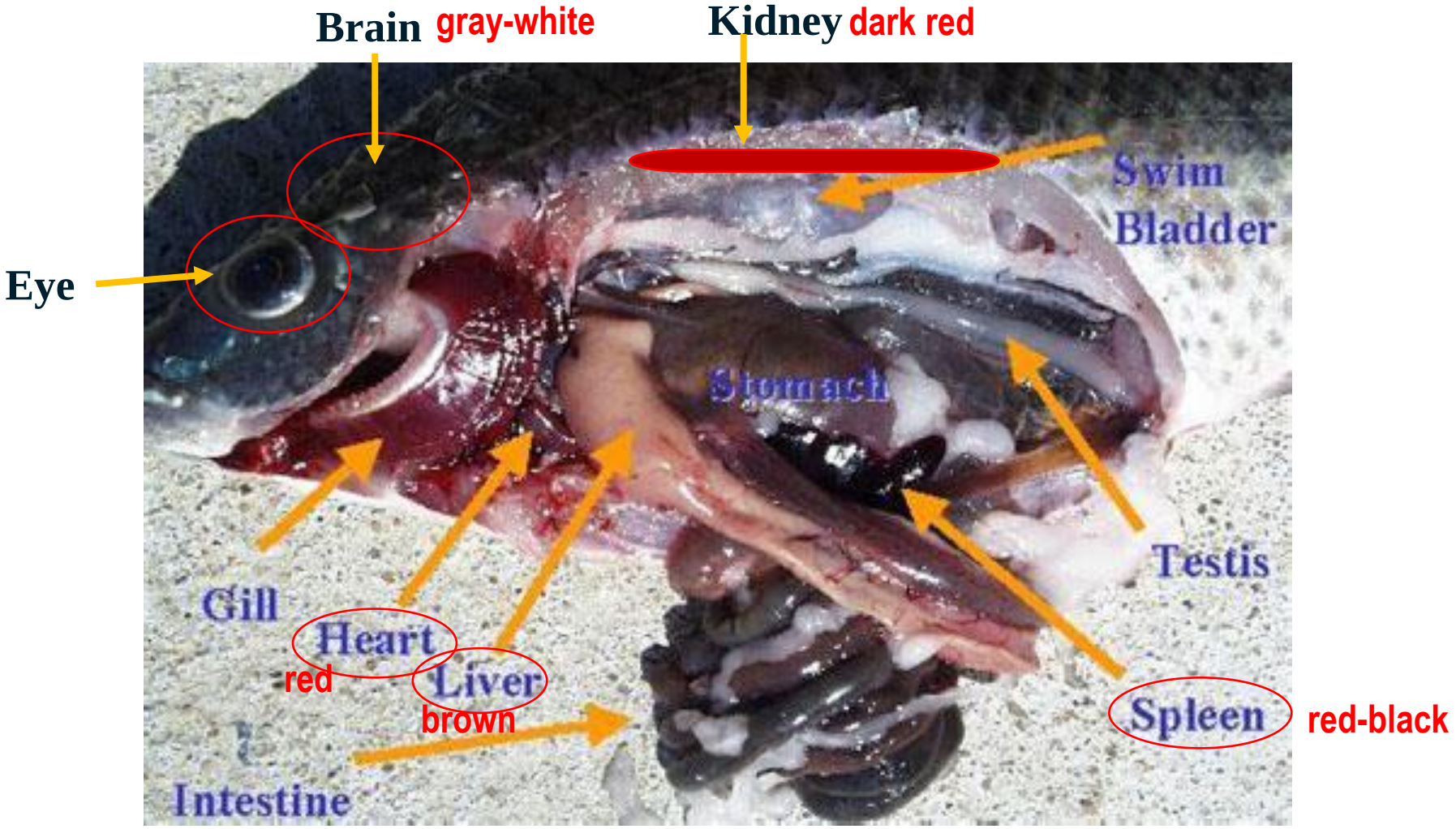
5. eyes (normal, exophthalmos, shrinking, cataracts, etc)

_____

6. photo/video: _____
7. skin scraping results: _____
8. gill scraping results: _____

Supplies for necropsy

Necropsy	☐fish nets ☐buckets ☐sedation chemicals (e.g. MS-222, 50-100 ppm) ☐measuring type/balance ☐sample jars, tubes or bags ☐sample ID labels ☐Permanent markers ☐95% ethanol	☐ laboratory gowns/latex groves/mask ☐dissecting kit (scissors, forceps, scalpels, etc) ☐dissecting tray ☐flame ☐sampling sticks, disposable razor blades ☐paper towel ☐trash bags



Tilapia tissue sampling for RNA extraction:

Fresh, frozen, or preserve in ethanol (70-95%), or other preserving solutions

Non-invasive: mucus

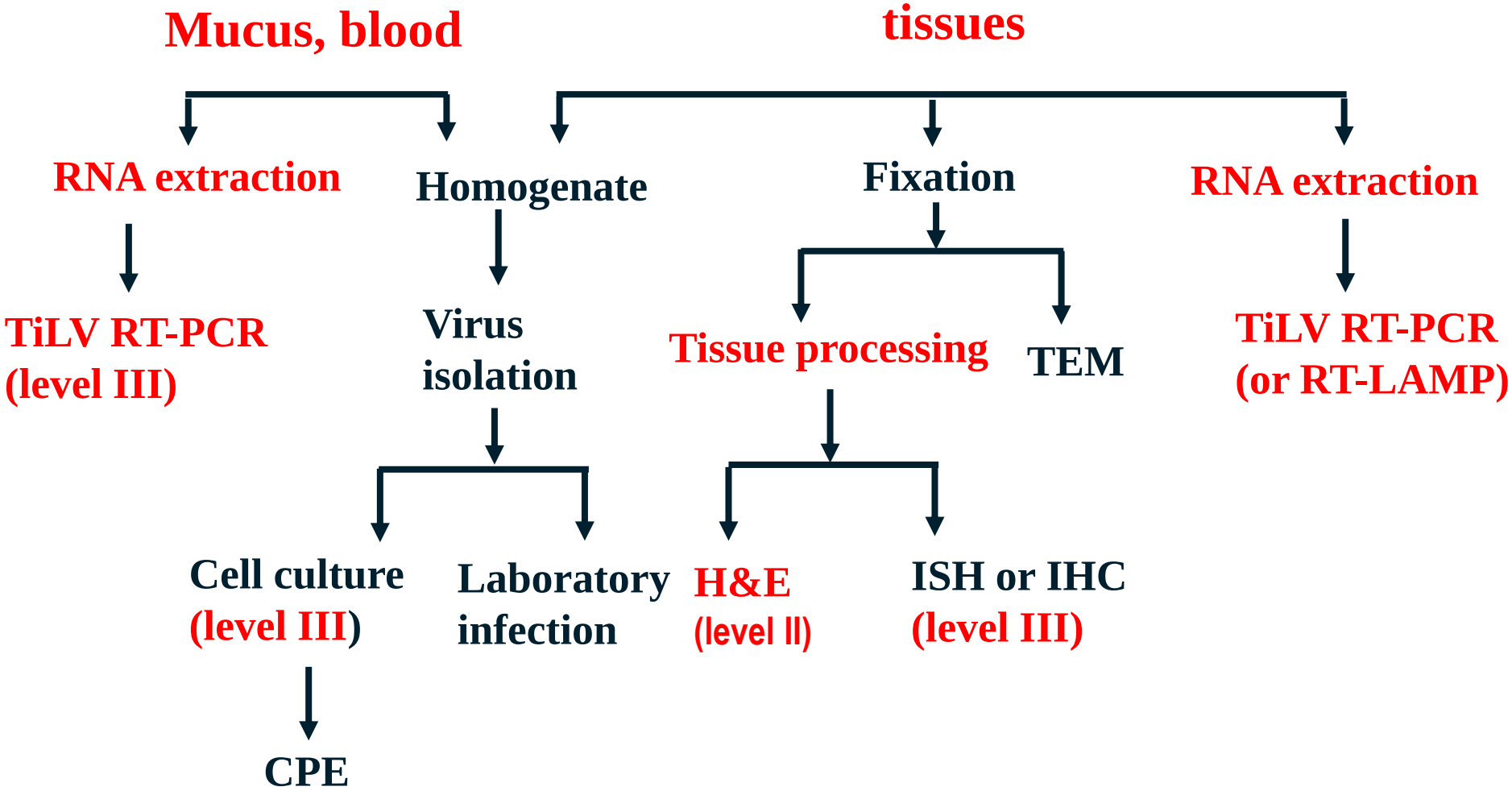
Data sheet for internal gross signs examination

Internal gross signs examination (Level I)	☐data sheet to record any abnormalities in the internal organs

Internal examination data sheet

1. visceral cavity (normal, acites, hemorrhage, etc)
2. Liver (normal, green, pale, watery, nodules, etc)
3. stomach (normal, empty, etc)
4. intestine (normal, empty, hemorrhage, fluid accumulation, etc)
5. spleen (normal, swollen, etc)
6. swimming bladder (normal, hemorrhage, etc)
7. kidney (normal, nodules, etc)
8. heart (normal, pale, etc)
9. brain (normal, reddness, etc)
9. gills (normal, hyperplasia, etc)
10. other tissues
11. photos/video

Fish samples for TiLV diagnosis (level II and III



Diagnosis usefulness of fish condition

Specimen	Histology	RT-PCR	Immuno assay	Virus isolation
Live fish	++	++	++	++
Dead fish (>6 h at room temperature)	-	-	-	-
Fresh dead fish in a plastic bag on ice for <6 h	+	+	+ (ELISA)	+
Frozen fish (-20°C, or dry ice)	-	++	++ (ELISA)	++
Formalin-fixed	++	-/+	++ (IHC)	-

Fish sample submission form

[Name of the diagnostic laboratory]				
Case number: Check in by: Date:	Sampled by: date:			
Name of the farm: Farm address:			Province:	Country:
Submitted by:	Address:			
Phone#:	Fax#		Email:	
Reporting name:	Address:			
Phone#	Fax#		Email:	
Billing name:	Address:			
Phone#			Email:	
Fish species:			Strain:	
Life stage: Age:			Sex: ☐male, ☐female, ☐not known	
Sample ID:			Number of fish:	
Test requested:	☐Histology	☐RT-PCR	☐others	
Sample type:	☐Whole fish	☐Liver	☐Brain	☐Spleen
	☐Kidney	☐Heart	☐Muscle	☐Gill
	☐Mucus	☐Cell culture	☐reproductive organs	☐Others
Sample condition	☐dead on ice (☐< 6 hr, ☐> 6 hr) ☐Dead >6 hr (room temperature)		☐Formalin-fixed ☐Glutaraldehyde (TEM)	☐Live fish ☐Frozen
Reasons for submitting the samples:				
☐Increasing mortality	☐Moribund	☐Surveillance	☐Health certificate	☐Others:
What treatment have been applied to this pond in the past 6 months? _____ Vaccination, ☐Yes, name of vaccine: _____; date: _____; ☐No Drug: ☐Yes, name of drug : _____; date: _____; ☐No				
Clinical sign: ☐Color change; ☐loss of scales; ☐skin erosion; ☐hemorrhage; ☐popped eyes; ☐shrinkage of eyes; ☐Abdominal swelling; others:				
Mortality: ☐Yes, date: _____; ☐No				
Abnormal behavior	☐gasping ☐Flashing	☐crowding in the water inlet or outlet	☐lethargic	☐swim erratically ☐swim in circles
Abnormal eating pattern	☐loss of appetite			

(A) Farm location and reporting
-farm owner(s), site
-person(s) by whom the samples were collected
-date that samples were collected
-name(s) to whom the report will be sent
-name(s) to whom a service fee will be charged

(B) Fish information: species
• Nile tilapia (*O. niloticus*)
• blue tilapia (*O. niloticus x O. aureus hybrids*)
• red tilapia (*Oreochromis sp.)*
• *Tilapia zillii* (wild cichlid in Africa and Middle East)
• *Sarotherodon galilaeus* (wild cichlid in Africa and the Levant)
• *Oreochromis aureus*
-strain (if known)
-life stage
-Sample ID by the sender

(C) Type of diagnostic tests to be performed

-gross signs (level I)

-histology (level II)

-RT-PCR (level III)

-viral isolation and cell-culture (level III)

-other

(D) Type of the samples

-whole fish (larvae, fry)

-tissues: liver, brain, spleen, kidney, heart, gills, muscle

-mucus, blood

-cell culture supernatant

-pondwater

-feces

(F) Purpose of testing

-increasing mortality
-moribund
-surveillance
-health certificate
-others

(G) History of treatment

-vaccination
-change water
-therapeutants
-others

(H) Gross signs (level I)

-body color change
-loss of scales
-skin erosion
-popped eyes (exophthalmos)
-shrinkage of eyes
-other

(I) Mortality

-% cumulative mortality (time-course)
-duration

(j) Abnormal behavior

-gasping (gills, environmental problems)
-flashing (skin irritation)
-scraping the body (parasite problems)
-crowding in the water inlet/outlet
-lethargic
-swim erratically
-swim in circles

(K) Abnormal feeding pattern

-loss of appetite
-others

Supplies and equipment for histology

Histology (Level II)	☐neutral buffered formalin (NBF) ☐fume hood ☐tissue processing machine ☐tissue embedding machine ☐microtome ☐staining solutions	☐compound microscope ☐microscope slides ☐coverslips

Supplies and equipment for PCR-based methods

PCR-based (level III)	**RNA extraction** ☐ RNA extraction kit ☐ Microfuge ☐ pipettors (50-, 1000- µl) ☐ filtered pipet tips ☐ Eppendorf tubes ☐ PCR tubes ☐ test tubes racks	**Conventional RT-PCR** ☐ PCR machine ☐ Gel electrophoresis apparatus ☐ Gel imaging system ☐ Pipettors ☐ RT-PCR enzymes kit ☐ TiLV primers ☐ electrophoresis buffer, molecular marker, gel loading dye, ethidium bromide ☐ positive control plasmid --- **RT-qPCR (real-time)** ☐ Real-time PCR system ☐ RT-qPCR enzymes ☐ TiLV real-time primers ☐ positive control plasmid

Food and Agriculture Organization of the United Nations

SUSTAINABLE DEVELOPMENT GOALS

Thank you for your attention!

Kathy Tang-Nelson

ktangnelson@gmail.com

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

This was also made possible with the support of the Norwegian Agency for Development Cooperation under the project GCP/GLO/979/NOR Improving Biosecurity Governance and Legal Framework for Efficient and Sustainable Aquaculture Production.

Norad