



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



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CHECKLIST #6

02 April 2021

Checklist 6: Diagnostic Testing General: Level I and Level II

Melba B. Reantaso

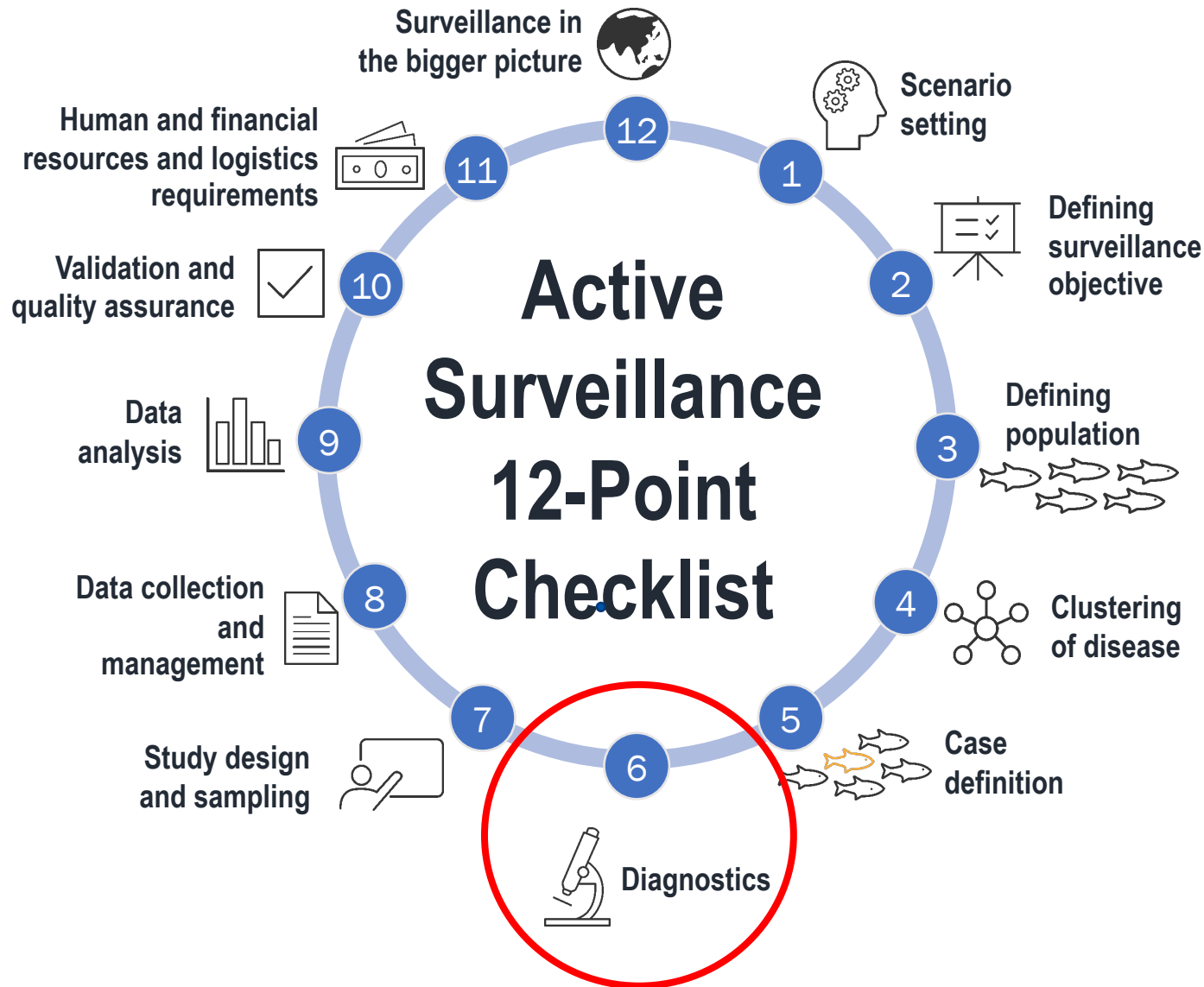
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TCP/INT/3707: Strengthening biosecurity (policy and farm level) governance to deal with Tilapia lake virus



Learning objectives

- to understand the requirements and criteria for Checklist 6
- to gain knowledge on the different levels of diagnostics in general
- to get to know the TiLV risk profile



Description of tests used
(procedures, interpretation of
results, sensitivity and
specificity), and
competent laboratories

Role of Diagnostics

Diagnostics play **two significant roles** in aquatic animal health management and disease control:

Screening of healthy animals to ensure that they are not carrying subclinical infections by pathogens of concern.

- This is commonly conducted on **samples of stocks or populations of aquatic animals destined for live transfer from one area or country to another**, and provides **protection on two fronts**:
 - it **reduces the risk that animals are carrying few, if any, opportunistic agents that might proliferate during shipping, handling or change of environment**; and
 - it **reduces the risk of resistant or tolerant animals transferring a significant pathogen to a population which may be susceptible to infection**.

Disease diagnosis of animals showing **signs of health deterioration** (such as spawning failure, growth or behaviour) or **clinical disease** (deformities, morbidity or mortality).

- **Accurate and rapid diagnosis** is essential for applying appropriate and effective management measures.



Role of diagnostics

Application of diagnostic analyses relies on a **broad array of techniques**:

- gross observation
- histology
- cell culture
- serology
- molecular testing
- genomic sequencing

The **choice of which approach** to be used for any diagnostic application depends on:

- the objective of surveillance
- available laboratory with competent equipment
- human and financial resources, and
- the quality management system for diagnostic tests.



Role of Diagnostics

When choosing the appropriate techniques: important considerations:

- **analytical sensitivity** (**limit of detection** for a disease agent) and
- **analytical specificity** (**ability to distinguish** the targeted disease agent from another) of each laboratory test
- ‘fit for use, fit for purpose’
- **diagnostic sensitivity** (test sensitivity to **correctly identify diseased individuals**) and
- **diagnostic specificity** (test specificity to **correctly identify non-diseased individuals**).

Role of Diagnostics

- **diagnostic sensitivity** (test sensitivity to **correctly identify diseased individuals**) and
- **diagnostic specificity** (test specificity to **correctly identify non-diseased individuals**).

COVID-19

Diagnostic test: **shows if you have an infection**

- Molecular test PCR: very sensitive, it targets the virus genetic material; nasopharyngeal samples; quick results; **shows active infection**
- Antigen test: less accurate; to see if there viral proteins; nasal swab; easy and simple; **may miss an active infection**

Antibody test (serology.blood serum): **shows if a person has antibodies** (produced when exposed to a viral infection); **do not show if one has current infection and should not be used as diagnostic test** but it can **identify who has been infected and has developed antibodies**; finger stick or draw blood



Role of diagnostics

- Diagnostic accuracy relies on a **solid case-history**.
- FAO has long promoted the use of **Levels I, II, and III** for disease diagnosis (FAO/NACA 2000, 2001).
- None of the **levels** function in isolation; each one **builds on the other, contributing valuable data and information for optimum diagnostic accuracy**.
- **Level I** provides the **foundation** and is the **basis for accurate interpretation of results** obtained from **Levels II and III laboratory findings**.



Diagnostic Levels, Associated Requirements and Responsibilities

Level	Activity	Work requirements	Responsibility	Technical requirements to support activities
I	Observation of animal and environment	Knowledge of normal (feeding, behavior, growth) of stock.	Farm worker/manager	Field keys
		Frequent / regular observation of stock.		Farm record keeping formats
	Gross clinical examination	Regular, consistent record-keeping and assistance (Levels II, III).maintenance of records – including fundamental environmental information.	Fishery extension officers	Equipment lists
				Model clinical observation sheets.
				Pond/Site record sheets.
		Knowledge contacts for health diagnosis Ability to submit and/or preserve representative specimens for optimal diagnosis (Levels II, III).	On-site veterinary support	Preservation/transportation guidelines for Levels II/III diagnoses
				Model job descriptions/skill requirements
			Local fishery biologists	e.g. Asia Diagnostic Guide for Aquatic Animal Diseases



Diagnostic Levels, Associated Requirements and Responsibilities

Level	Activity	Work requirements	Responsibility	Technical requirements to support activities
II	Virology	Laboratories with basic equipment and personnel trained/experienced in aquatic animal pathology	Fish biologists/ technicians	Model laboratory record-keeping system Protocols for preservation/ transport of samples to Level III Model laboratory requirements/ equipment/ consumables lists
	Bacteriology	Keep and maintain accurate diagnostic and laboratory case records	Aquatic Veterinarians	
		Ability to preserve and storage specimens for optimal Level III diagnoses	Parasitologists/ technicians	Model job descriptions/ skill lists Access to Level II and Level III specialist expertise
	Mycology	Knowledge of/ contact with different areas of specialization within Level II.	Mycologists/ technicians	e.g. Asia Diagnostic Guide for Aquatic Animal Diseases, OIE Diagnostic Manual for Aquatic Animal Diseases Regional General Diagnostics Manuals
	Histopathology		Bacteriologists/ technicians	
		Knowledge of who to contact for Level III diagnostic assistance	Histopathologists/technicians	



Diagnostic Levels, Associated Requirements and Responsibilities

Level	Activity	Work requirements	Responsibility	Technical requirements to support activities
III	Virology	Highly equipped laboratory with highly specialized and trained personnel	Virologist/ technician	Model laboratory requirements/ equipment/ consumables lists
	Electron microscopy	Keep and maintain accurate diagnostic and laboratory case records.	Ultrastructural histopathologist/ technicians	Model job descriptions/ skill requirements
		Preserve and store specimens.		Contact information for reference laboratories
	Molecular biology	Maintenance of contact with people responsible for sample submission.	Molecular biology scientists/ technicians.	Asia Diagnostic Guide for Aquatic Animal Diseases, OIE Diagnostic Manual for Aquatic Animal Diseases, General molecular and microbiology diagnostic references
Immunology				



Level 1: Includes farm/production site observations, record-keeping and gross clinical signs – such information forms the basis for accurate results from Levels II and III diagnostic analyses.

Observations: examples of abnormal behavior (any sign deviating from normal behavior) usually exhibited when fish is under stress

Gross observations	fish swimming near the surface, sinking to the bottom, loss of balance
Behaviour	flashing, cork-screwing air gulping (non air-breathers
Surface observations: different external organs	oxygen deprivation leads to gulping, listlessness, belly-up or rolling motion - this can be due to blood or gill impairment.
Internal observations: internal organs	flashing can indicate surface irritation, e.g., superficial secondary infections of surface lesions.
	Cork- screw and other bizarre behaviour may also indicate neurological problems that may be disease related



Level 1: Includes farm/production site observations, record-keeping and gross clinical signs – such information forms the basis for accurate results from Levels II and III diagnostic analyses.

RECORD Keeping including stock records

Environmental observations

It is critical to establish, and record, normal behaviour and appearance to compare with observations made during disease events. Record-keeping is, therefore, an essential component of *effective* disease management.

open water, ponds, cage and net culture systems

Gross observations

weather

water temperature

For hatcheries, critical information that should be recorded include:
feeding activity, growth, mortalities

oxygen

salinity

turbidity (qualitative evaluation or Secchi disc)

algal blooms

For pond or net/cage sites, observations which need to be recorded include:
growth, fouling, mortalities

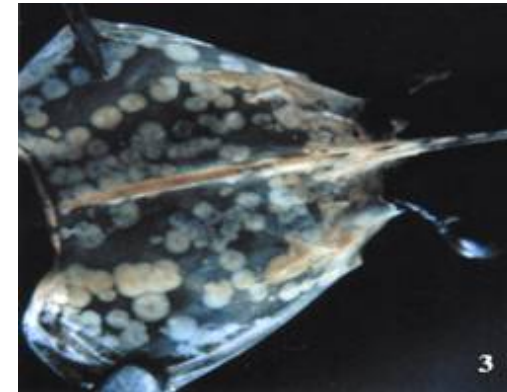
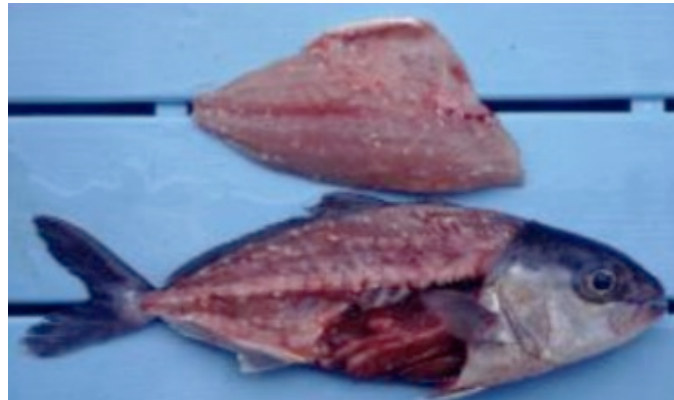
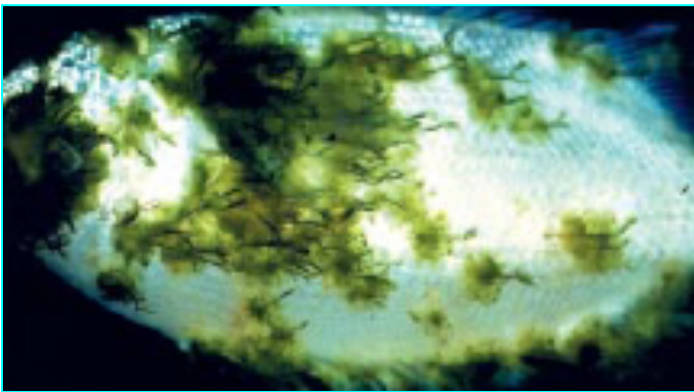
human activity (handling, neighbouring land use/water activities)

pH

All movements of fish into and out of a hatchery or site should be recorded, including: the source of the broodstock/eggs/larvae/juveniles and their health certification the volume or number of fish condition on arrival date and time of delivery and name of person responsible for receiving the fish date, time and destination of stock shipped-out from a hatchery or site.

Level 1

- Includes farm/production site observations, record-keeping and gross clinical signs – such information forms the basis for accurate results from Levels II and III diagnostic analyses.





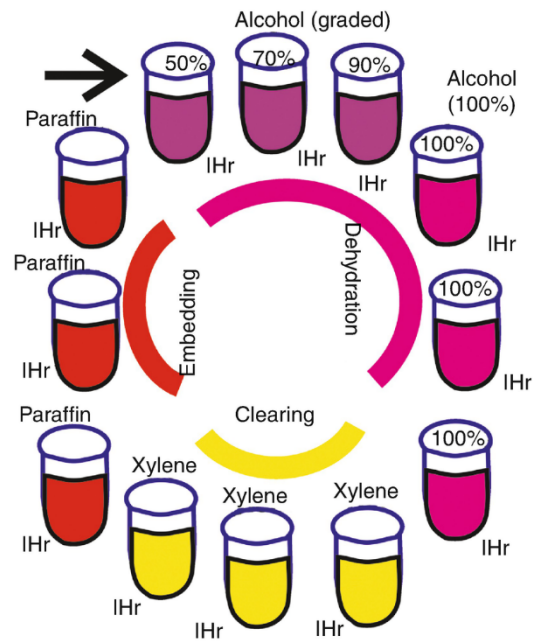
Level II: Includes the equipment and experience to undertake analyses that can detect and/or identify a range of pathogens.

Level II laboratories can do parasitology, histopathology, bacteriology and mycology.



Lab type	What it does	Process
Histopathology	microscopic examination of tissue in order to study the manifestations of a disease	Collection of sample, appropriate fixation, processing, embed in wax, sections in glass slide, staining (most common is H&E, specialized stains)

Fish necropsy
 Collect tissue sample from moribund fish
 Fix (formalin)
 Change to alcohol
 Process



Dey, 2018

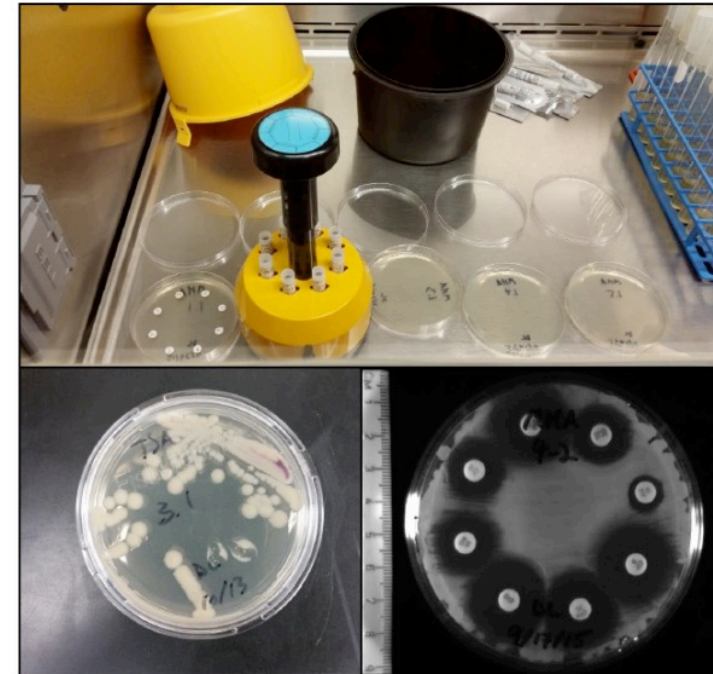
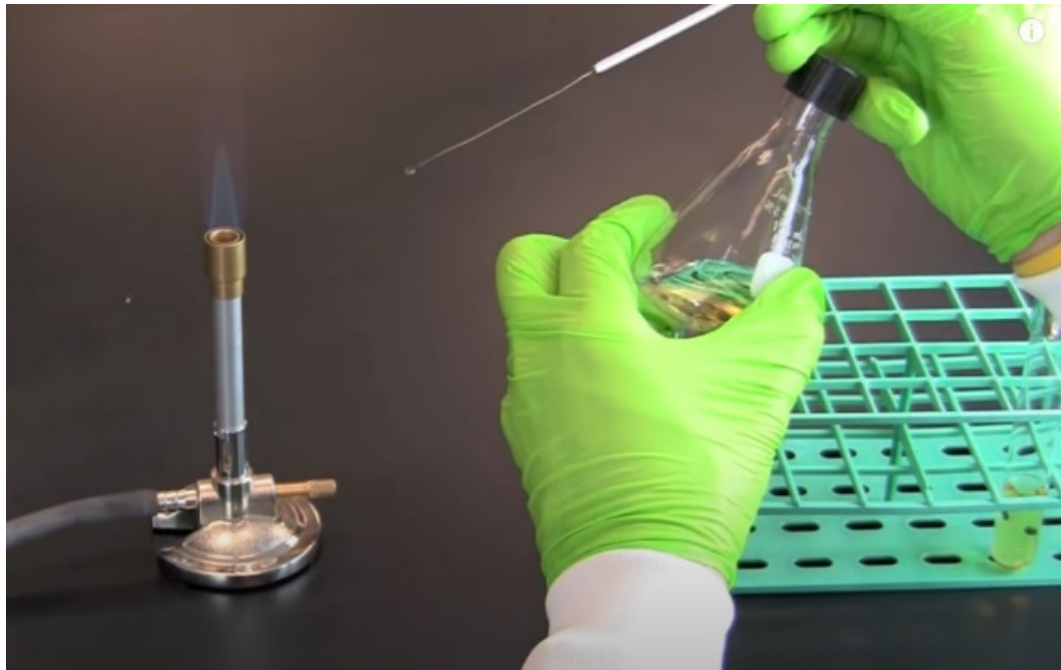




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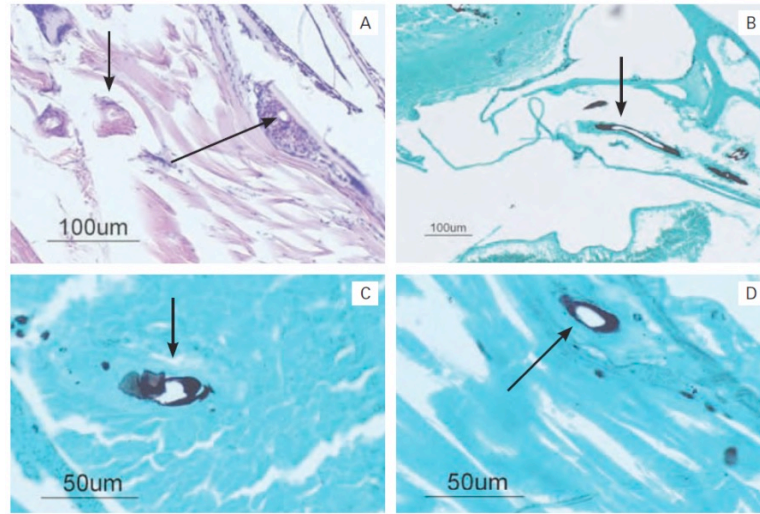
Level II laboratories can do parasitology, histopathology, bacteriology and mycology.

Lab type	What it does	Process
Bacteriology	identification, classification, and characterization of bacterial species	Collection of sample (lab or field), inoculate in agar plate, culture of bacterial isolates and identification Aseptic techniques very important to prevent contamination

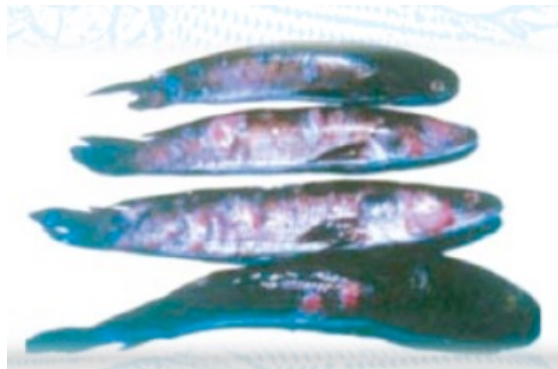




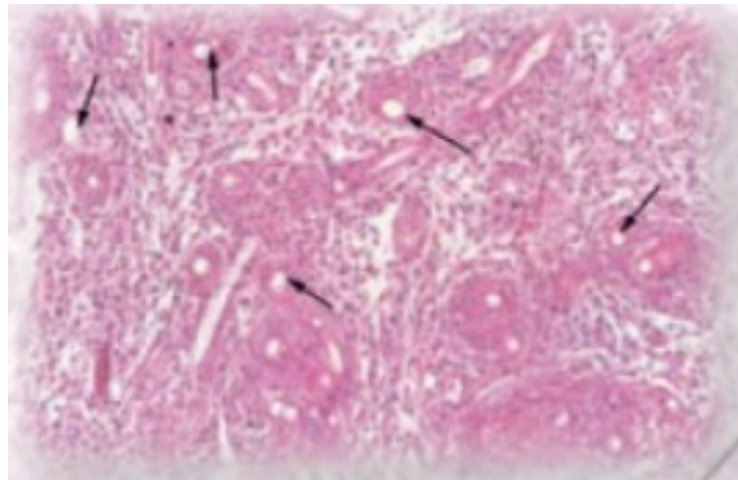
Silver barb, Botswana



H & E and Grocott;s stain: mycotic granuloma



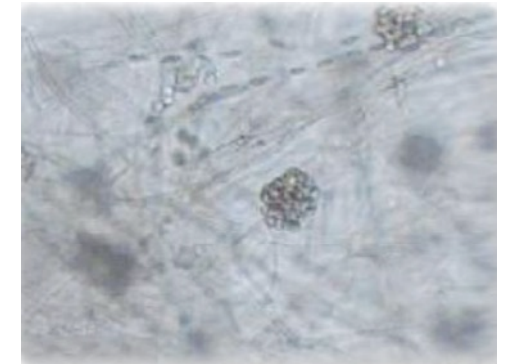
Snakehead, Philippines



H&E, mycotic granuloma



Aphanomyces sporangia



Aphanomyces
sporangium

Granuloma: aggregation of
macrophages – a response
to an infection
Macrophages are
specialized cells involved in
detection and destruction
of harmful organisms

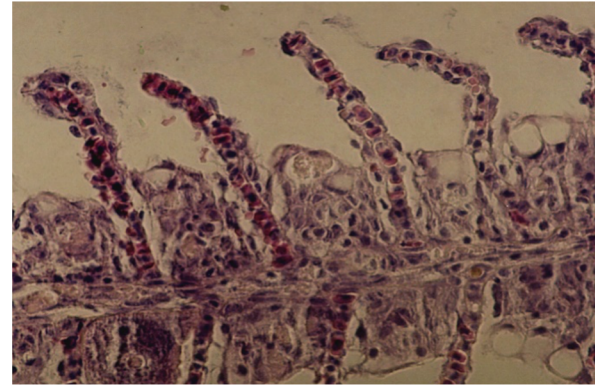
Koi herpesvirus: Level I,II,III



Mass mortality of
common carp,
Indonesia



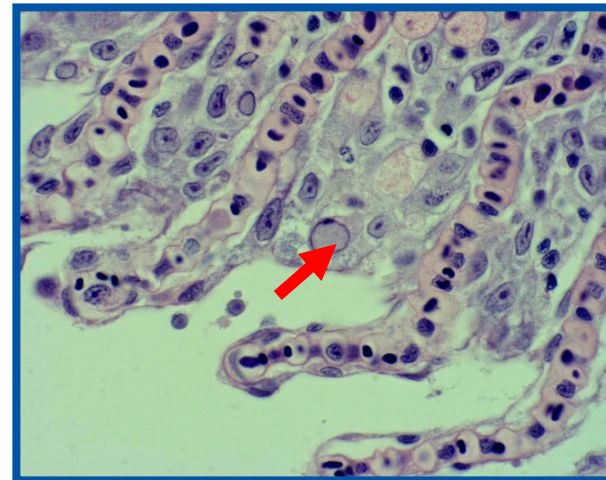
Darkened and blistered skin
and gill damage, common carp



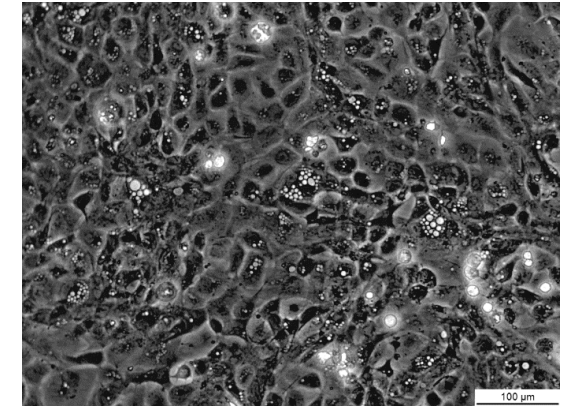
Swelling of the tips of gill
primary lamellae



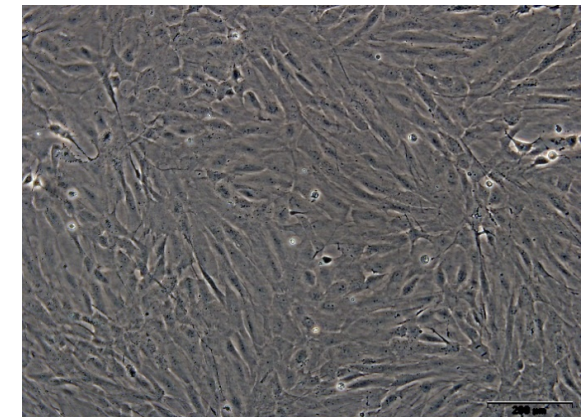
Excessive mucous in the gills,
koi carp



Presence of inclusion bodies

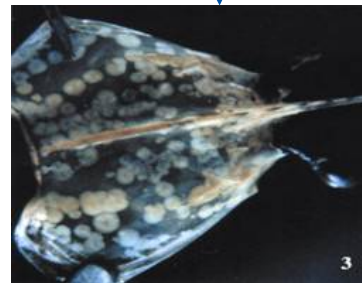
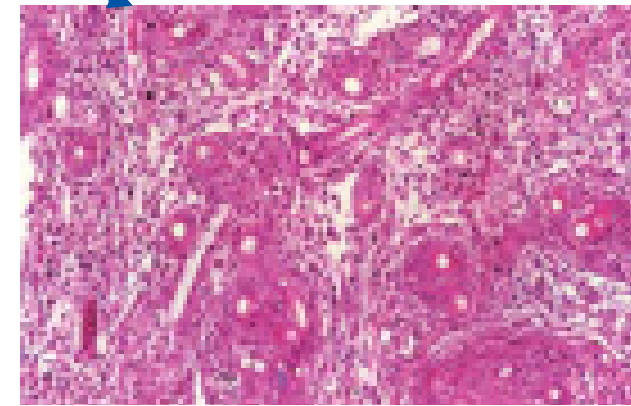
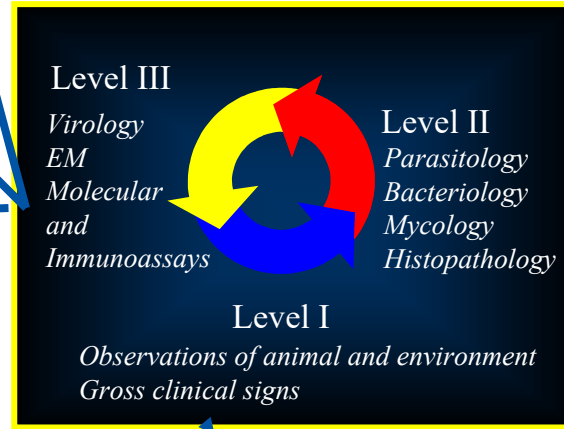
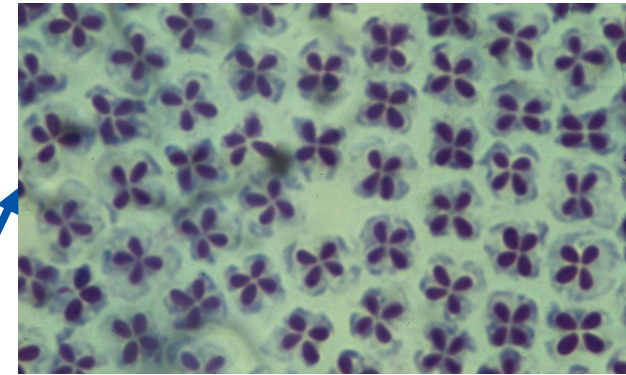
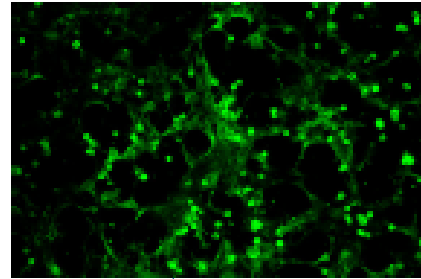
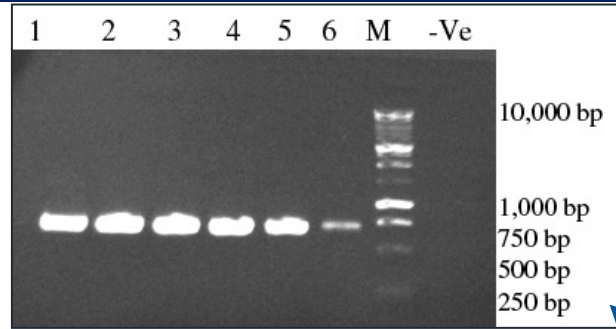


Cytopathic effect (CPE) – structural
changes in host cell due to viral
infection: extensive vacuolation in
infected KF-1 cells (upper photo);
normal KF-1 cells (lower photo)



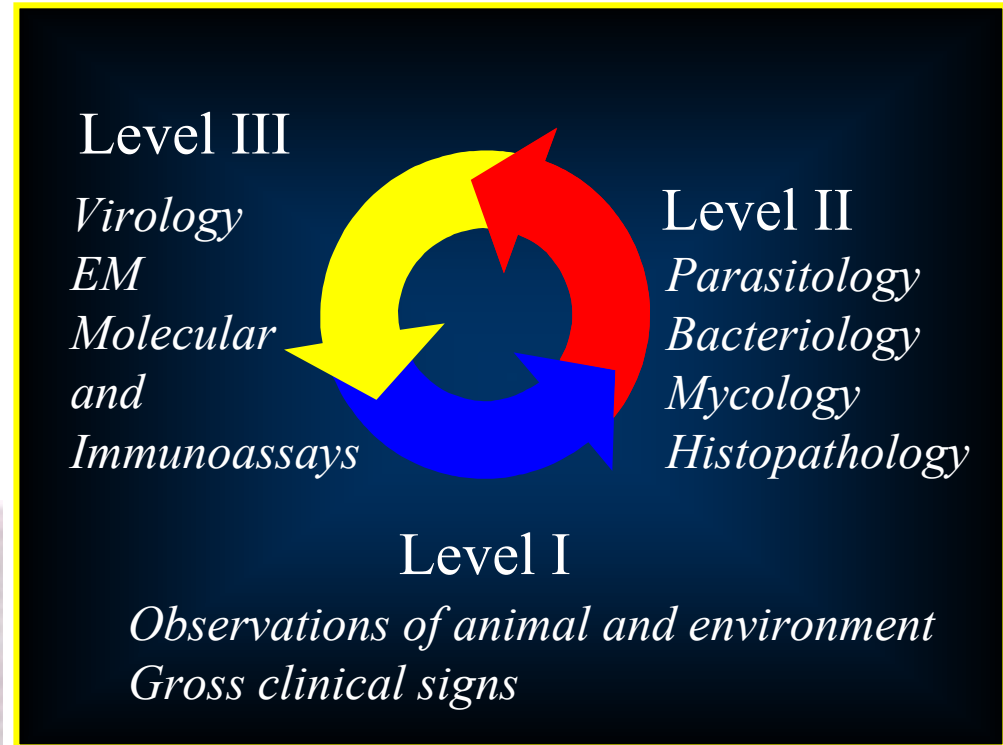
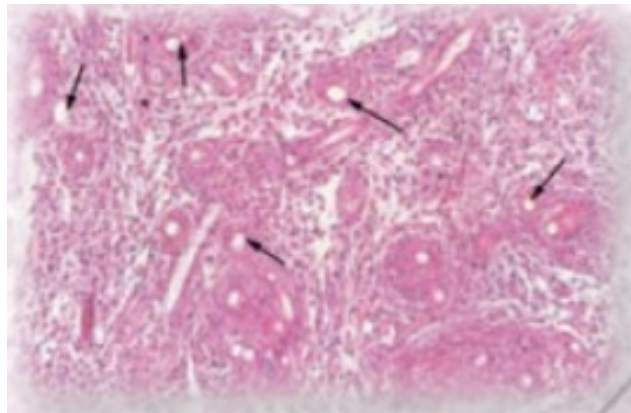
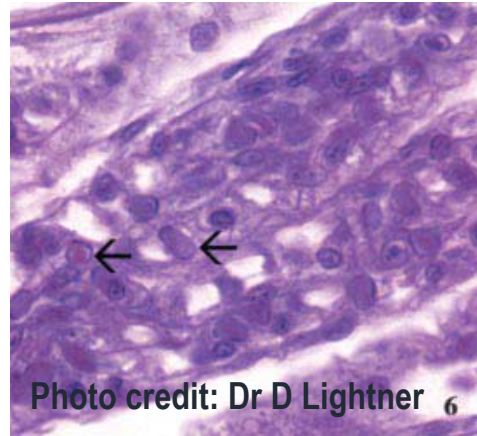
Levels of Diagnostics

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Levels of Diagnostics





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Thank you for your attention!

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Norad

TCP/INT/3707:

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