

## **GENERAL PROCEDURE FOR DISEASE DIAGNOSIS**

Diagnosis is a label given for a medical condition or disease identified by its signs, symptoms, and from the results of various diagnostic procedures. The term "diagnostic criteria" designates the combination of signs, symptoms, and test results that allows the clinician to ascertain the diagnosis of the respective disease. Disease diagnosis is refers to the various procedures and techniques used to identify the nature of disease and to precisely pinpoint the primary and secondary pathogens involved. Disease diagnosis is an integral part of aquatic health management.

### **Steps of diagnosis**

1. Case history
2. Clinical signs
3. Post Mortem examination
4. Lab exam- Microbiological diagnosis
5. Histopathological examination
6. Microbiological diagnosis
7. Molecular methods to detect and characterize the agent's nucleic acid
8. Biological test

### **1. CASE HISTORY**

#### **1) FISH**

##### **Mortality**

- Mortality rate
- Time-early morning or sudden
- Species involved
- Size and age od dead fish

#### **All run facilities**

- Sudden change in routine management
- Usual routine for feeding as well change in diet quantity, quality or forms ( fry, fingerling or brood stock). As well source of protein percentages
- Prior treatments, including medications, have been given
- Abnormal behaviour or functions of fish.

#### **2) ENVIROMENT**

##### **(A) Rearing unit**

- Type, size and location of facilities
- System of fish management
- Stocking rate/ stocking time
- Depth of water

- Presence of aquatic plants or vegetation
- Presence of natural enemies (dogs, birds, etc)
- Habitat utilization
- The surrounding of the rearing unit-human habituation infected neighbour farms.

### **(B) WATER-**

- Water source – sewage, underground or surface water
- Water exchange rate
- Physical examination of water
  - Colour, transparency, presence of algae
  - Turbidity
  - Smell- ammonia, organic matter
- Chemical examination of water
  - Dissolved oxygen
  - Ammonia, Nitrate etc
  - pH
  - Hardness
  - Heavy metals

## **2. Clinical signs**

### **1. Behaviour examination**

#### **(A) general examination**

- If a client submit live fish to the laboratory, aeration should be directly placed into the container that holds the fish
- Examination of the fish
  - I.** Swimming and movement behaviour
  - II.** Escape reflex- sick fish are easily caught
  - III.** Position of the fish
    - Flashing or itching (rubbing against object suggesting skin problems)
    - Pipping (staying near the air- water interface to obtain more oxygen, suggesting gill problems)
    - Sick fish may exhibit other behavioural signs including staying near the surface of the water = respiratory distress.
    - Scrapping the body or holding the fins close to the body ('clamped') because of parasite irritation
    - Showing various behavioural abnormalities
    - Sick fish often congregate together, separating themselves from healthier
    - Different fish species inhabit different parts of the water column (surface, bottom, shoreline, etc), and this position often changes with sickness.

## **(B) Clinical examination-**

Precautions during examination

1. Fish should be carefully handle
2. Not allow fish to come in contact with hard objects that affects slim
3. Examine fish on moist surface
4. Use naked eye, stereo microscope or magnifying lens.

### **2. External Examination**

- small fish can be examined in moist palm, leaves etc.
- large fish, put on the right side on moist glass or plastic. Put the fish on their right sides and held them by left hands. Save eyes with the palm and with other hand support tail.

- **Body inspection**

Color of the body

- Sick fish are often abnormally colored, compared with the healthier
- Fishes in breeding condition have more brilliant colors than non breeding fish
- Darkness over whole body--- chronic disease, bad water quality
- Hemorrhagic lesions on body surface or fins.....Reddening of the body is usually caused by hemorrhage, which can result from systemic bacterial or viral infections
- Parasites or other irritating conditions may also elicit a thickening of the skin, leading to a whitish or bluish skin color. This change might be highly localized (pinpoint to larger foci) or cover nearly the entire body

### **3. Post Mortem Examination**

- Symptoms and signs of disease
- Swollen kidney, liver, inflamed internal organs
- Pale, eroded gills, excess mucus on gill

**4. Microbiological diagnosis-** Bacterial diseases can be identified by various microbiology methods. The samples are taken from the fish under aseptic condition and they are first grown in non-selective media, then the various microbial diagnostic methods are used. The different microbiological diagnostic methods are

- 1. Staining methods-** Various staining methods are used to identify various bacterial pathogens.

**Gram stain:** The Gram stain classifies bacteria according to whether they retain crystal violet stain (gram-positive — blue) or not (gram-negative — red) and highlights cell morphology (eg, bacilli, cocci) and cell arrangement (eg, clumps, chains, diploids).

**Acid-fast and moderate (modified) acid-fast stains**

## **Fluorescent stains**

**India ink (colloidal carbon) stain**

**Wright's stain and Giemsa stain**

**Trichrome stain (Gomori-Wheatley stain) and iron hematoxylin stain**

## **2. Motility test**

There are three methods to see the motility of the bacteria and they are

- i. Hanging Drop Method
- ii. Semi Solid Agar Method (Craigie)
- iii. Three Coverslip Method

**3. Culturing in selective medium-** Certain bacteria have the capacity to grow in selective media. This property of bacteria is used to identify the bacterial pathogen. Only particular kind of bacteria can grow in selective media. Example for selective medium is TCBS for *Vibrios*.

**4. Biochemical test-** Various biochemical test are used for the identification of bacteria based on the character of a particular bacteria to give its positive and negative result for a particular biochemical test.

- I. The Indole Test
- II. The Methyl Red and Voges-Proskauer Tests
- III. Catalase Test
- IV. The Citrate Test
- V. Oxidase Test
- VI. Nitrate Reduction Test
- VII. Urease Test
- VIII. Triple Sugar Iron agar etc.

**5. Histopathology** – Histology is a branch of biology that involves the microscopic examination of thin, stained tissue sections in order to study their structure and function and in the case of histopathology, to determine changes which may be due to pathogens and disease.

## **6. Immunological Diagnosis**

Antigen — Antibody reactions are highly specific and sensitive. This forms the basis of immunodiagnosics. These tools are used for the qualitative and quantitative estimation of the pathogens and/or the protective antibody.

1. Agglutination Test,
2. ELISA,
3. Dot ELISA,
4. Latex Agglutination Test and
5. Fluorescent Antibody Test.

## **7. Molecular diagnosis**

Molecular techniques are potentially faster and more sensitive than culture, serology, and histology methods that are traditionally used to identify fish pathogens. During the last 15 years or so, molecular techniques have been increasingly employed to diagnose fish diseases. These techniques include polymerase chain reaction (PCR), restriction enzyme digestion, probe hybridization, in situ hybridization, and microarray. Pathogens can be detected from asymptomatic fish by molecular diagnostic techniques so disease outbreak could be prevented.