Second Interregional Workshop of FAO TCP/INT/3501

Update on the strategy planning for Infectious myonecrosis (IMN) disease

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Components of a contingency plan

- **Technical plans**
  - **Disease strategy manuals (one for each high priority disease)**
  - **General procedures manuals**
  - **Enterprise manuals**
  - **Job descriptions**
- **Support plans**
  - **Financial**
  - **Resource**
  - **Legislation**
  - **Other agencies**
- **Operational capability**
  - **Management manuals**
  - **Diagnostic resources**
  - **Field personnel**
  - **Training resources**
  - **Awareness and education**
  - **Response exercises**

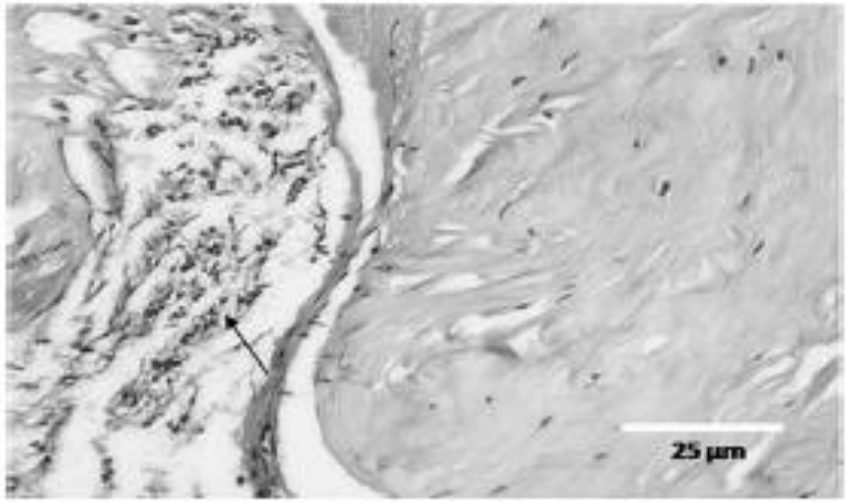
See: http://www.fao.org/docrep/009/a0090e/A0090E10.htm

Chapter 1. Introduction

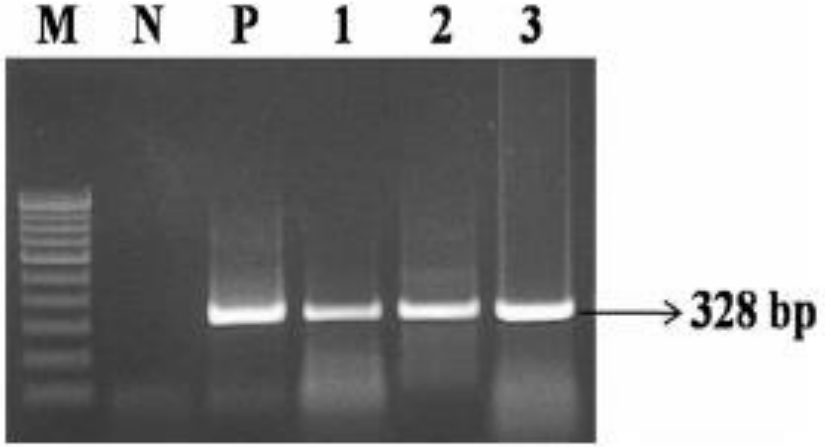
- **IMN discovered in 2002 in NE Brazil, emerged in 2006 in Indonesia, 2016 in India,**
- **The causative agent was identified as a virus (IMNV) in 2003,**
- **Morality: 40-70%**
- **Economic losses during 2002-2011: > 1 billion USD**
- **Brazil, Indonesia, India: 27% of global shrimp production**

Studies on the occurrence of infectious myonecrosis virus in pond-reared *Litopenaeus vannamei* (Boone, 1931) in India

A S Sahul Hameed | S Abdul Majeed | S Vimal | N Madan | T Rajkumar | S Santhoshkumar | S Sivakumar



Histology showing coagulative necrosis and infiltrated hemocytes



IMNV RT-PCR, positive found in *P. vannamei* from farms. Mortality: 20-50%

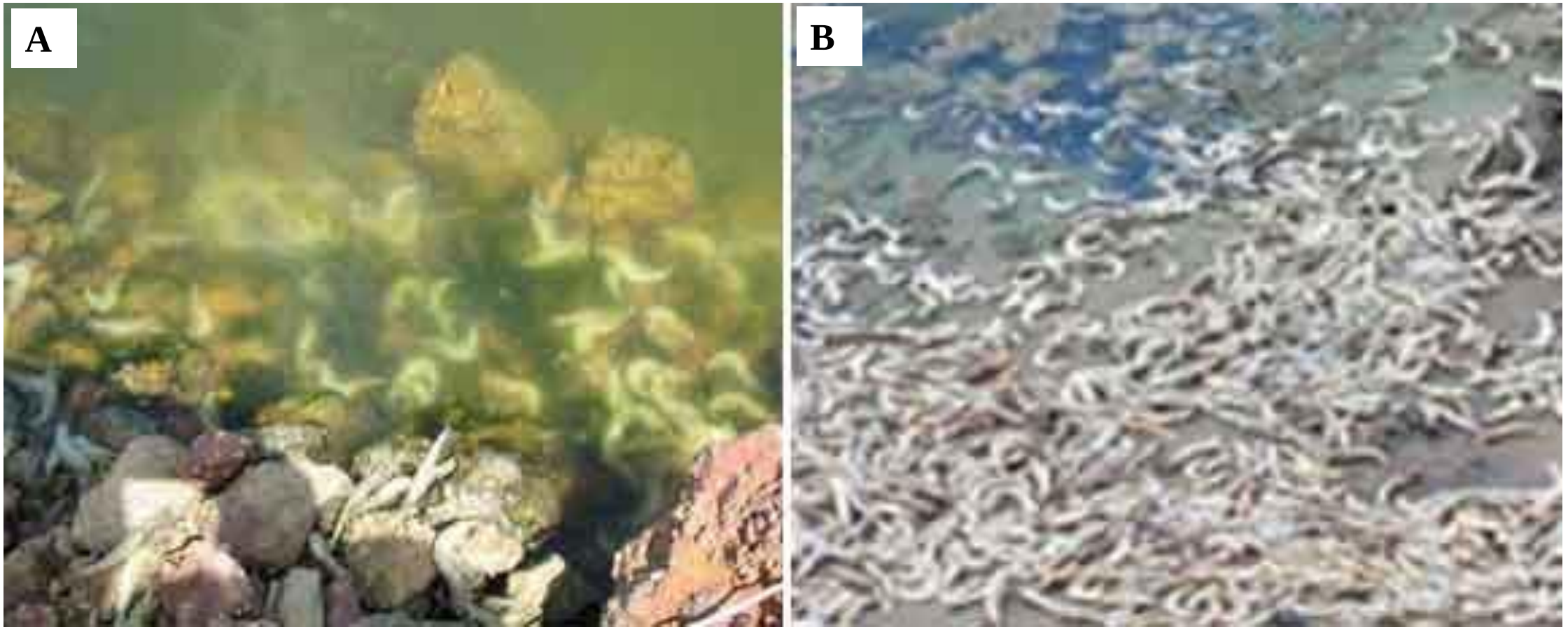


Figure 1. Massive mortality of *Penaeus vannamei* caused by infectious myonecrosis cultured in farms located in (A) north eastern Brazil in 2002; (B) East Java Indonesia in 2006.

Chapter 2 : The nature of IMN

2.1 Etiology

- **Totivirus (40 nm)**
- **One segment of dsRNA (8.2-kb)**
- **2 overlapping ORFs**
  -ORF1: structural proteins, including MCP
  -ORF2: RNA-dependent RNA polymerase
- **Receptor: Laminin receptor protein (Lamr)**

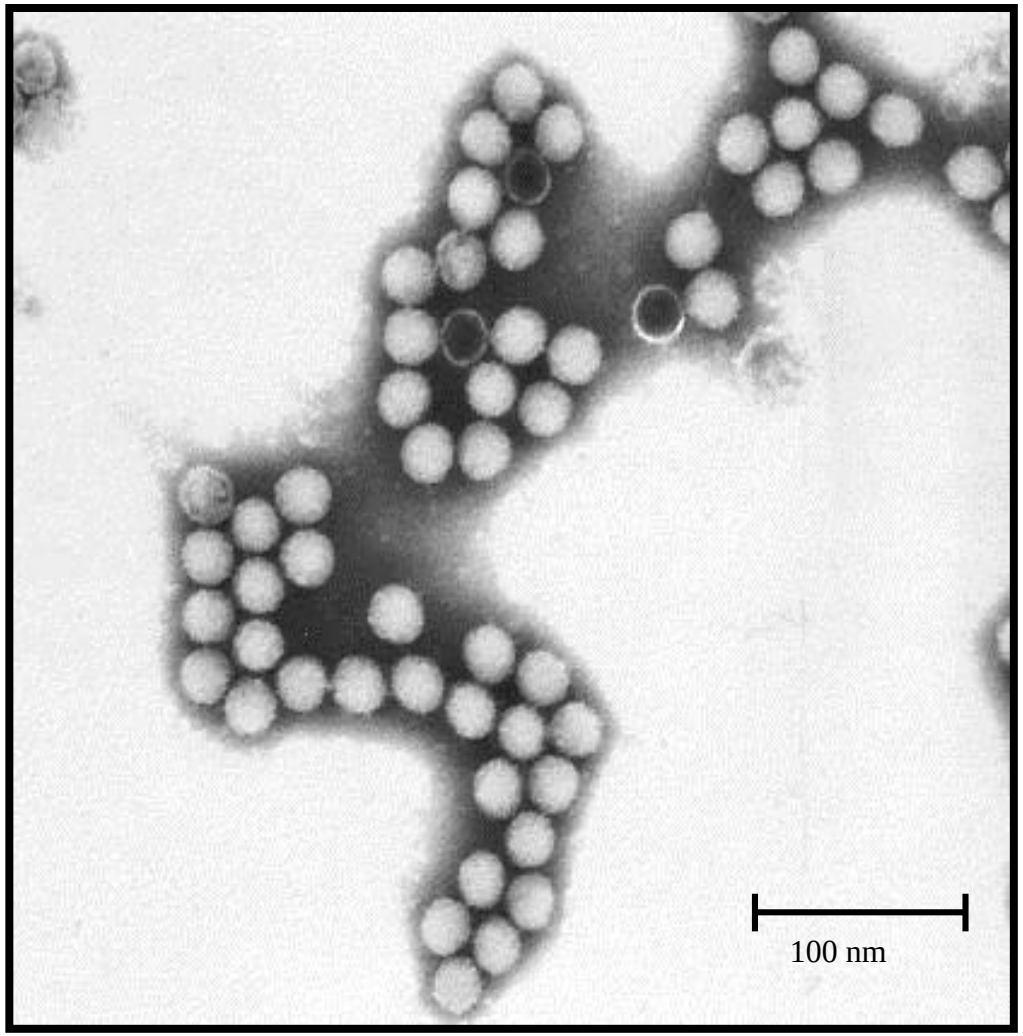


Figure 2. Transmission electron micrograph of IMNV; virions are icosahedral in shape and 40 nm in diameter.

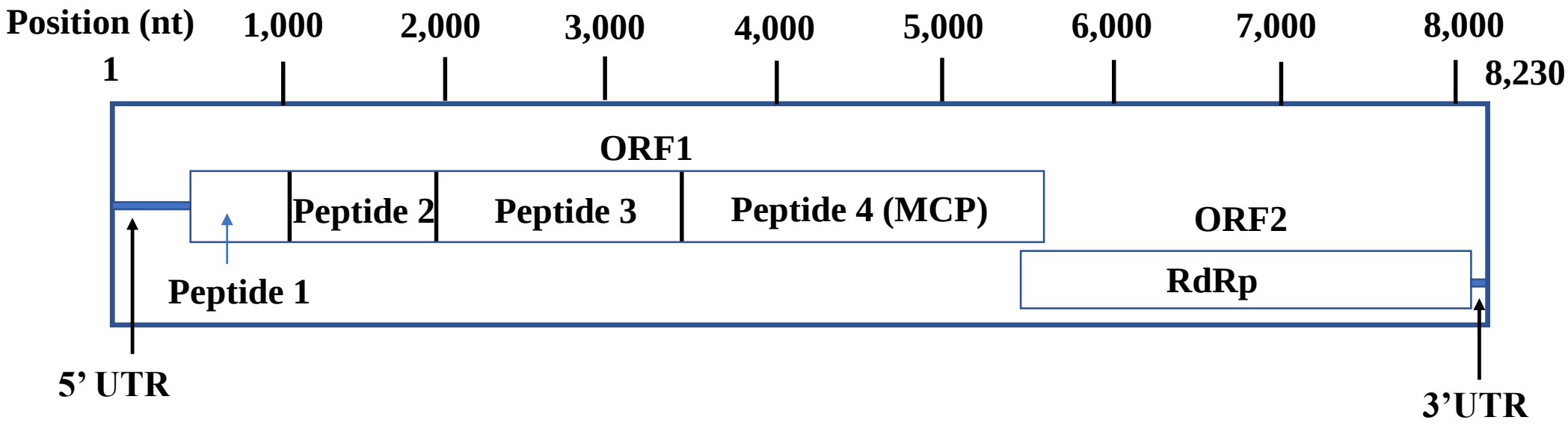
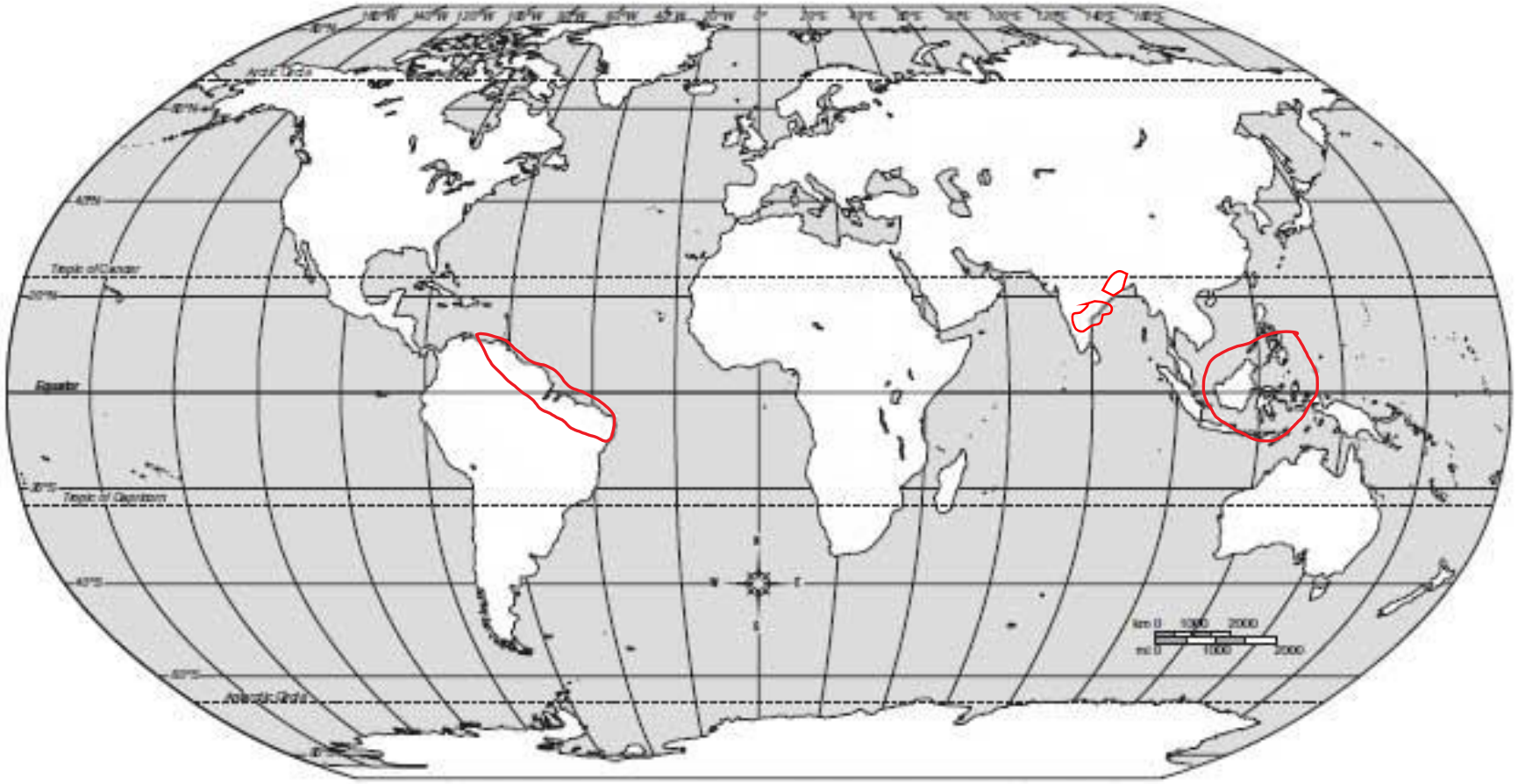


Figure 3. IMNV genome (dsRNA) organization: 5' and 3' UTRs. ORFs, major capsid protein (MCP) and RNA-dependent RNA polymerase (RdRp).

2.2 Susceptible species: brown tiger prawn (*P. esculentus*), banana prawn (*P. merguiensis*), Pacific white shrimp (*P. vannamei*) and black tiger shrimp (*P. monodon*)

2.2.1 Species with incomplete evidence for susceptibility; Pacific blue shrimp (*P. stylirostris*), and southern brown shrimp (*P. subtilis*).

2.3 Global distribution: Brazil (2002), Indonesia (2006), India (2016)



Chapter 3. Diagnosis of infection with IMNV

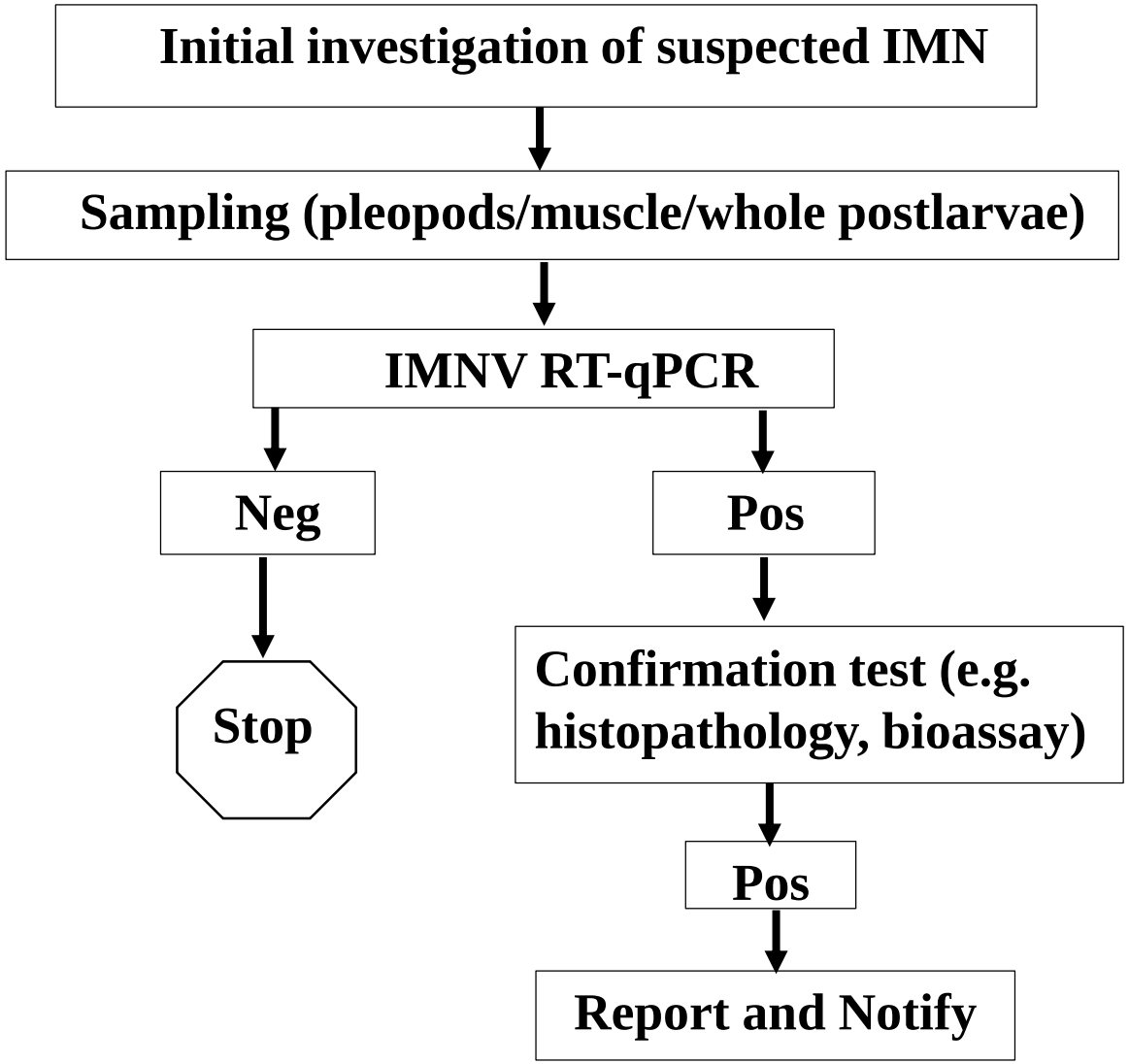
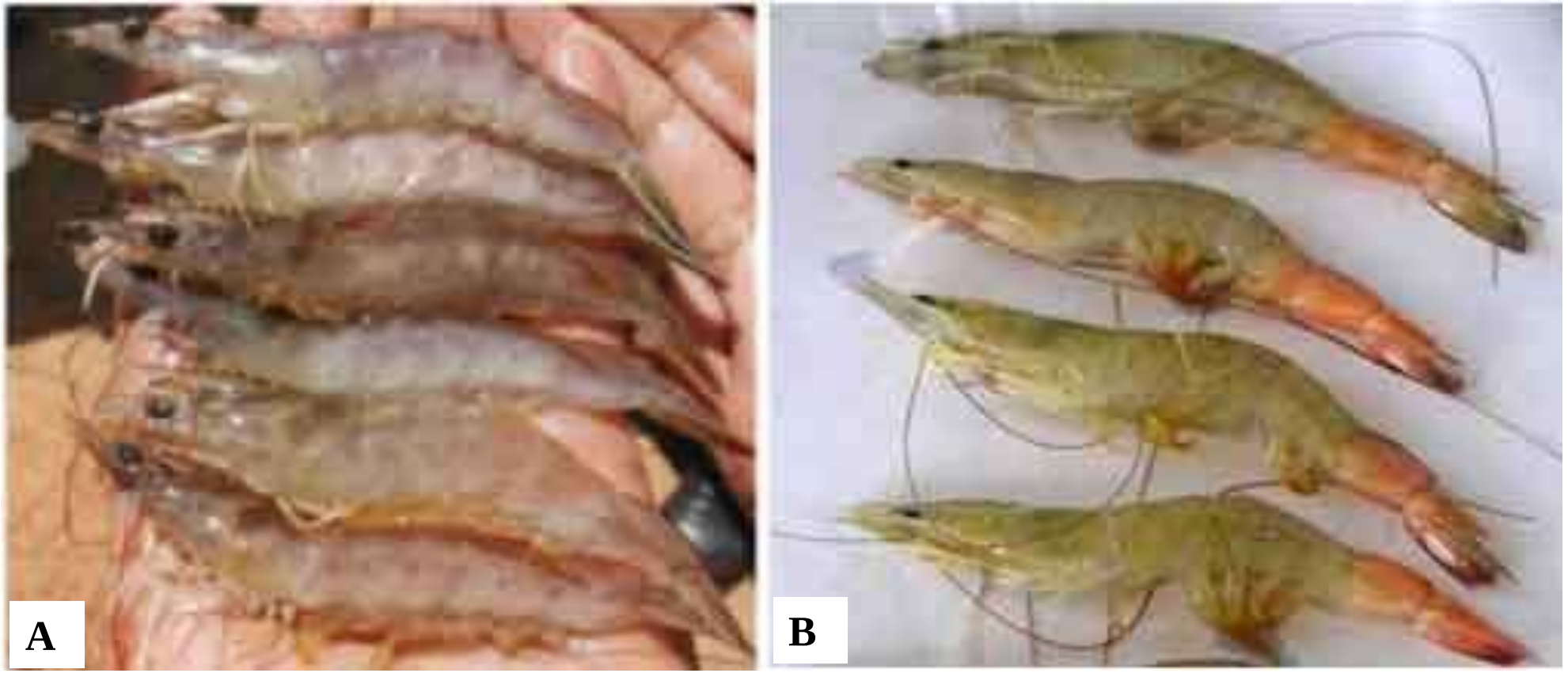


Figure 4. Diagnostic flowchart

3.1 Gross signs: whitish, opaque, tail muscle; chronic infection, moderate mortality, lethargic

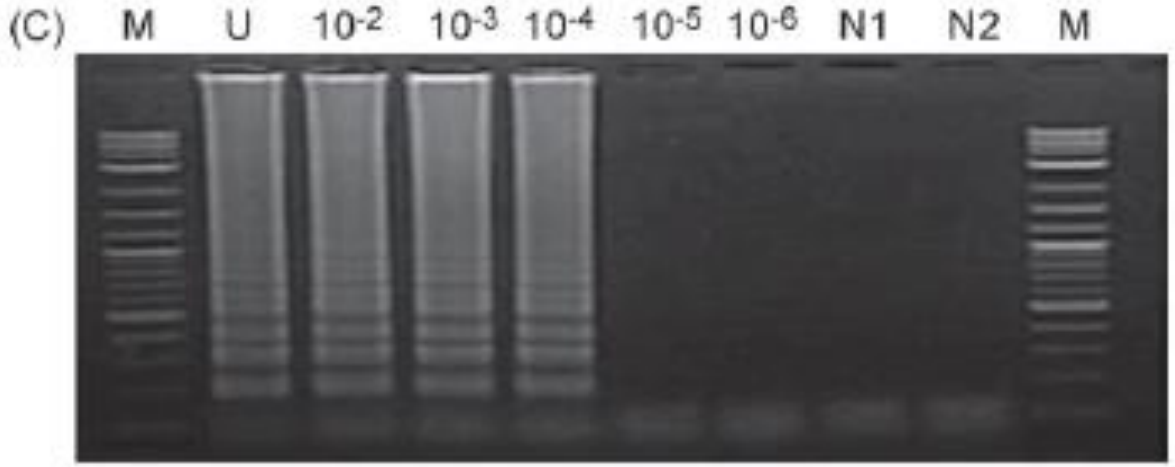


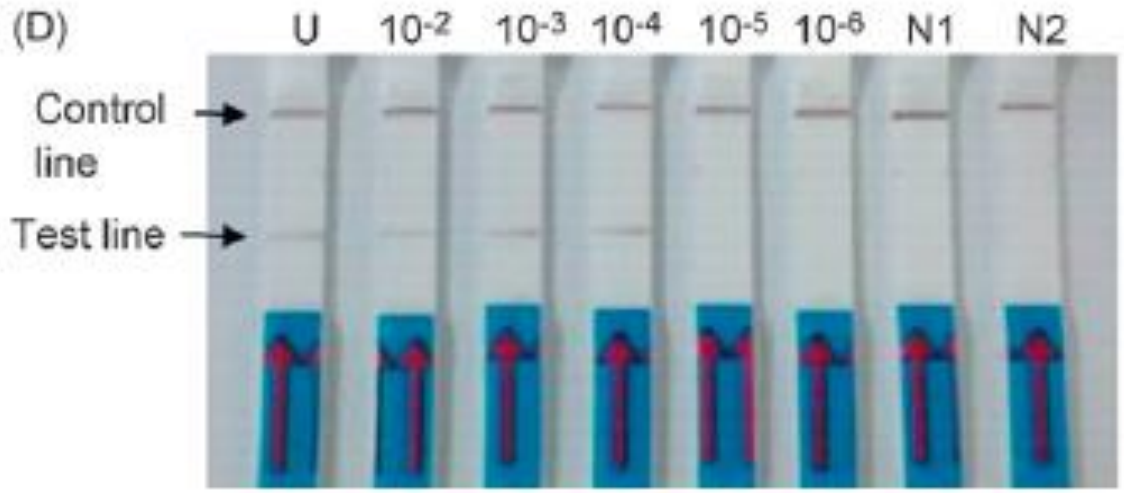
IMNV-infected *P. vannamei* (A) exhibiting various degrees of skeletal muscle necrosis, visible as an opaque, whitish discolouration in the abdomen; (B) showing reddened necrotic tails, similar to the colour of cooked shrimp.

3.2 Field diagnostic methods

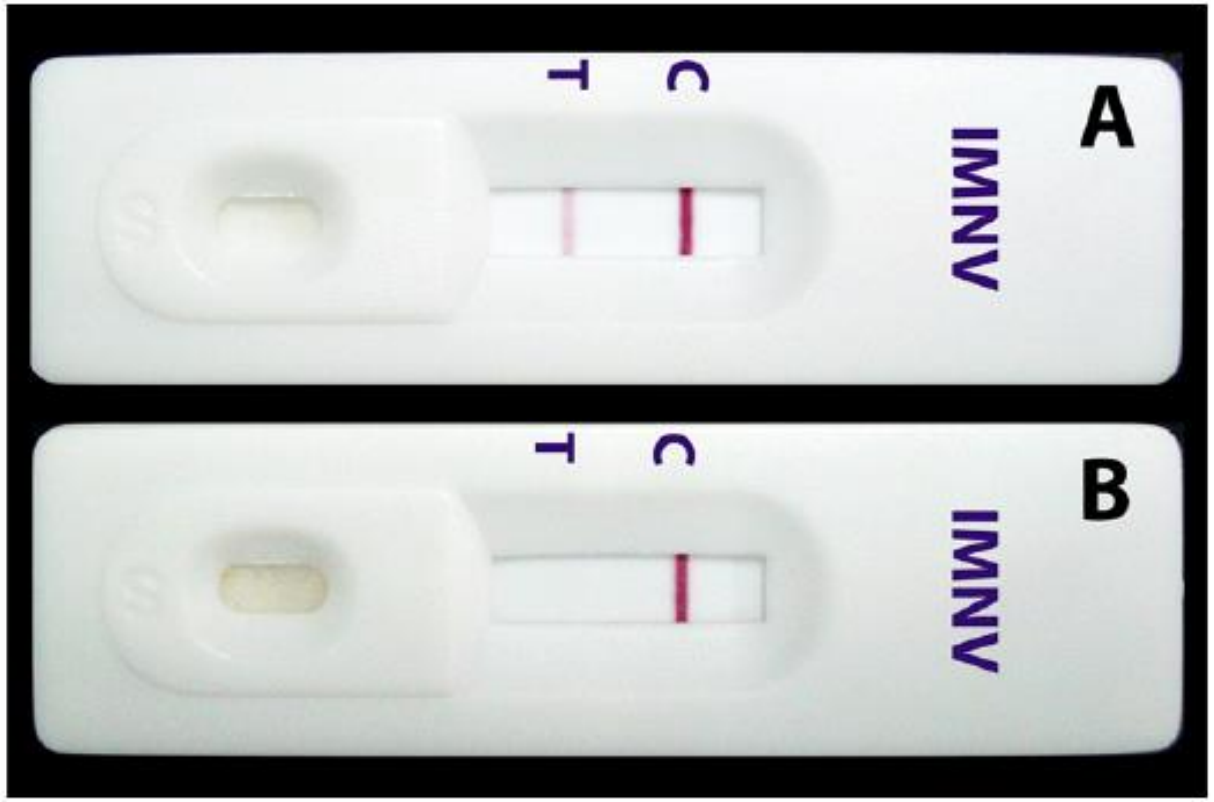
3.2.1 Rapid on-site diagnostic assays

- **RT-LAMP**





Lateral flow dipstick



IMNV LFIA
(lateral flow immunochromatographic assay)

Hand-held IMNV RT-qPCR

3.4 Laboratory methods

-histopathology

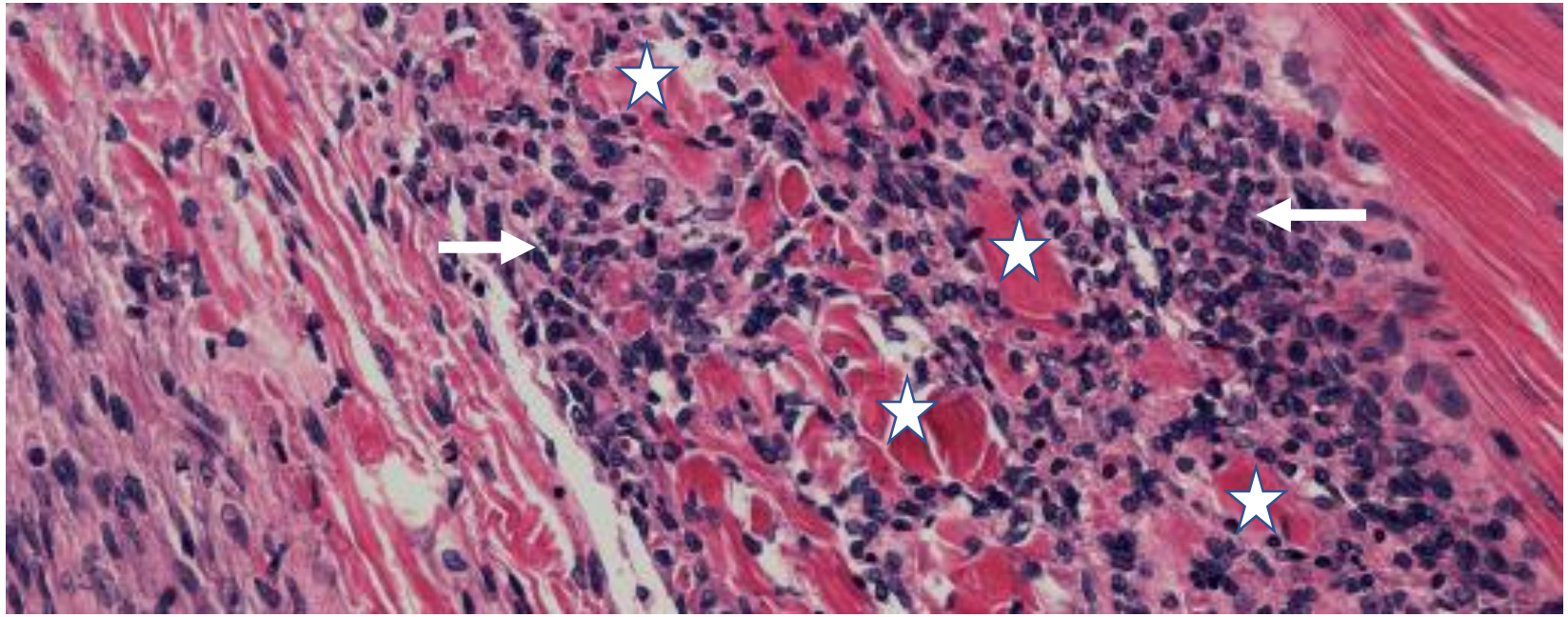
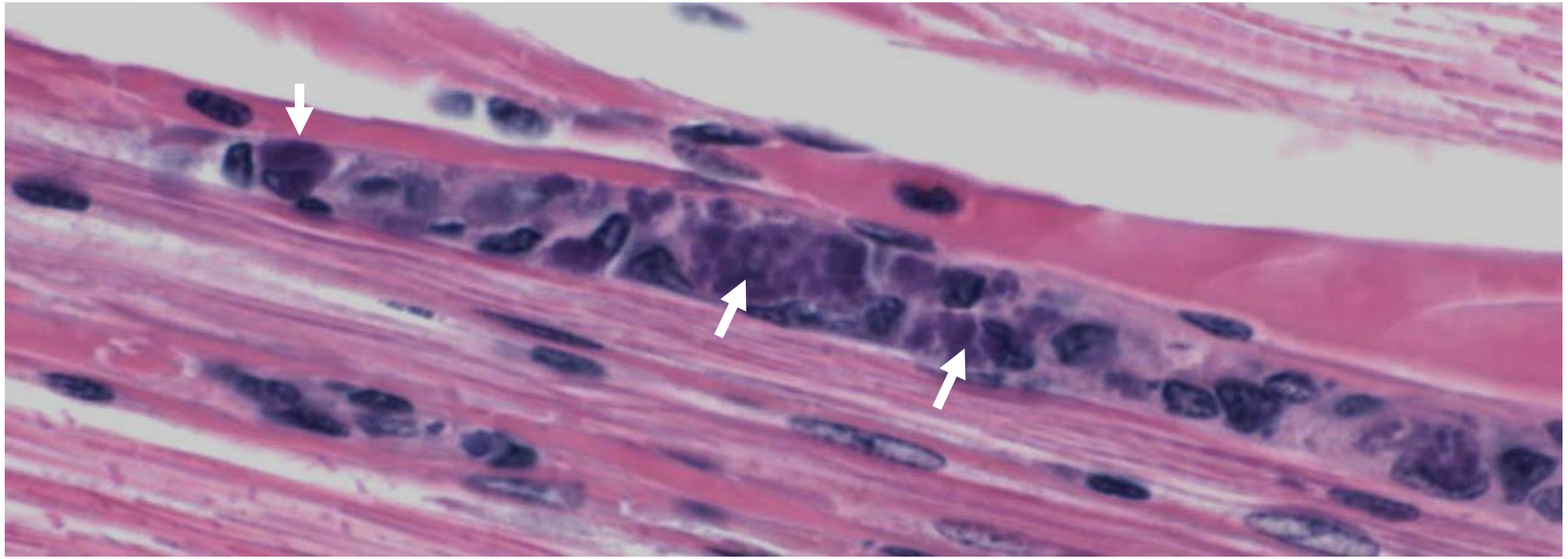
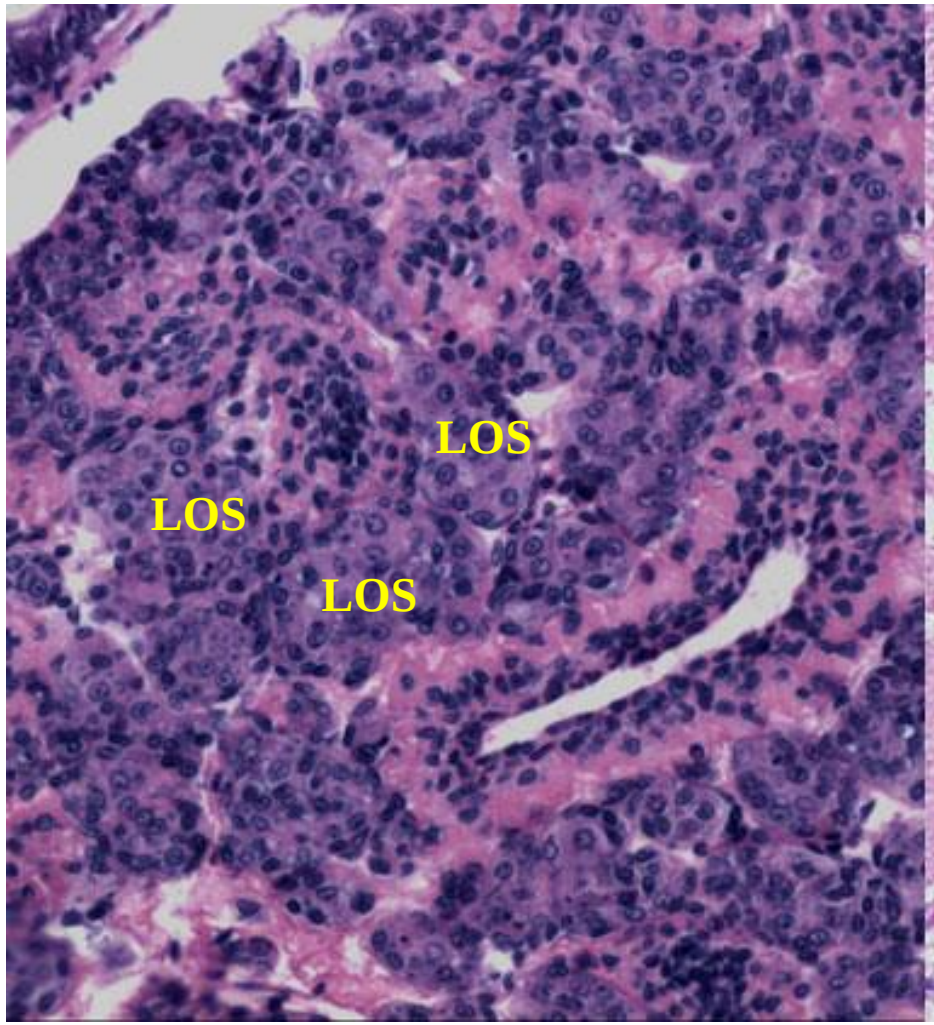
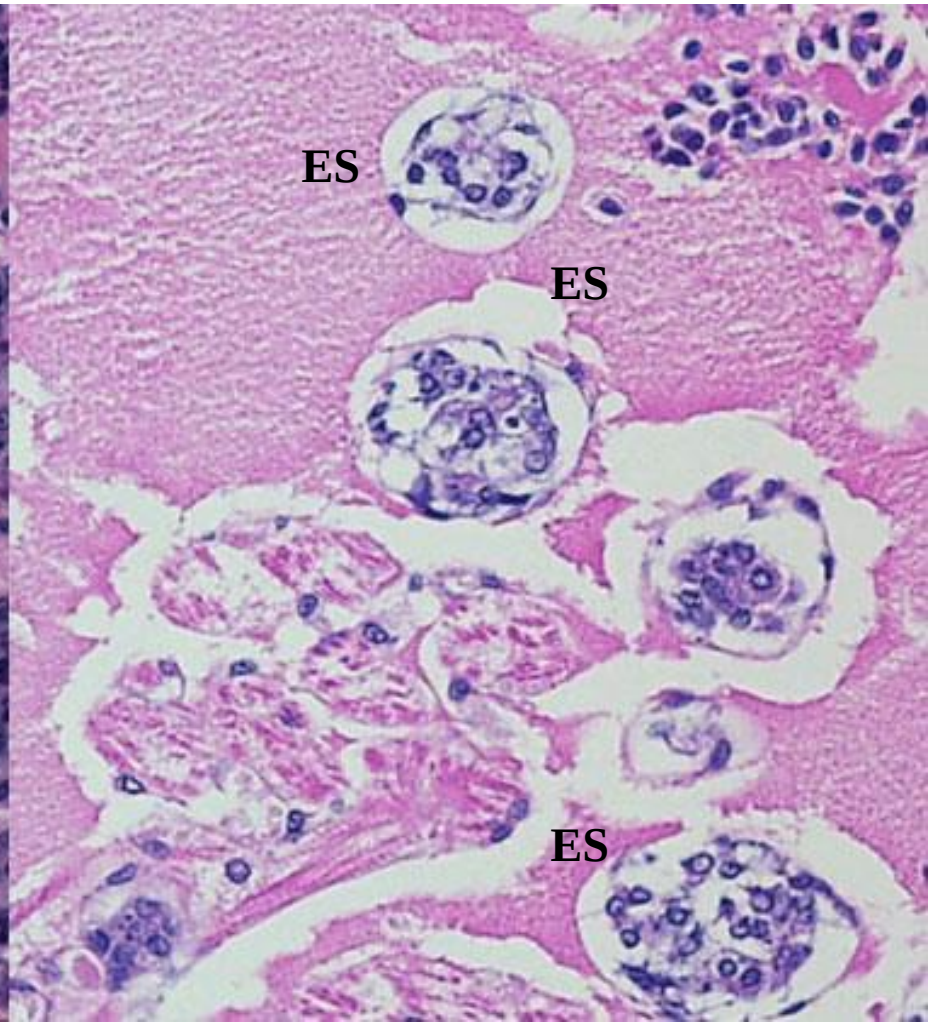


Figure 6A. focal acute coagulative muscle necrosis with infiltrated hemocytes



cytoplasmic basophilic inclusion bodies





lymphoid organ spheroids; ectopic spheroids

-IMNV-RT-PCR

Since IMNV genome is dsRNA, the heat denaturation step (95-99^{o}C for 5-10 min) must be performed prior to the setup of RT-PCR (RT-qPCR).

Chapter 4. PREVENTION AND TREATMENT

4.1 Vaccination: None; the development of “nanovaccine” (based on RNAi) in the laboratory studies.

4.2 Resistance and immunity: innate immunity

-Humeral responses: Toll like receptors, prophenoloxidase (proPO) activating system; production of anti-microbial peptides;

-Cellular responses: apoptosis, encapsulation, phagocytosis and melanisation

-JAK-STAT signalling pathway

Chapter 5. EPIDERMIOLOGY

5.1 Persistence in the environment: no data

5.2 Mode of transmission: horizontal and vertical (IMNV is detected in eggs and overies)

5.3 Vectors and reservoir hosts: *Artemia franciscana*, seabirds, *P. subtilis* (wild shrimp)

5.4 Factors influencing disease transmission and expression: salinity, temperature (hot season)

Chapter 6: Principles of control and eradication

- **6.1 Methods for control and elimination**
  - **Quarantine and movement controls**
    -should establish appropriate zone and compartment designations

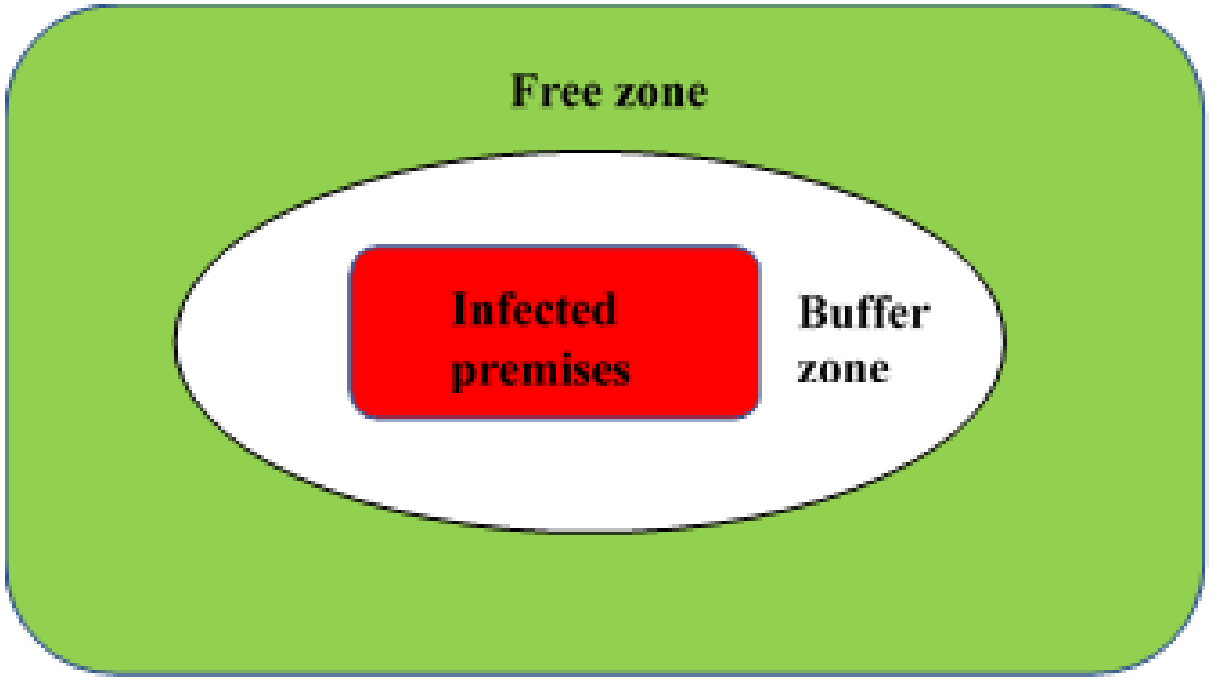


Figure 6. Designation of zone, area, and premise in the IMNV outbreak response.

Movement controls from the infected areas should include:

- Bans on the movement of **live and uncooked (raw) shrimp** from the infected premises into IMNV-free areas
- Restrictions or bans on releasing shrimp and **pond water** from the infected premises into aquatic environments
- Bans on using **uncooked shrimp** in the infected premises as **baits for fishing**
- Bans on discharging of **processing-plant effluent** without any treatment within the infected premises
- Restrictions on harvest and then transportation of shrimp in the infected premises to off-site **processing plants**
- **Control of disposal** of dead shrimp
- **Control of seabird** access to live and dead shrimp within the infected premises
- Restrictions or bans on the use and movement of **equipment and vehicles** between farms within the infected premises

• 6.1.2 Tracing

- if the infected shrimp were moved to other areas
- if the IMNV infection has spread to other areas
-to identify the sources of the IMN disease

- **live shrimp,**
- **uncooked (fresh and frozen) shrimp,**
- **live feed, such as *Artemia* biomass,**
- **effluent and waste products from processing plants and farms,**
- **vehicles and farm materials**

- **6.1.3 Surveillance:**

  (a) to detect the early occurrence of IMN disease

  (b) to determine its prevalence in populations

  (c) used in the process of maintaining, and certification of, farms or areas, freedom from IMN disease.

  -Diagnosis: histology and RT-PCR (RT-qPCR)

  -Rapid on-side diagnostic assays: RT-LAMP, LFIA, hand-held POCKIT (RT-qPCR)

- **6.1.4 Use of IMNV-free postlarvae**
- **6.1.5 Use of IMN-resistant shrimp: resistant line of *P. vannamei*, *P. stylirostris*, *P. monodon***

- **6.1.6 Use of probiotics**

-enhance the innate immunity

-improve the water quality

- **6.1.7 Disinfection of shrimp and shrimp products**

-60^{o}C for >20 min

-eggs washing

- **6.1.8 Emergency harvest**

- **6.1.9 Destruction of infected shrimp**

-approved chemicals
-avoid spillage or escape to the environment
-keep the seabird away

- **6.1.10 Disposal of hosts**

-burial, remote from shrimp ponds
-incineration

• **6.1.11 Decontamination of infected farms**
-chlorine (50 ppm) for 4 days
-lime, drying (e.g. 2 months)

• **6.1.12 Vector control**
-seabird, crab

• **6.1.13 Environmental considerations:**
-develop a management plan to prevent the transmission of IMNV to wild hosts

6.1.14 Public Awareness

-To support the response strategy by coordinating government agencies and stakeholders to provide unified messages to the local, national and international audiences,

-To address the concerns relating to food safety, public health, the environment,

- To address issues related to regional commerce, continuity of business, and international trade,

-To disseminate information through workshops, agency technical reports, industry bulletins, social media

6.2. Control, containment, and eradication options

6.2.1 Eradication

(a) Prohibiting imports of potentially infected shrimp,

(b) Screening imports of live or frozen commodity shrimp as well as other susceptible species (e.g. *Artemia* sp.) or potential carriers,

(c) Destruction and safe disposal of all shrimp at infected farms,

(d) Disinfection of pond and reservoir water,

(e) Decontamination of pond bottom, equipment, supplies, and facility surfaces through drying, application of bleach, lime or other appropriate chemical agents.

6.2.2. Containment and zoning

(a) Establishing infected and free zones.

(b) Prohibiting movement of infected shrimp, un-cooked shrimp products, or any contaminated materials into IMNV-free areas.

(c) Establishment of well monitored buffer zones where spread of IMNV can be detected before the IMNV-free zones are affected.

6.2.3. Control and mitigation of disease

(a) implementing more rigorous methods of eliminating potential vectors

(b) control the movements of live shrimp from the infected premises

(c) reduce the stress factors

6.3 Trade and industry considerations

6.3.1 Domestic markets: place restrictions on transporting or marketing some products between infected and disease free areas.

6.3.2 Export markets

In countries where IMNV is exotic, import conditions such as requiring imports to be certified IMNV-free; and testing by commerce inspection organizations to reject shrimp batches that are IMNV(+)

7. Policy and Rationale

- **7.1 Overall Policy**

(1) to eliminate IMNV from the country if possible

(2) to prevent re-emergence of IMNV

(3) to prevent the spread of the disease to farmed or wild populations outside of infected areas

(4) to minimize the impact of the disease on commercial production

(5) to prevent loss of domestic and international markets for locally farmed shrimp

(6) to ensure that stake holders and the public are informed of the issues involved in preventing the introduction or spread of IMNV through improper importation or movement of shrimp products

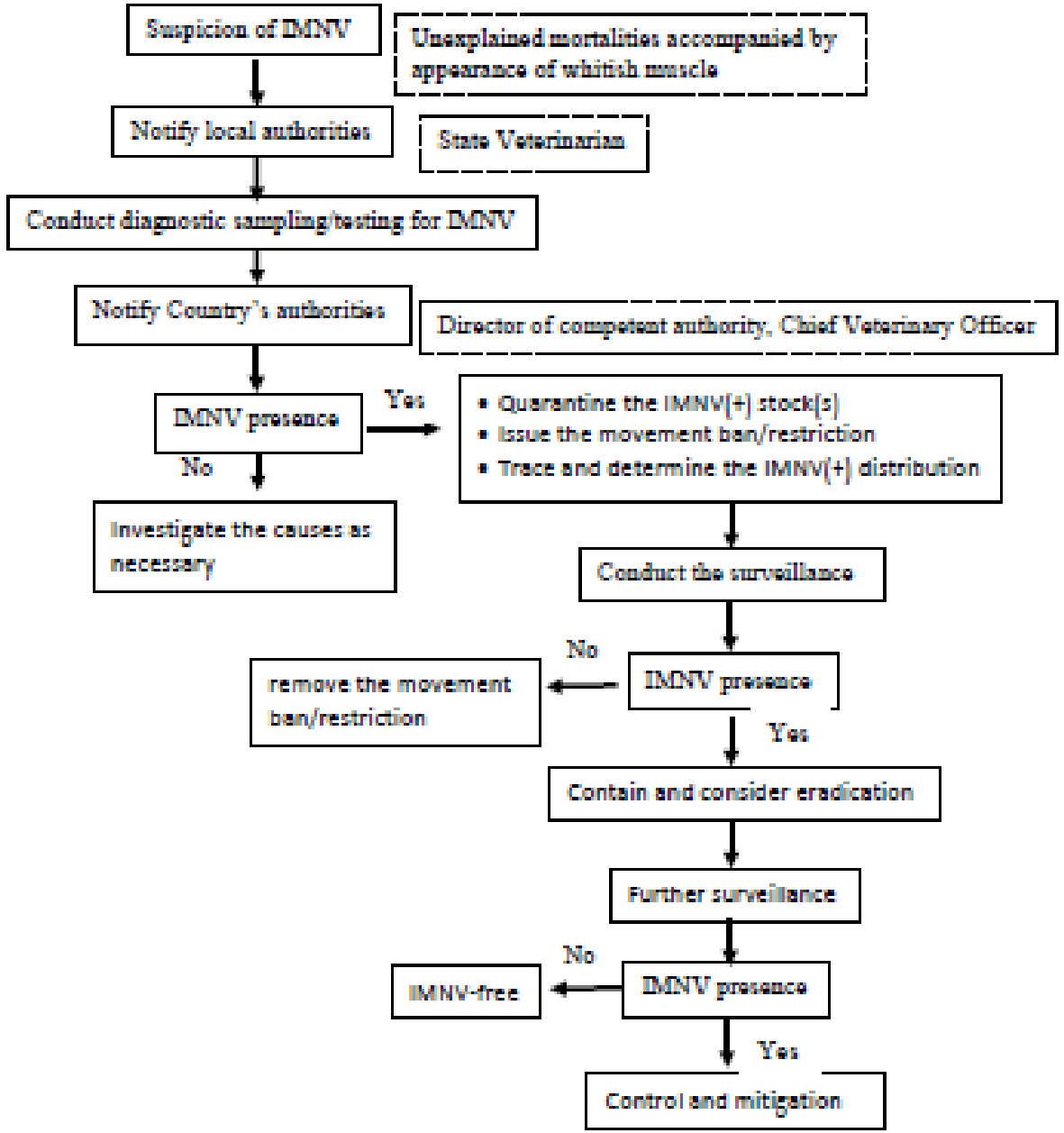


Figure 8. IMNV responses flow chart.

- **7.4 Strategies for eradication and control**
- **7.4.1 Eradication from production facilities**

-Diagnosis and surveillance

-Disposal of diseased stock and disinfection

-Restocking with specific-pathogen-free stocks

-IMNV-free declaration

IMNV has been eliminated

-based on a series of surveys of farmed and wild populations of susceptible species over at least a two-year period
-2 years of basic biosecurity
-There should be 2 surveys per year to be conducted 3-4 more months apart,
-provide a greater than 95% confidence with a prevalence of 2 % or lower,
-Shrimp to be sampled are preferred to display any clinical signs, such as whitish muscle
-with the involvement of the competent authority of the country.

- **7.4.2 Containment and movement control**

-Restrictions on movement of shrimp products

-Restrictions on water discharge

-Prevention of spread by seabirds or other wildlife

-Surveillance

- **7.4.3 Management and mitigation**

-Manage facilities restored to IMNV-free status:

stock SPF shrimp and high levels of biosecurity

-Manage farms showing low levels of IMNV infection:

reduce stress factors, the use of best management practices

7.5 Capacity building

-improving capacity of diagnostic laboratories

-improving National competency of aquatic animal health management

7.6 Social and economic effects

-farmers may fall into debt

-devastating to the communities depending entirely on incomes from shrimp farming

7.7 Funding and compensation

-adequate funding by government

-individual producer

-shrimp-grower associations