Developing an active surveillance for TiLV: 12-point checklist

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12-point checklist

#	Element	#	Element
1	Defining surveillance objective/purpose	7	Study design and data analysis methodology
2	Definition of population	8	Data flow and management
3	Clustering of disease	9	Validation
4	Case/outbreak definition	10	Quality assurance
5	Sampling	11	Human and financial requirements
6	Diagnostics/laboratory testing	12	Putting surveillance in the bigger picture (biosecurity, animal health, aquaculture, food safety/security, One Health)

12-point checklist

Step	Description	Criteria
1	Defining surveillance objective/ purpose	1.a. Set with respect to disease 1.b. Set with respect to disease presence 1.c. Set with respect to level of certification 1.d. Set with respect to timeframe
2	Definition of population	2.a. Includes definition of the population of interest 2.b. Includes definition of the targeted population 2.c. Includes definition of the study population (population used for sampling) 2.c.1.Inclusion criteria are set and described 2.c.2.Exclusion criteria are set and described
3	Clustering of disease	• Distribution of disease is considered and described • Distribution of disease is accounted in sampling/survey design and data analysis
4	Case/outbreak definition	• Case/outbreak definition are included: clinical, laboratory, epidemiological

12-point checklist

Step	Description	Criteria
5	Sampling	• Used/described sampling frame • Described sampling method • Defined sampling units • Explained consideration regarding sample size • Describe tissues/fluids used as sampling material • Describe sample selection process
6	Diagnostics/testing	• List and description of tests used (procedures, interpretation of results, Se/Sp) • List of laboratories included
7	Study design and data analysis methodology	• Survey design described • Risk assessment used and described • Methods of data analysis described
8	Data flow and management	• Data forms • Data base • Compatibility and transparency • Consistency, quality and resolution of data

12-point checklist

Step	Description	Criteria
9	Validation	Done by statistical estimation of the level of confidence (Se of surveillance program) Confirmed by pilot trial Done by expert/external evaluation (peer-review)
10	Quality assurance	Audit and corrective measures
11	Human and Financial Requirements	Included and described (e.g. personnel, cost of sampling, cost of laboratory tests, analysis of data, etc.)
12	Putting surveillance in the bigger picture (biosecurity, animal health, aquaculture, food safety/security, One Health)	Surveillance as an essential component of aquatic animal health/aquatic biosecurity strategies, aquatic animal health protection programmes or disease control plans, One Health platform within the context of aquaculture

1	**Defining surveillance objective/purpose**	**1.a. Set with respect to disease** **1.b. Set with respect to disease presence** **1.c. Set with respect to level of certification** **1.d. Set with respect to timeframe**

TiLV surveillance scenario	Aim/purpose of EUS surveillance	Countries
Infected country (one or more TiLV cases reported in previous two years)	To measure TiLV prevalence at national level in wild and farmed populations during 2018/2019 To identify possible risk factors for spreading TiLV in order to develop a disease control program To establish a transparent (according to OIE requirements for emerging disease) reporting system	Indonesia Malaysia Peru Philippines VietNam
Unknown status (no reported cases and no previous surveillance activities, however considered at risk)	To investigate presence/absence of TiLV in wild and farmed fish To secure early detection of TiLV	Mozambique Myanmar Sri Lanka
Considered free (no reported cases in previous surveillance activities)	To confirm present status of TiLV in country To secure early detection of TiLV	Brasil China

2	Definition of population	2.a. Includes definition of the population of interest 2.b. Includes definition of the targeted population 2.c. Includes definition of the study population (population used for sampling) 2.c.1.Inclusion criteria are set and described 2.c.2.Exclusion criteria are set and described

- listing of all susceptible wild fish species , present or likely present in rivers, lakes and other water bodies as well as a list of farmed fish species
- mapping the geographical distribution of rivers, lakes and water bodies and organization into zones based on water flow and/or the provinces or districts and neighbouring counties involved.
- mapping of population data and information (i.e., which species occur in which water bodies)
- list of farm/ponds to establish a sampling framework - sampling size may be determined
- 3-5 fish species are susceptible; all or the most susceptible should be identified and targeted..

TiLV surveillance scenario	Definition of population
Infected country (one or more TiLV cases reported in previous two years)	• All susceptible fish species (juvenile and young adults) in rivers and lakes • Wild fish • All farmed susceptible fish species (number of fish farms/establishments described, if they exist)
Unknown status (no reported cases and no previous surveillance activities, however considered at risk)	• All susceptible fish species (juvenile and young adults) in rivers and lakes • All farmed susceptible fish species
Considered free (no reported cases in previous surveillance activities)	• All susceptible fish species (juvenile and young adults) in rivers and lakes • All farmed susceptible fish species in rivers andlakes • All farmed susceptible fish species

3	Clustering of disease	• Distribution of disease is considered and described • Distribution of disease is accounted in sampling/survey design and data analysis

TiLV occurs mostly at water temperatures ranging between xx-xx°C and after (some identified risk factors or environmental conditions). These risk factors and environmental conditions have to be considered in determination of the sampling period. Seasonal occurrence of TiLV is an important factor in the planning of the sampling period. TiLV has not been reported from, e.g. broodstock (??), so only susceptible stages (e.g., juveniles and adults) need to be sampled

Country	Which month of the year			
	Q1	Q2	Q3	Q4
Brasil				
China				
Indonesia				
Malaysia				
Mozambique				
Myanmar				
Peru				
Philippines				
Sri Lanka				
Vietnam				

4	Case/outbreak definition	Case/outbreak definition are included: Clinical, Laboratory, Epidemiological

A case definition *is a set of standard criteria for deciding whether an individual study unit of interest has a particular disease or other outcome of interest. The study unit may be an individual animal or a group of animals such as a pond of shrimp, a cage of fish, an entire farm or a village.*

The choice of a particular case definition will depend on the objectives and methods used in the investigation. No matter what case definition is used, it will not be perfect. In fact, **case definitions are subject to the same types of errors as screening and diagnostic tests in general**, i.e. they are subject to **random (lack of precision)** and **non-random (false negative and false positive)** errors.

False negative results are due to lack of sensitivity, while **false positive results are due to lack of specificity.**
In any test system, there is always a trade-off between sensitivity and specificity – as we increase one, there is

The **purpose of a case definition** is to assure that the surveillance will focus on the disease of concern, TiLV, and not any other disease showing similar clinical signs. The following case definition has been adopted for purpose of EUS surveillance:-
Suspect TiLV case: A fish showing clinical signs similar to the ones associated with TiLV followed by positive finding of xxx pathology in the xx organ.
Suspect TiLV location/farm: A location/farm where one or more suspect EUS fish have been found.
Confirmed TiLV case: Histopathology or viral isolation or PCR.
Confirmed TiLV location/farm: A location/farm where a case of TiLV has been confirmed.

5	Sampling	• Used/described sampling frame • Described sampling method • Defined sampling units • Explained consideration regarding sample size • Describe tissues/fluids used as sampling material • Describe sample selection process

Wild fish population: non-random spatial sampling can be used.

Spatial sampling is similar to random sampling, but instead of selecting individuals from the sampling frame, random locations can be selected from an area.

Samples should be from randomly selected locations.

If from one river catchment with the same species, environmental conditions and disease risk, fish may be randomly sampled at sites along the river.

Sampling locations can be determined by measuring the length of the river and randomly selecting numbers along that length. This may be adapted to conform with administrative divisions.

5	Sampling	• Used/described sampling frame • Described sampling method • Defined sampling units • Explained consideration regarding sample size • Describe tissues/fluids used as sampling material • Describe sample selection process

Farmed fish population, random sampling from the sampling framework (i.e. list of registered/approved/known farms or ponds) can be used.
If data on all registered fish farms exist, one stage sampling can be used (simple random selection of xx farms from a list of all farms) and at each selected farm, samples to include 160 captured fish.
Sample size depends on number of fish farms, for example, if there are 100 farms, 59 of them need to be randomly selected for the sampling (see step 8 Methodology, concerning sample size).
Two-stage sample will be used if the number of farm is unknown. In this case, a first-stage sampling is needed to randomly select district/villages/farm and followed by a second-stage sampling from randomly selected ponds.
In terms of sampling units , this can be a pond (or group of ponds) or a farm if all fish in the same unit share same environmental condition. In such a case, a positive finding of one case of EUS will be interpreted as that the farm is infected regardless how many ponds are present in the farm.
Sample size is calculated using epi calculators .
For **wild fish populations**, **320** fish should be sampled from selected locations and **for farmed fish**, **160** fish from a pond/farm.

6	Diagnostics/testing	• List and description of tests used (procedures, interpretation of results, Se/Sp) • List of laboratories included

- Diagnostic laboratory is an important part of a general surveillance system.
- In order to support a general surveillance system, laboratories should be capable of diagnosing a wide range of different diseases. If countries have limitations in diagnosing all diseases.
- Under such circumstances, a two-level system may be used to overcome the problem. The first level is represented by laboratories with relatively simple, but general diagnostic capabilities.
- The second level is one or more specialized laboratories (sometimes contracted from abroad) to assist in more complex diagnosis. This second level laboratory may serve as a reference laboratory.
- The following tests are recommended:
  - clinical signs and gross pathology: red spots or small to large ulcerative lesions on the body;
  - microscopic pathology: pathological observations in sections of liver, brain, spleen, etc.
  - isolation of pathogen and
  - PCR, etc.

7	Study design and data analysis methodology	• Survey design described • Risk assessment used and described • Methods of data analysis described

When the survey is finished, the prevalence of disease can be calculated and expressed as the percentage of infection. The methodology of surveillance design is a tool that gives confidence in the interpretation of surveillance data (set as 95% level of confidence). In practical terms, this means that results gained from selected sample can be confidently interpreted for the whole population.

Some details are provided below:

Wild fish population: The surveillance is designed to provide **95% confidence** of detecting the presence of TiLV, if present in **1% of the fish population** or more using a diagnostic test with **95% sensitivity (Se)** and **100% specificity (Sp).** The value of 10% (design prevalence) is selected to be practical and to reflect the current knowledge of TiLV in participating countries

Farmed fish population: The surveillance is designed to provide *95% confidence* of detecting the presence of TiLV in farms if TiLV occurs in **5% of the farms** or more and if TiLV occurs in **2% of the farmed** fish population or more within a farm using a diagnostic test with **95% sensitivity (Se)** and **100% specificity (Sp).**

8	Data flow and management	• Data forms • Data base • Compatibility and transparency • Consistency, quality and resolution of data

Data collection should be done (before sampling) using separate questionnaires for wild and farmed fish depending on population sampled.

After sampling and inspection of fish in a sample, if clinical signs of TiLV are observed, 2 copies of the laboratory form will be completed and 1 copy sent together with fish samples to diagnostic laboratory.

All data including laboratory results will be entered into an excel data sheet.

It is of key importance to secure traceability of data (farm or population data with laboratory results).

Correct data recording in the field is critical.

Surveillance questionnaires are developed and collected data (for wild fish sampling, cultured fish and diagnostic sample) need to be entered into excel sheet.

In order to secure proper data collection, each country will translate the questionnaire into local language. However for post surveillance analysis, countries are requested to develop the English language excel sheet and create consistent data form and databases.

9	Validation	Done by statistical estimation of the level of confidence (Se of surveillance program) Confirmed by pilot trial Done by expert/external evaluation (peer-review)

This step is done throughout the whole process from the design until the actual implementation.

The surveillance design and implementation plan need to be reviewed by experts and other relevant project proponents.

10	Quality assurance	Audit and corrective measures

There are administrative and procedural activities which need to be done in order to avoid problems and if problems or mistakes occur, corrective measures can be introduced. These will guarantee good quality implementation of the surveillance plan.

These activities include:

- national surveillance team (NST) established;
- training and education of NST on TiLV pathogen biology, pathology, diagnostics and surveillance; data collection and a questionnaire described and explained clearly and common understanding achieved;
- diagnostic laboratory accredited in line with ISO 17025, if possible; trained field and laboratory personnel;
- a clear standard operating procedures developed and used during implementation,
- aseptic technique procedures for minimizing contamination from potential areas of sample collection developed and made clear to the sampling teams;
- sampling teams closely supervised; and a pilot survey will be conducted as a sampling exercise .

11	Human and Financial Requirements	Included and described (e.g. personnel, cost of sampling, cost of laboratory tests, analysis of data, etc.)

In this step, several forms need to be completed. These include, e.g.

- checklist of field logistics/operational requirements (e.g.
  - surveillance team
  - diagnostic team
  - field support team
  - communication
  - work plan
  - finance
- and submitted for approval by project proponents as basis for generating financial support for its implementation.

12	Putting surveillance in the bigger picture (biosecurity, animal health, aquaculture, food safety/security, One Health)	Surveillance as an essential component of aquatic animal health/aquatic biosecurity strategies, aquatic animal health protection programmes or disease control plans, One Health platform within the context of aquaculture

This last step puts TiLV active surveillance in line with overall national strategies for enhancement of aquaculture biosecurity and aquatic animal health, aquaculture and international trade as well as the One Health platform. Within this framework, domestic producers may benefit from increased revenue and reduced disease-related losses.

National fishery/aquaculture authorities and/or veterinary services will enhance their competence and gain trust, and the society will benefit as whole by contributing to national economy, public health and country recognition in world trade.