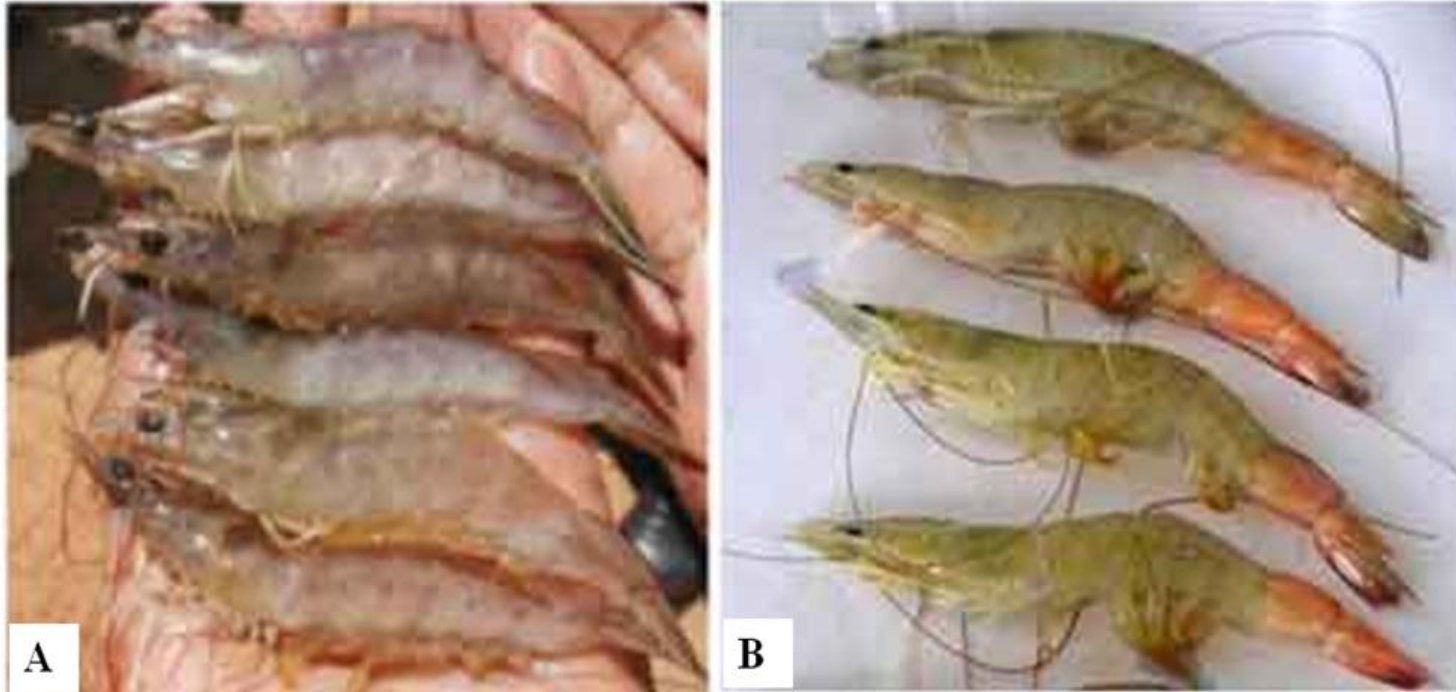


Second Interregional Workshop of FAO TCP/INT/3501

**Diagnostic Methods for the Detection
of IMNV: Gross signs, RT-PCR, RT-
qPCR**

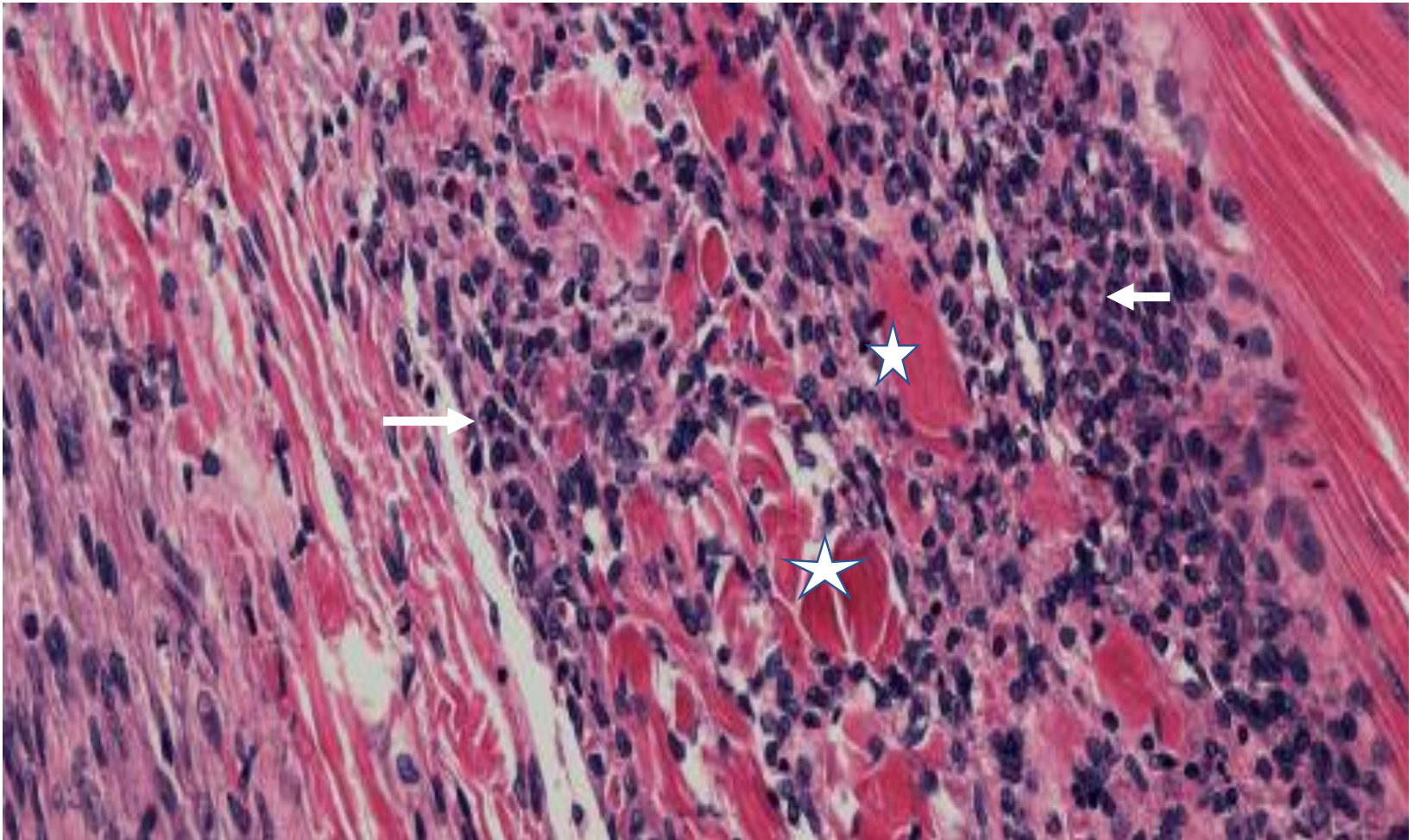
Gross signs for IMNV



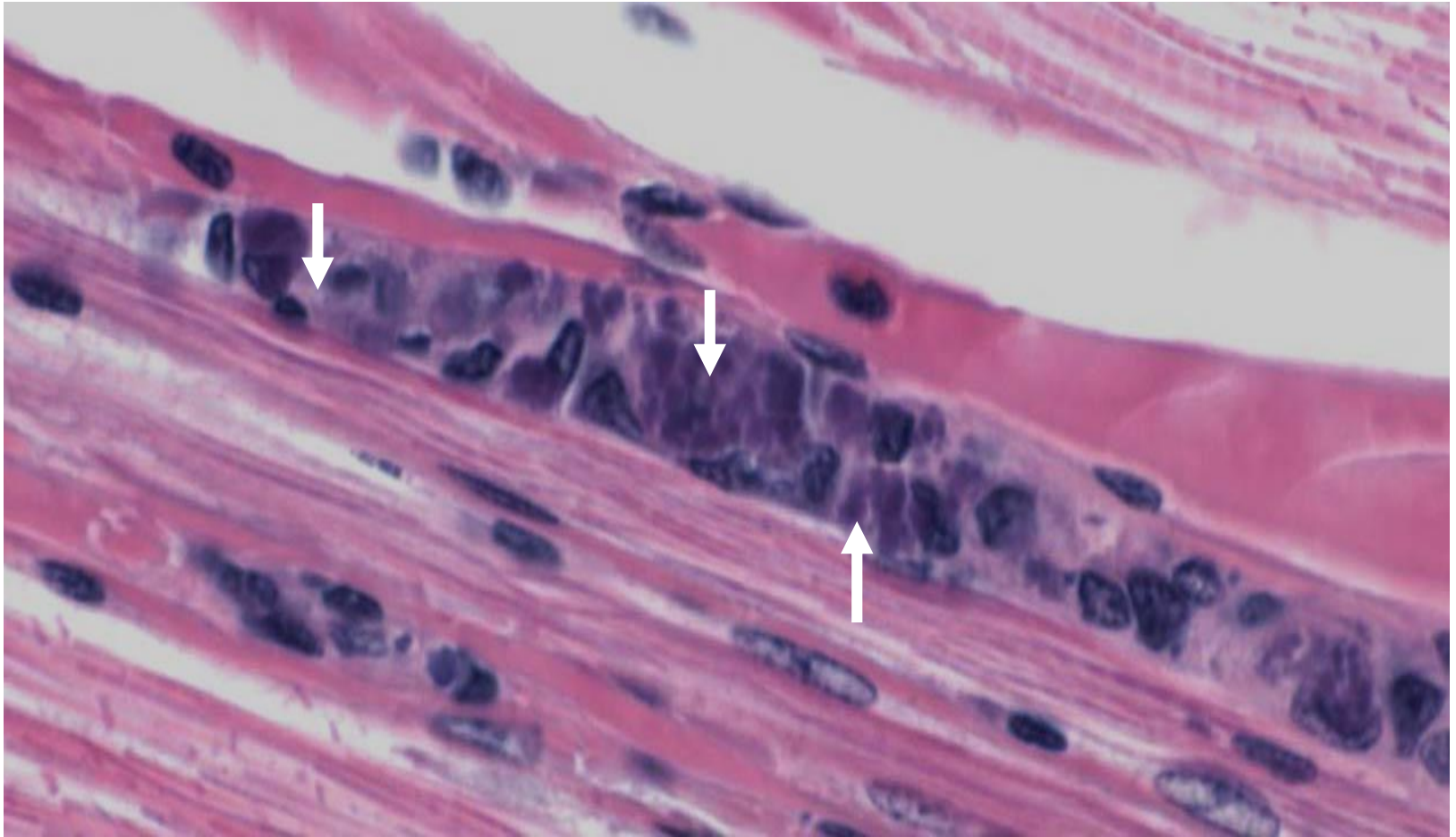
(A) Farmed *P. vannamei* from an outbreak in Brazil, exhibiting various degrees of skeletal muscle necrosis, visible as an opaque, whitish discolouration in the abdomen; (B) farmed *P. vannamei* from an outbreak in Indonesia, showing reddened necrotic tails, similar to the colour of cooked shrimp.

**IMN in young
juvenile
*P. vannamei***

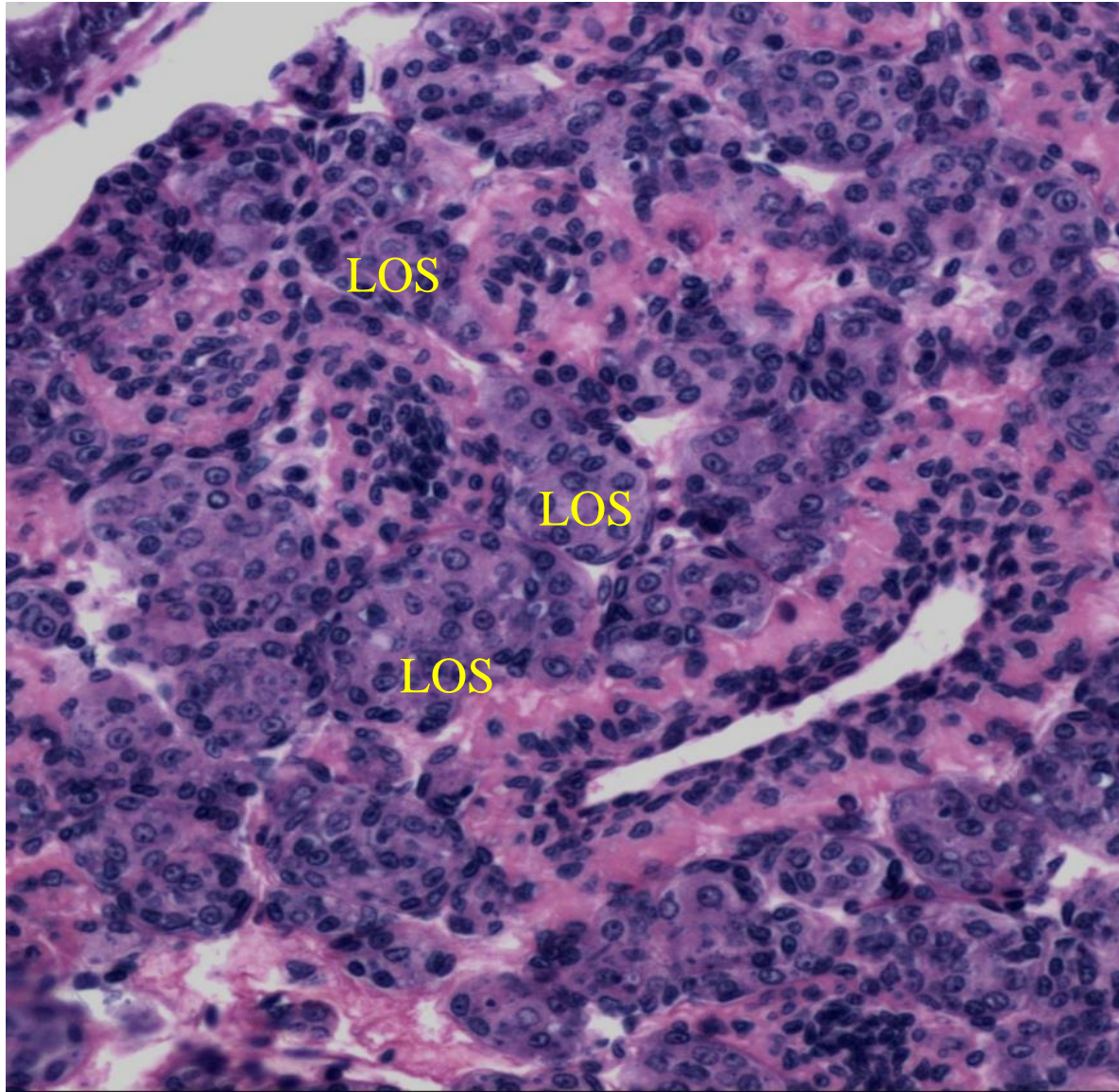




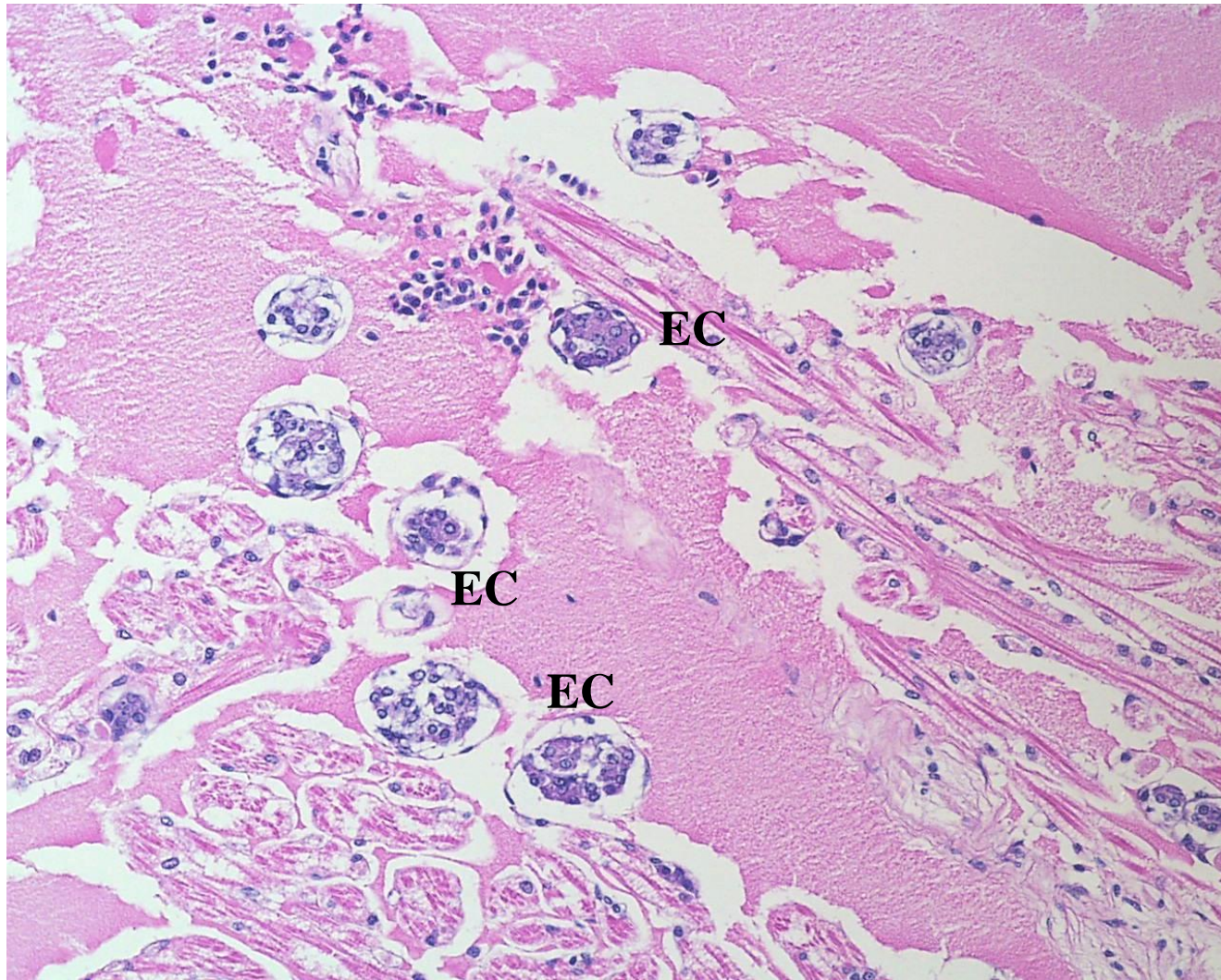
Histopathological characterization of IMNV infected *Penaeus vannamei*, H&E stain. (A) Coagulative necrosis (stars) of skeletal muscle accompanied by haemocytic infiltration (white arrows)



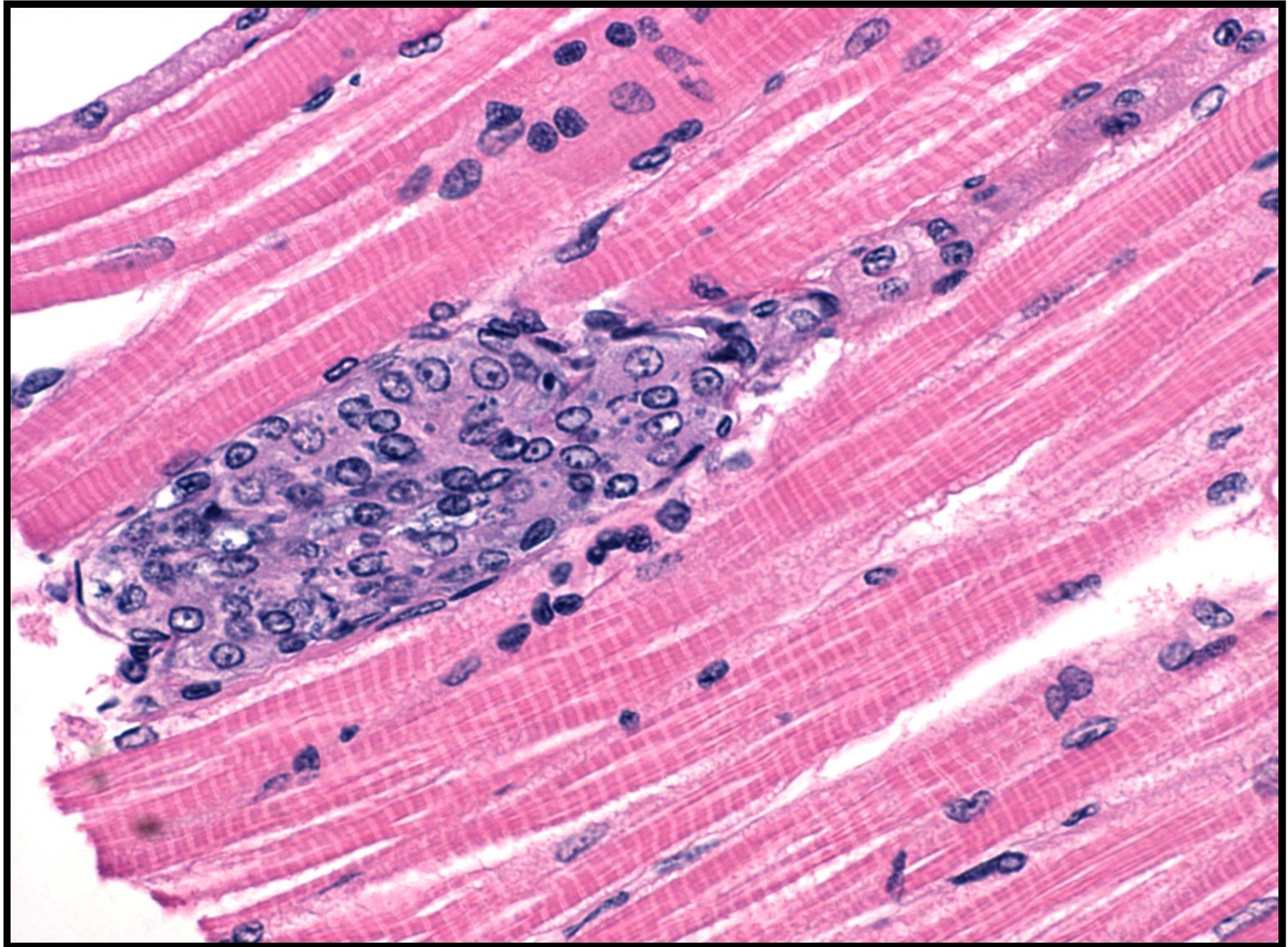
pale basophilic to dark basophilic inclusion bodies are evident in the muscle cells



Presence of lymphoid organ spheroids (LOS) in the IMNV-infected *P. vannamei*



Presence of Ectopic spheroids (EC) in the heart of IMNV-infected *P. vannamei*



Ectopic spheroids (EC) in IMNV-infected *P. vannamei*

Penaeid shrimp showed white tail gross signs

- 1. *Penaeus vannamei* nodavirus (PvNV)**
- 2. Cotton shrimp disease**
- 3. Unknown etiology, e.g. an muscle necrosis disease in Ecuador**
- 4. Environmental factors (hypoxia, crowding, or sudden changes in temperature or salinity)**
- 5. Rumors of IMNV in Thailand and China, not able to confirm**

PVNV (*penaeus vannamei* nodavirus)



P. vannamei with IMN-like gross signs (from Belize, 2003)

Penaeus vannamei specimens from Belize in 2005

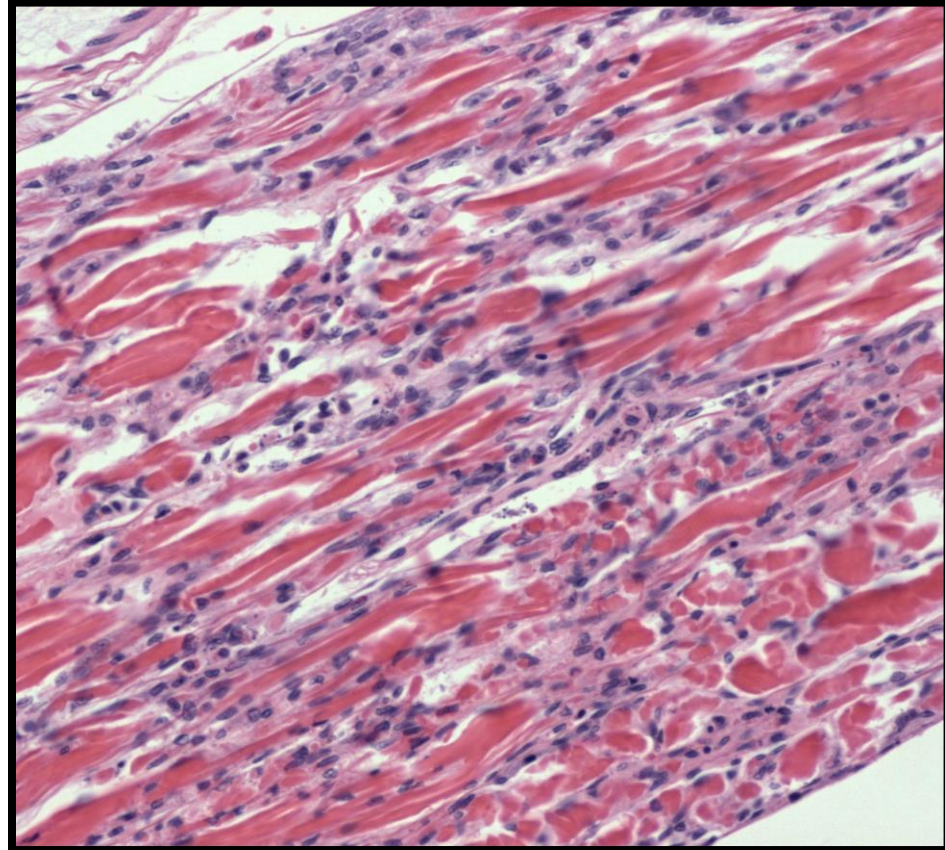
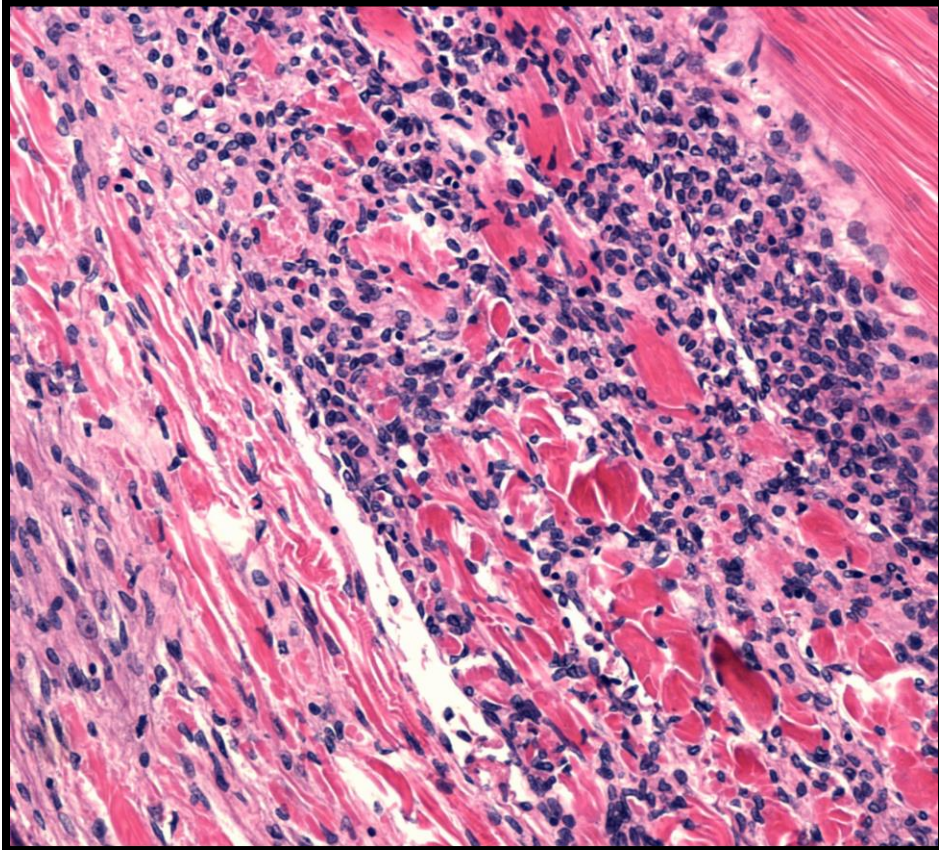
- Significant **mortalities** noted.
- Affected shrimp presented **opaque** muscle.
- Histopathology consistent with **IMNV** infection:
 - Acute, subacute & resolving **myonecrosis**.
 - Significant formation of **lymphoid organ spheroids**.

Gross Signs – experimental infection *PvNV*



IMNV - skeletal muscle

***PvNV* - skeletal muscle**



**Totivirus, monosegmented
dsRNA genome**

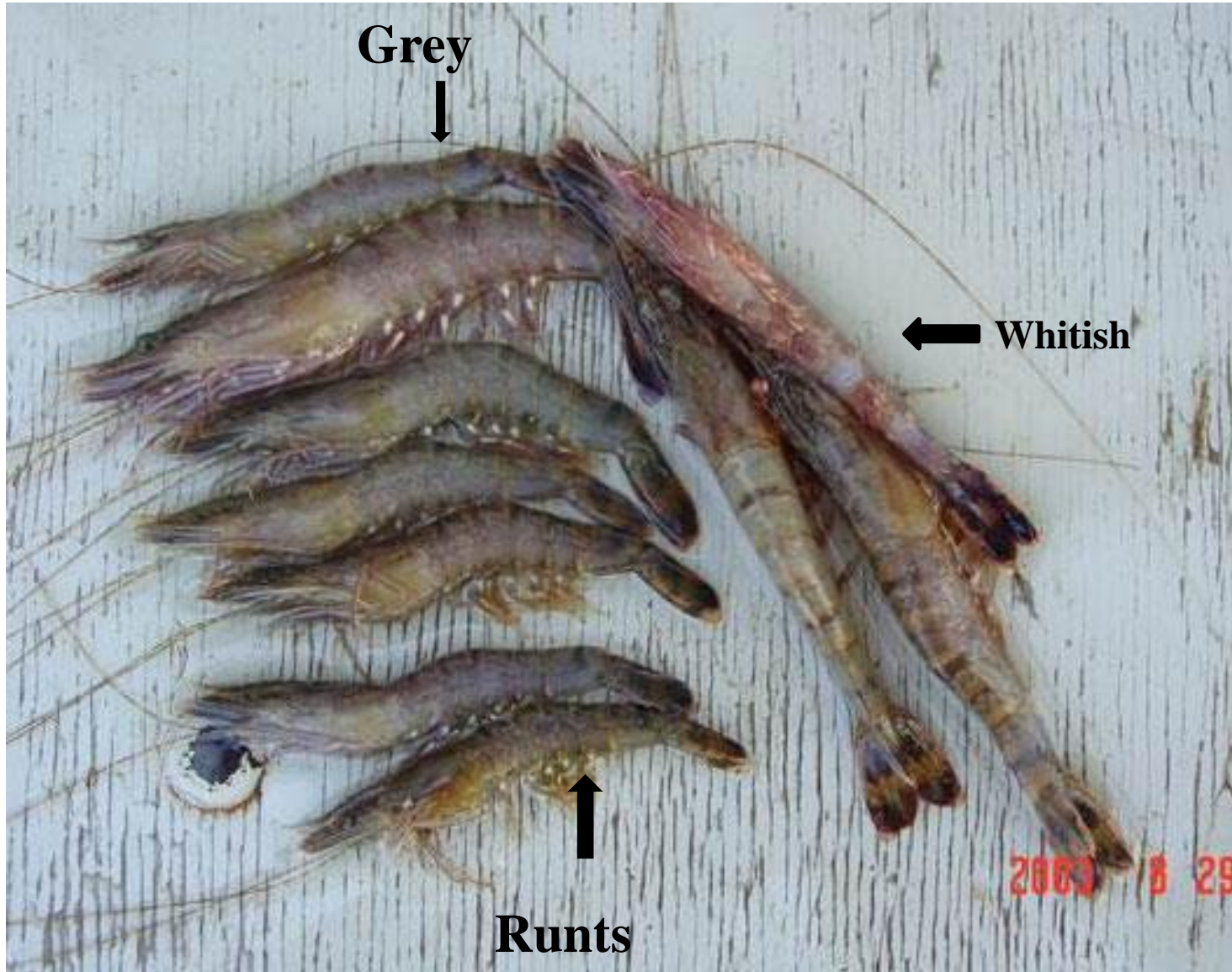
**Nodavirus: 2-segmented,
ssRNA(+) genome**

Clinical signs of cotton shrimp disease

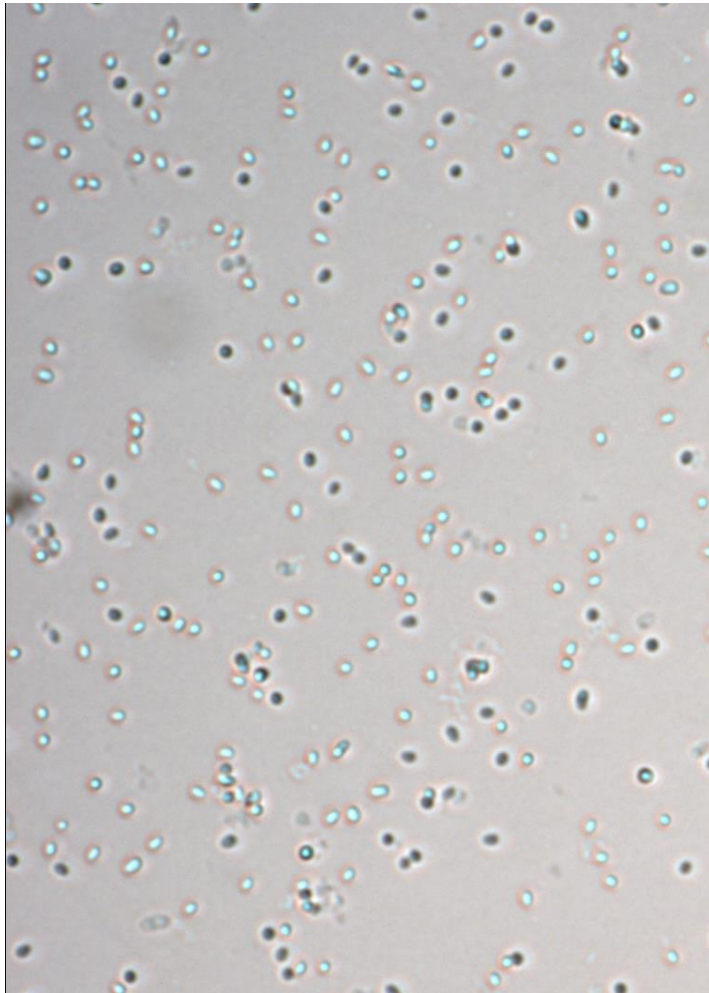


- **Lethargy and increased mortalities**
- **Opaque white patches under the carapace**
- **Shrimp appear stressed with expanded chromatophores.**

Cotton shrimp disease *Perezia* s

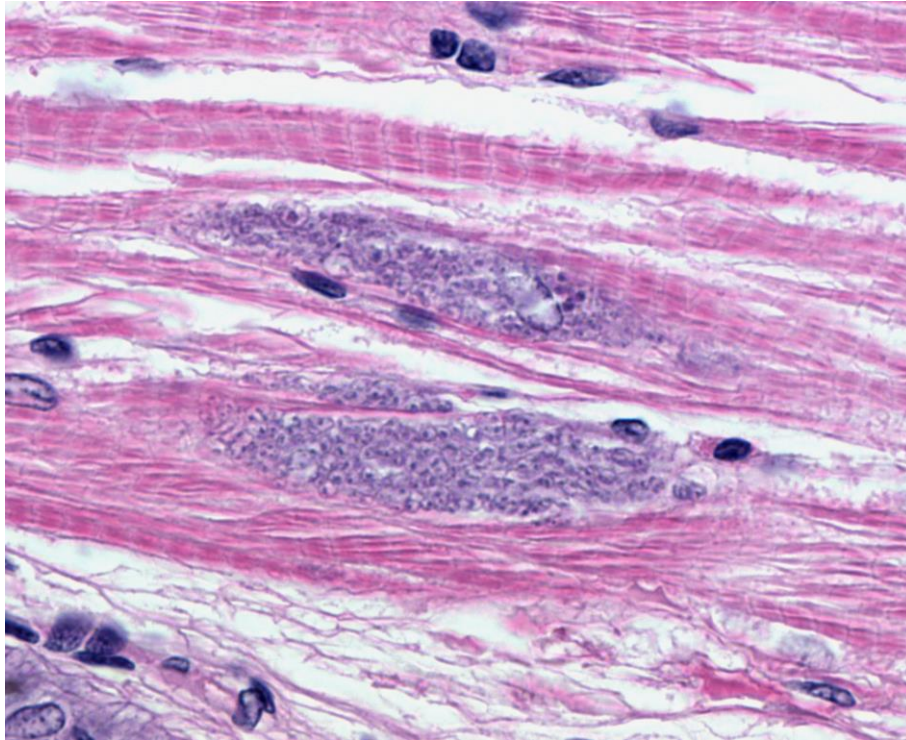


Perezia sp. microsporidia



- **Cotton shrimp disease**
 - **Muscle (& other tissues) infected with microsporidia**
- **Spore size: 1-2 μm , oval shaped**

**Spores in muscle smear,
P. monodon, Madagascar**

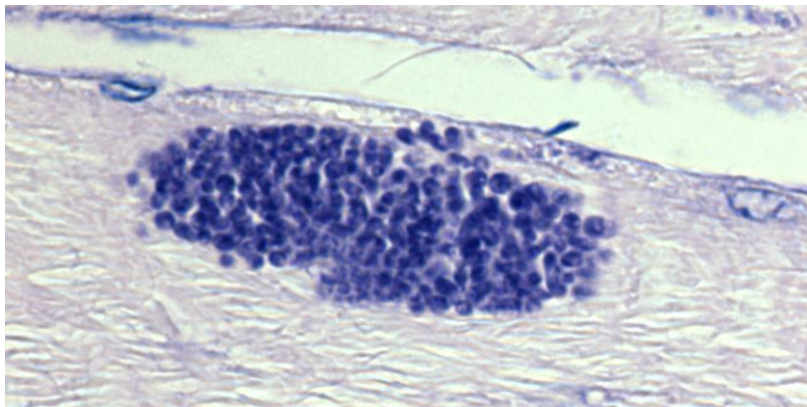


Histology of tail muscle

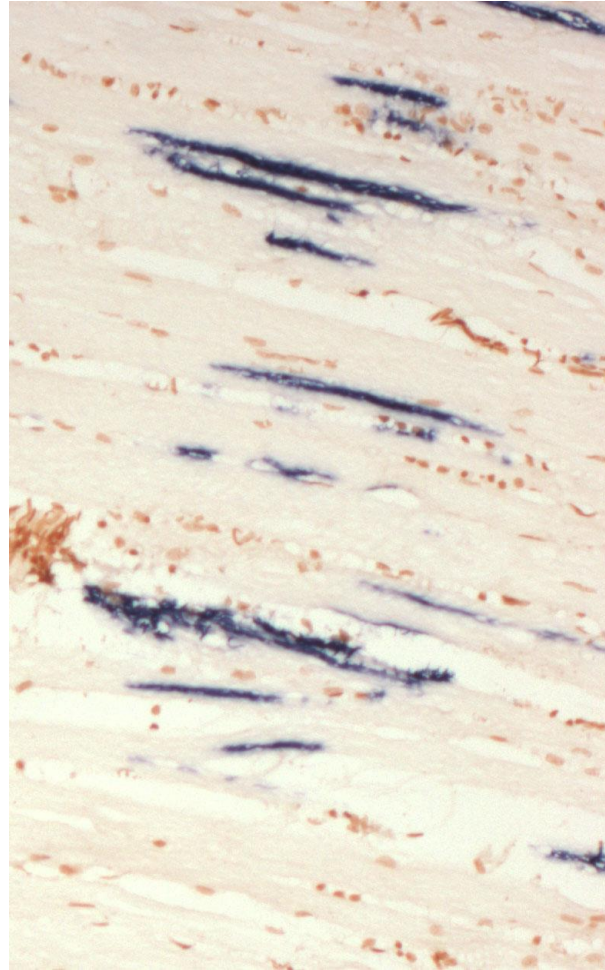
- The presence of cytoplasmic inclusions in the tail muscle:

- Top: H&E stain

- Bottom: Giemsa stain



In situ hybridization with a Dig-labeled SSU rDNA probe



Melena et al; Infectious muscle necrosis etiology in the Pacific White Shrimp
(*Penaeus vannamei*) cultured in Ecuador. Braz J Vet Pathol, 2012, 5(1), 31 - 36

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BJVP
Brazilian Journal of Veterinary Pathology
ISSN 1983-0246

Original Full Article

Infectious muscle necrosis etiology in the Pacific White Shrimp (*Penaeus vannamei*) cultured in Ecuador

José Melena^{1*}, Javier Tomalá², Fanny Panchana¹, Irma Betancourt¹, César Gonzabay¹,
Stanislaus Sonnenholzner¹, Yasuji Amano³ & Jean-Robert Bonami⁴

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²Universidad Estatal Península de Santa Elena (UPSE), Avenida principal La Libertad – Santa Elena, La Libertad, Ecuador

³Instituto Nacional de Higiene y Medicina Tropical “Leopoldo Izquieta Pérez”, Guayaquil, Ecuador

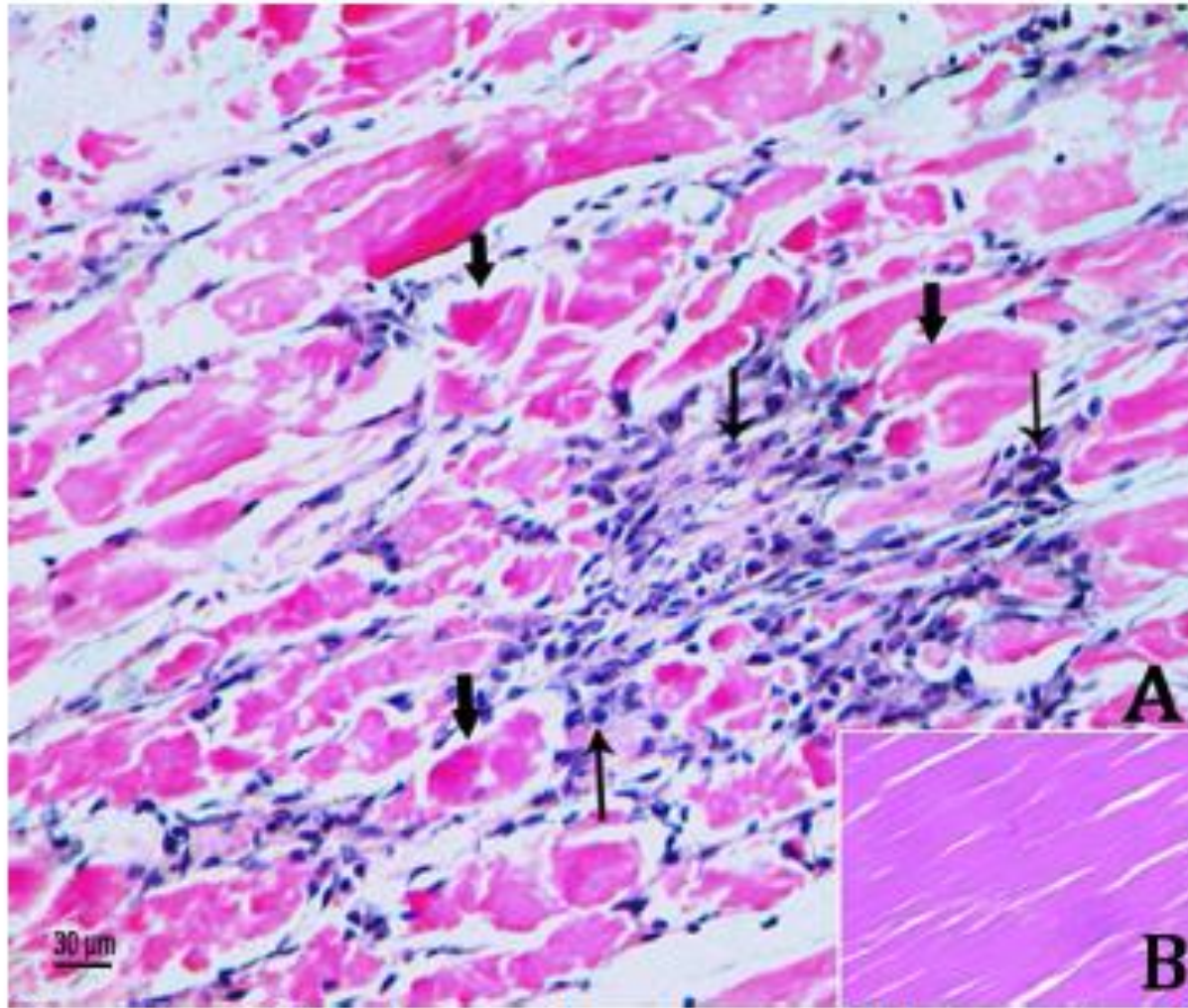
⁴Pathogens and Environment, UMR 5119 EcoLag cc 093, CNRS/UM2, Place E. Bataillon, 34095 Montpellier Cedex 5, France.

IMNV-suspected *P. vannamei* cultured in Ecuador



Figure 1 - Diseased *P. vannamei* shrimp obtained from commercial grow out ponds and used as starting material (inoculum), exhibiting skeletal muscle necrosis as an opaque, whitish, discoloration of several abdominal segments (arrows).

Muscle necrosis disease in Ecuador



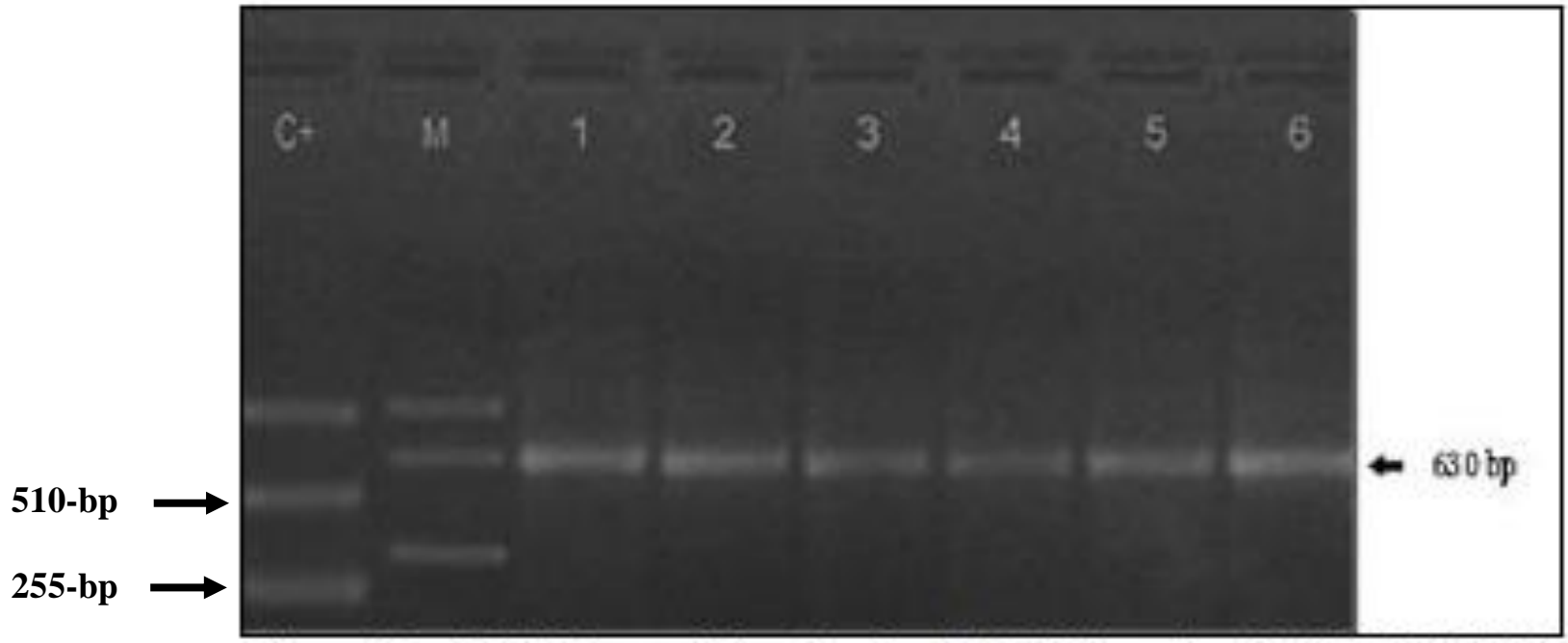
Extensive coagulative necrosis and hemocytic infiltration in skeletal muscle obtained from diseased *P. vannamei* shrimp cultured in Ecuador.

Muscle necrosis disease in Ecuador



Gross signs of muscle necrosis observed in challenged *P. vannamei*, opaque, whitish discolorations in the tail, with emphasis on the last abdominal segments.

IMNV RT-PCR detection using a commercial kit



The detection of IMNV in the shrimp (lanes 1-6) injected with tissue homogenates prepared from IMNV-suspected shrimp cultured in Ecuador.

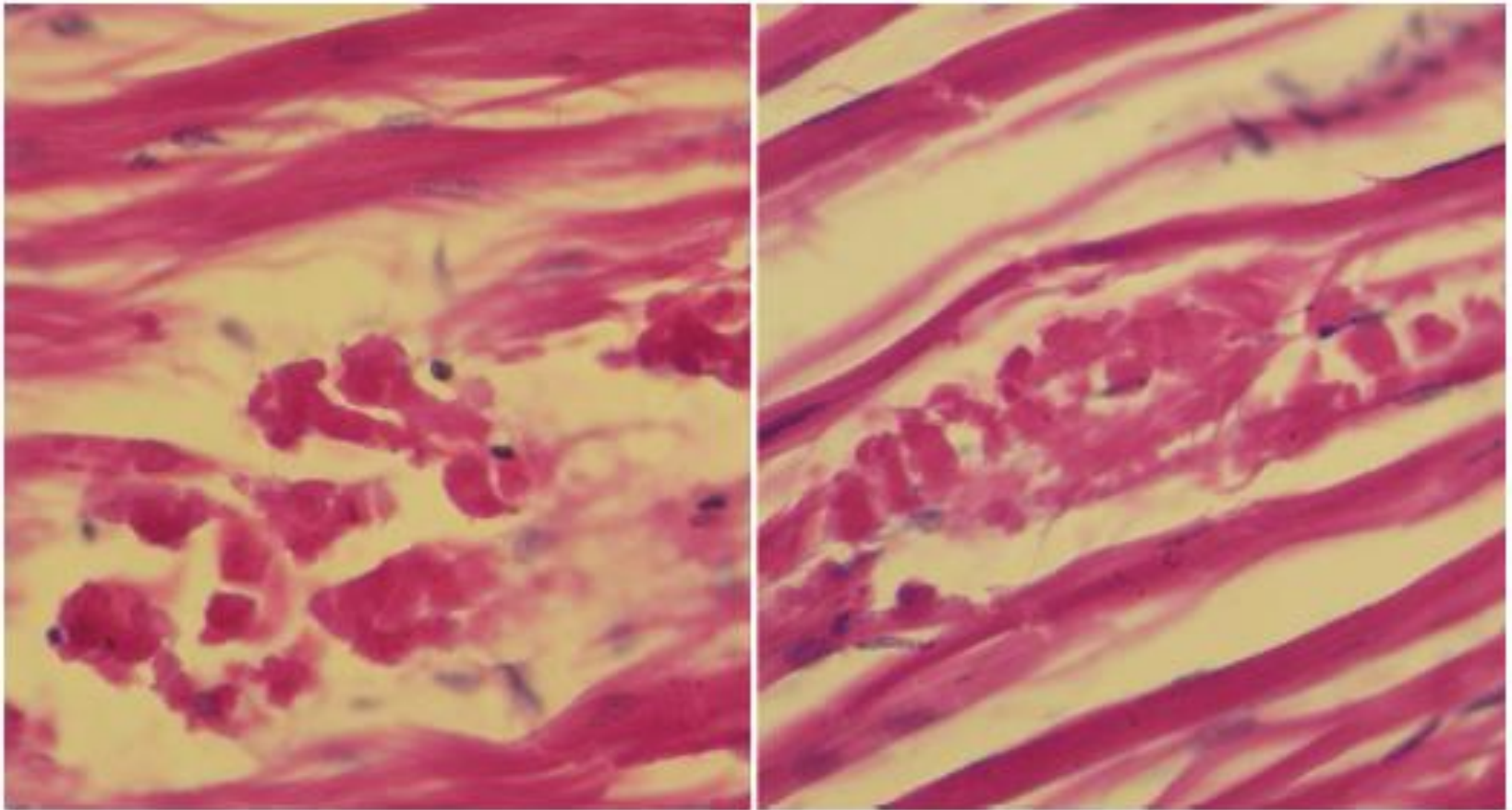
RESEARCH

Open Access

False rumours of disease outbreaks caused by infectious myonecrosis virus (IMNV) in the whiteleg shrimp in Asia

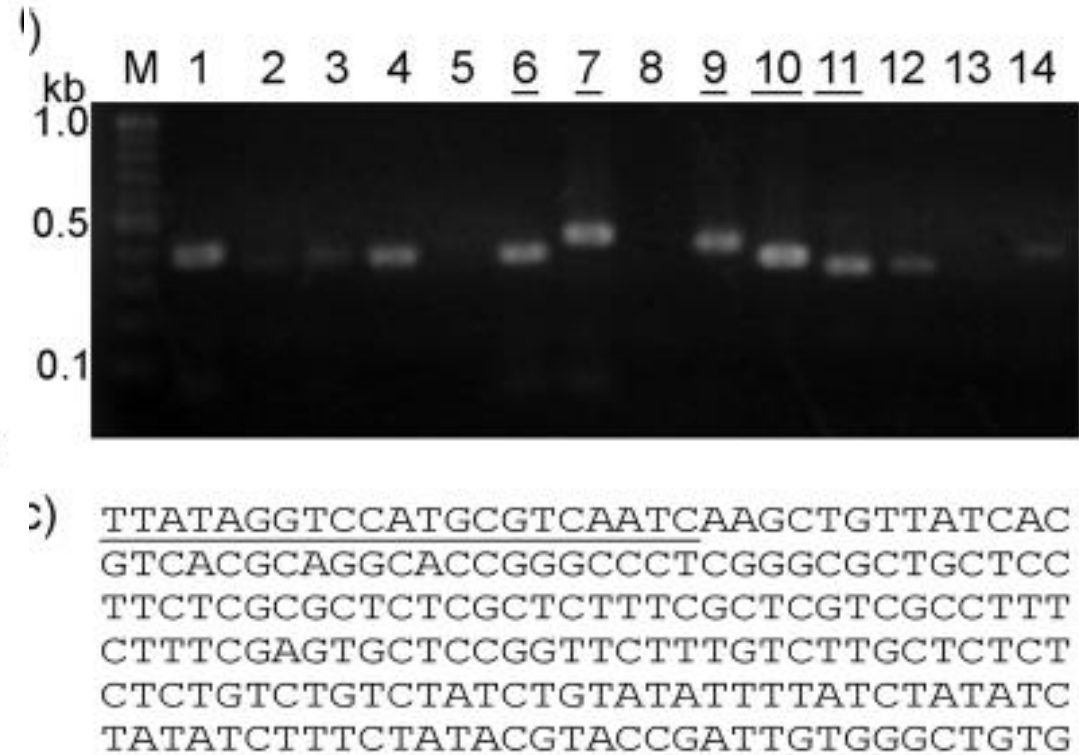
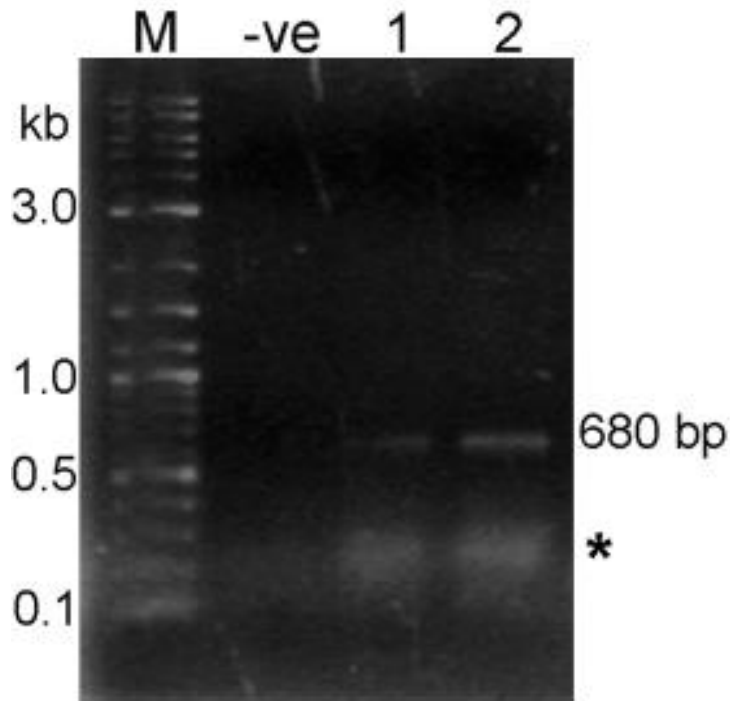
Saengchan Senapin^{1,2†}, Kornsunee Phiwsaiya^{1,2†}, Warachin Gangnonngiw^{1,2} and Timothy W Flegel^{1,2,3*}

False rumors of IMNV in Asia



Histology showing coagulative necrosis; lack of inclusions and infiltrated hemocytes


False rumors of IMNV in Asia

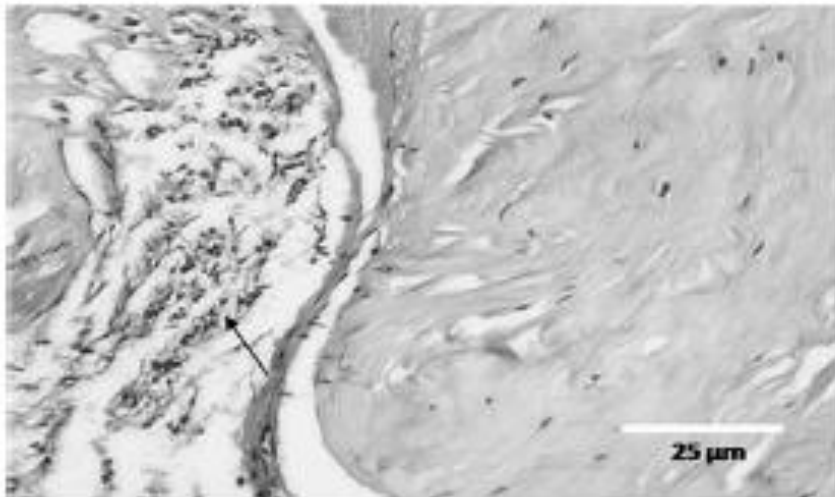


IMNV was not detected by RT-PCR, amplicon sequencing showed non-specific reactions

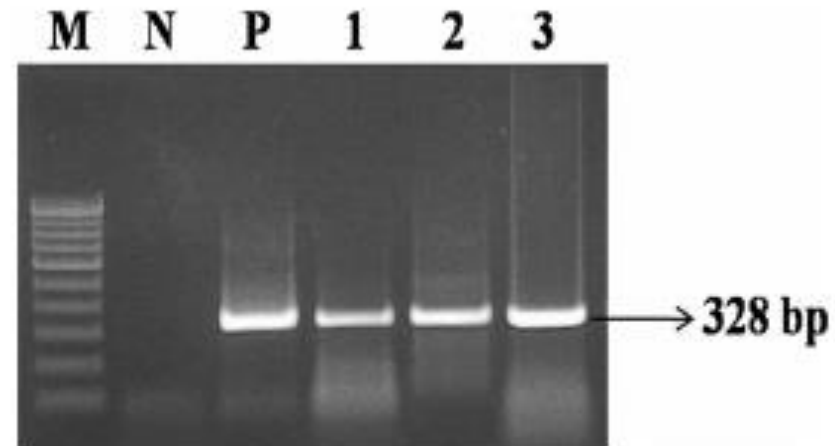
IMNV in India_West Bengal

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



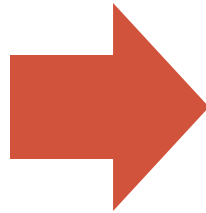
IMNV RT-PCR, positive found in *P. vannamei* from farms. Mortality: 20-50%

Diagnostic methods

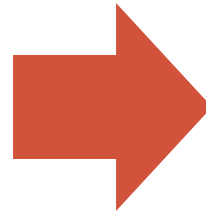
- **Nucleic acid-based**
- **PCR (or RT-PCR):**
 - **targets and amplifies** a pathogen's DNA (or RNA) from a DNA sample
- **Real-time PCR (quantitative PCR, qPCR):**
 - **quantifies** DNA (or RNA) templates (copy numbers)

Conventional PCR

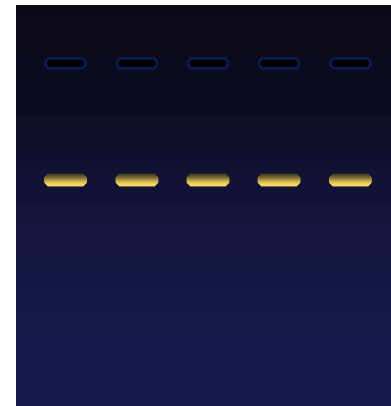
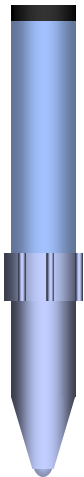
Sample
prep



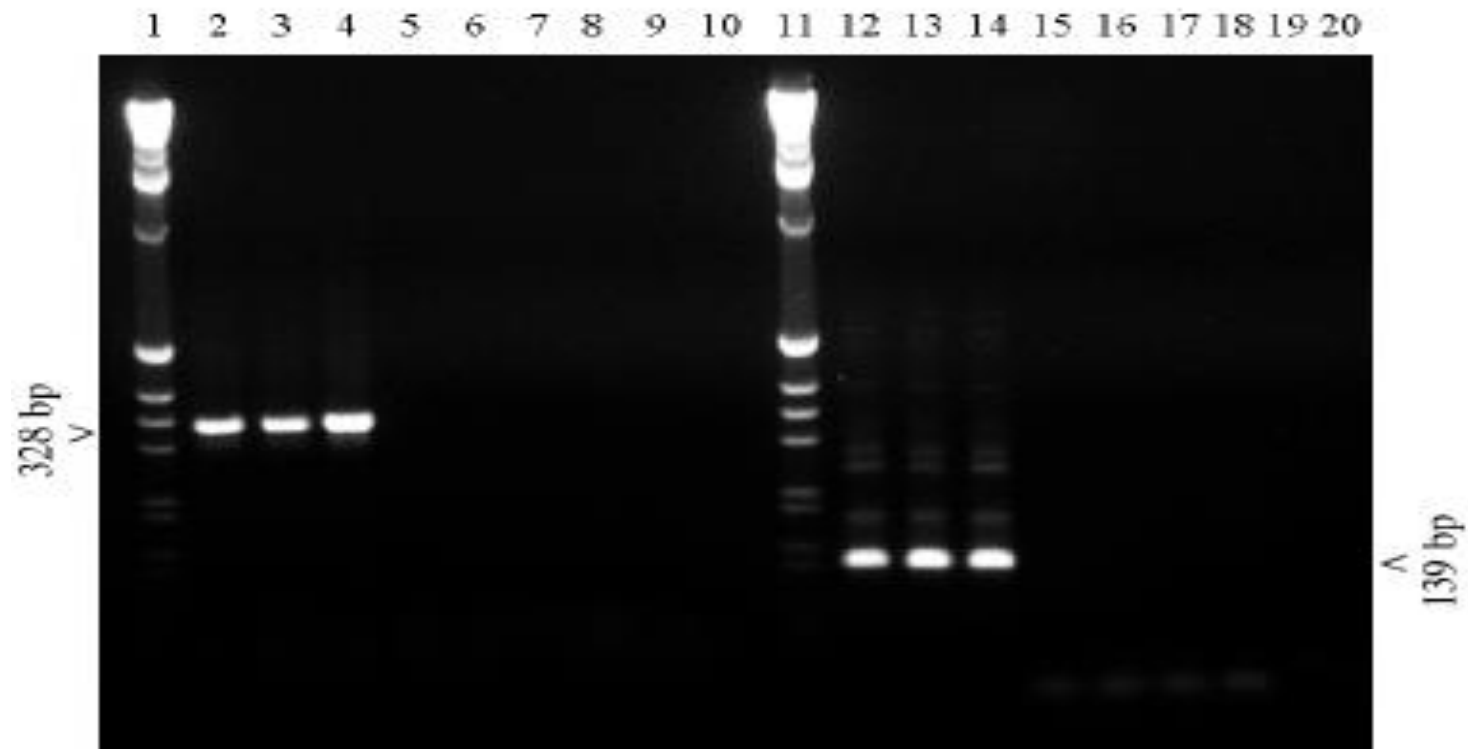
PCR



Endpoint Analysis



Detection of infectious myonecrosis virus (IMNV) of penaeid shrimp by reverse-transcriptase polymerase chain reaction (RT-PCR)



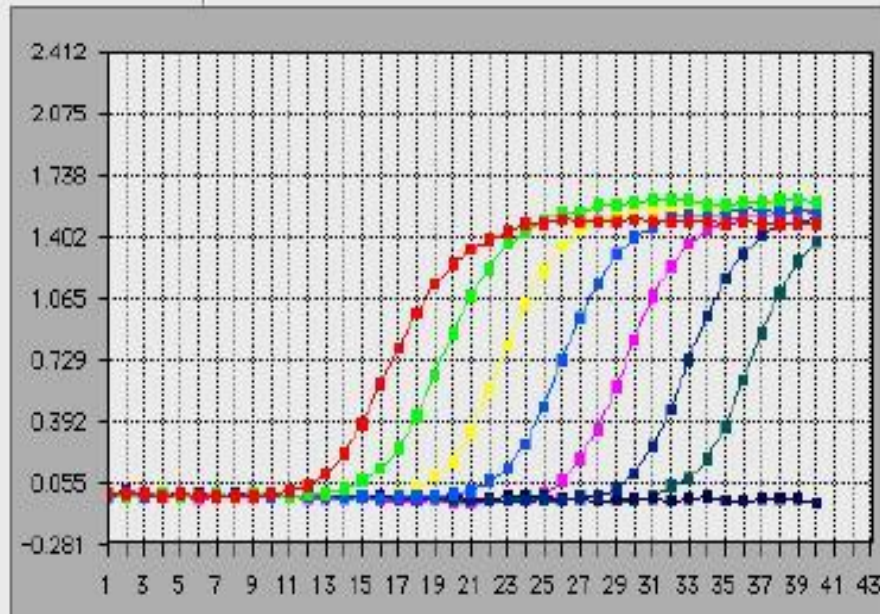
**L2 & 12: Brazil IMNV; L3 & 13: pleopods, L4 & 14: muscle
L5 & 15: TSV; L6 & 16: YHV; L7 & 17: IHNV; L8 & 18: WSSV; L9 & 19: SPF**

Real-time RT-PCR

- **Real-Time Vs Conventional PCR**
- **PCR**
 - **Post PCR processing**
 - **Based on the band intensity on the agarose gel (semi-quantitative)**
 - **Detect end-point of the reaction**
- **Quantitative PCR (Real-Time)**
 - **The fluorescence is monitored during each PCR cycle to provide an amplification plot allowing the user to follow the reaction.**

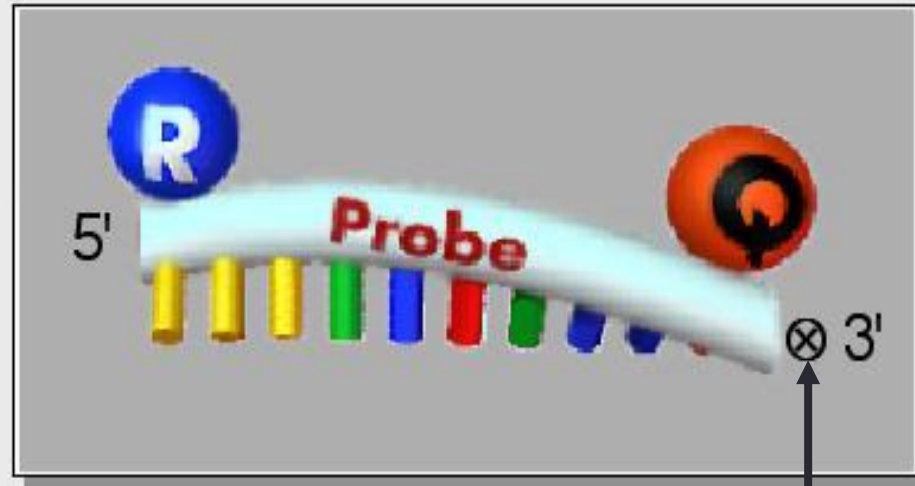
Real-Time PCR detection

The automatic detection of PCR product growth throughout the amplification process.



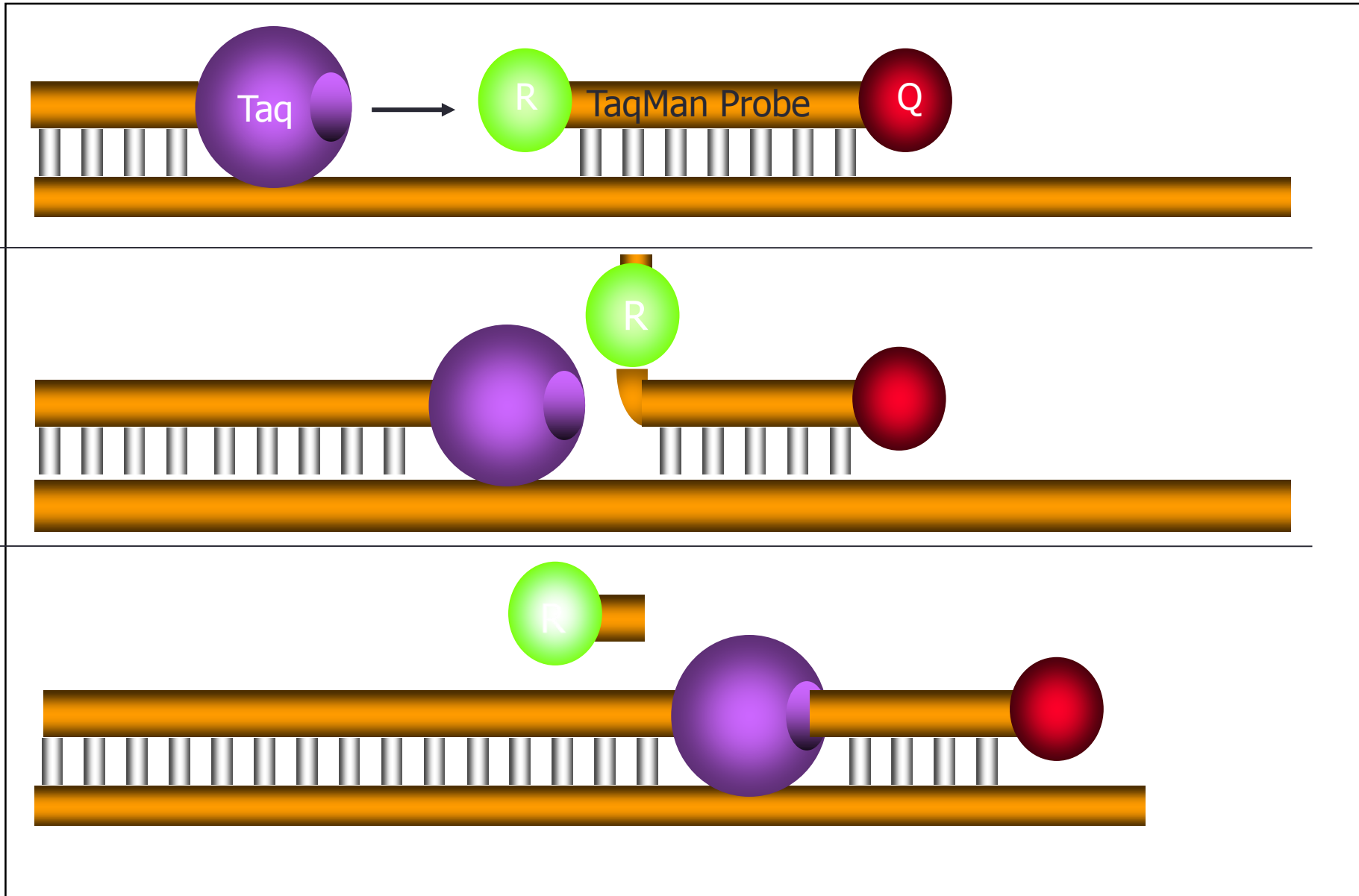
- **Monitors the progress of PCR reaction in real-time**
- **Collects exponential amplification phase data**
- **Amplification and detection in a single tube, eliminates post-PCR steps**

TaqMan probe



- Hybridises with the target amplicon
- Is 3' terminally blocked (cannot be extended by the polymerase)
- Has two fluorescent dyes attached:
 1. Reporter (R)
 2. Quencher (Q)

5'-Nuclease Activity of Taq Polymerase



Ct = Threshold Cycle

The cycle number at which the fluorescence emission exceeds a fixed threshold

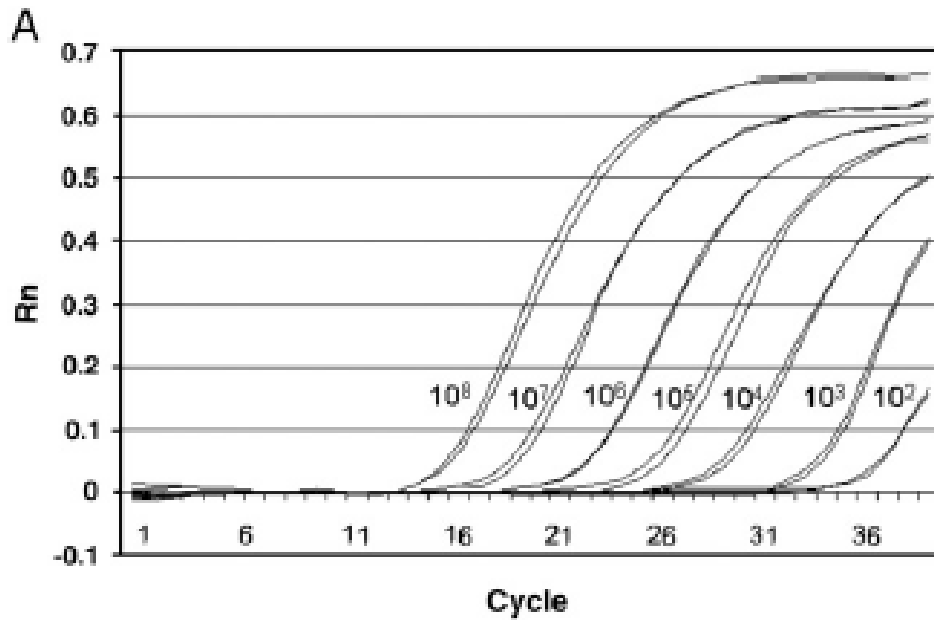
Real-time reverse transcription polymerase chain reaction assay
using TaqMan probe for detection and quantification
of *Infectious myonecrosis virus* (IMNV)

Primer/Probe	Sequence (5' to 3')
IMNV412F	GGACCTATCATACATAGCGTTGCA
IMNV545R	AACCCATATCTATTGTCGCTGGAT
IMNV-P1	FAM-CCACCTTTACTTTCAATACTACATCATCCCCGG-TAMRA

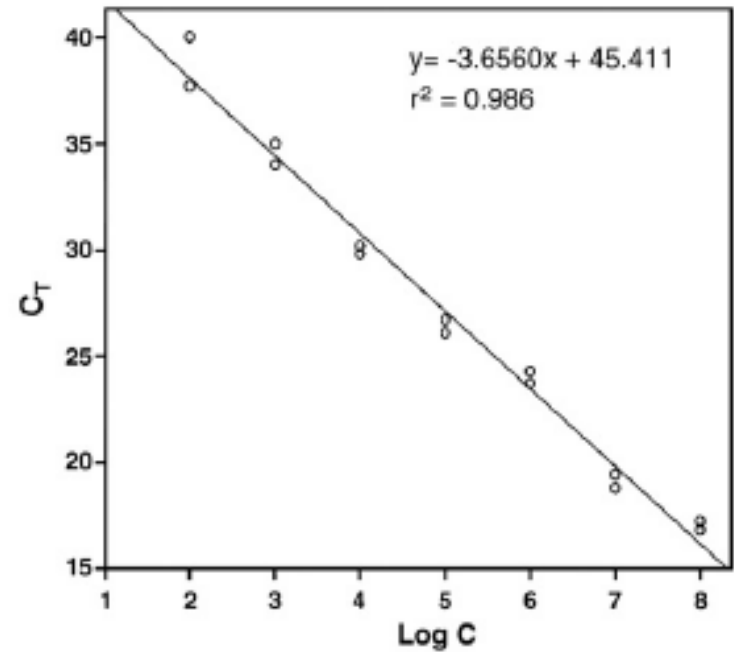
IMNV RT-qPCR

- **2 X TaqMan Master mix**
- **RT enzyme**
- **IMNV primers**
- **TaqMan probe**
- **RNA template**
- **Cycling profile**
- **42°C, 5 min**
- **95°C, 10 s**
- **40 cycles of 95°C/5 s and 60°C/30 s**

IMNV RT-qPCR

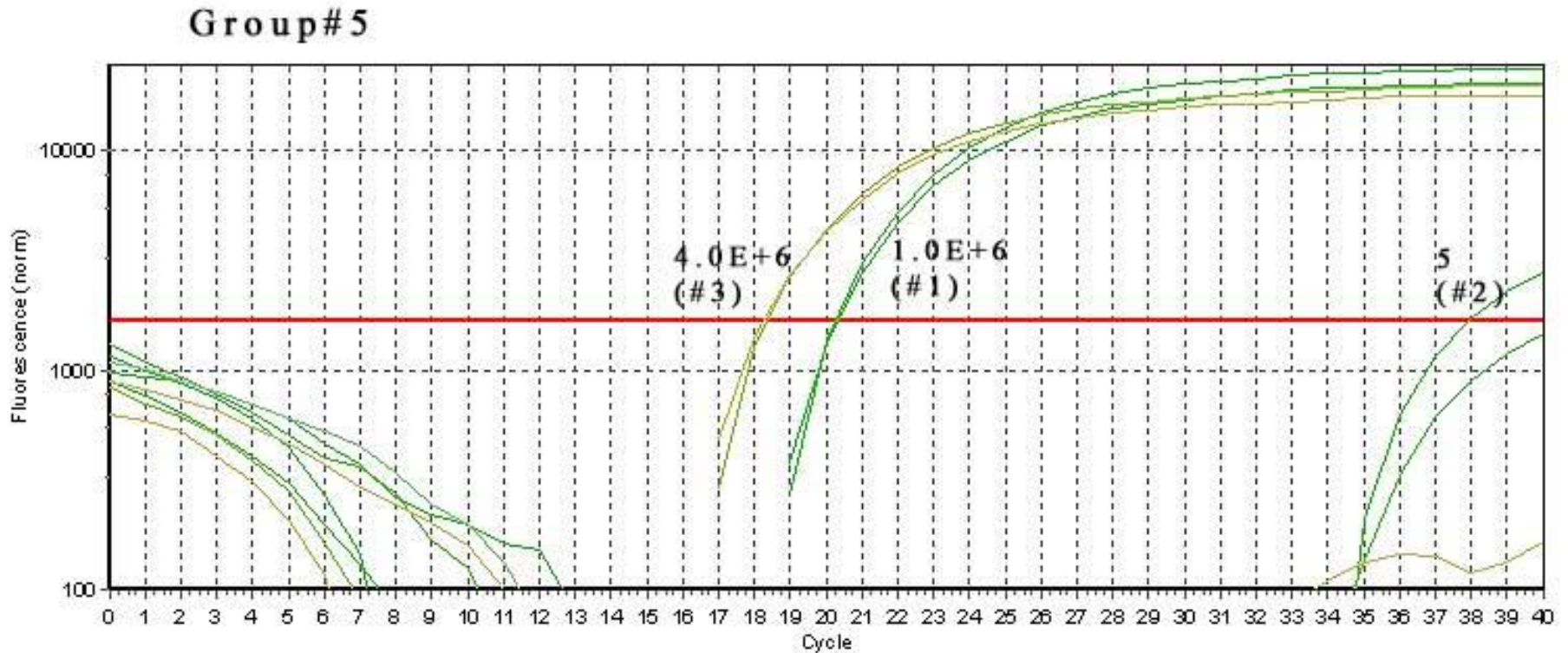


Amplification plot



Standard curve

Group #5



Sample#1 and #3: WSSV-infected tissue

Sample #2 and #4: SPF *P. vannamei*