Biosecurity & Health in US Indoor Shrimp Farming

Arun K Dhar, PhD

Aquaculture Pathology Laboratory

The World Organization for Animal Health (OIE) Reference Laboratory

USDA-APHIS Approved & ISO 17025 Accredited Laboratory

The University of Arizona, Tucson, Arizona, USA

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Agenda

- Introduction of Aquaculture Pathology Laboratory
- What is Biosecurity & Why Biosecurity?
- Components of Biosecurity

- Shrimp Diseases
  - Bacterial Disease- Acute Hepatopancreatic Necrosis Disease (AHPND)/ Early Mortality Syndrome (EMS)
  - Viral Disease- White Spot Syndrome (WSD)
  - Fungal Disease- *Enterocytozoon hepatopenaei* (EHP)

- Perspective in Disease Prevention/Management

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Two Campuses, One Lab

- Main Campus

- West Campus

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Aquaculture Pathology Laboratory: Missions

- **Shrimp Disease Research- Main Campus**
  - Disease diagnostic services to shrimp industry- composed of three units: Histopathology, PCR & Microbiology
  - Educational & Training Services: Annually--*Shrimp Pathology Short Course.*
  - Conducting Inter-laboratory Calibration- Proficiency/ Ring Test
  - Basic research in shrimp virology, microbiology & genomics.

- **Shrimp Research Facility- WCAC**
  - Disease challenge study, testing therapeutics, feed & feed additives.
  - *Building diagnostic capabilities for fish diseases*

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BIOSECURITY: Excluding Shrimp Pathogens in the Mixed of Microbial Milieu

Healthy Harvest

GENETICS:
Genetically superior
SPF/SPR stock

Biosecurity:
Farm Management

NUTRITION:
High Quality
Balanced Diet

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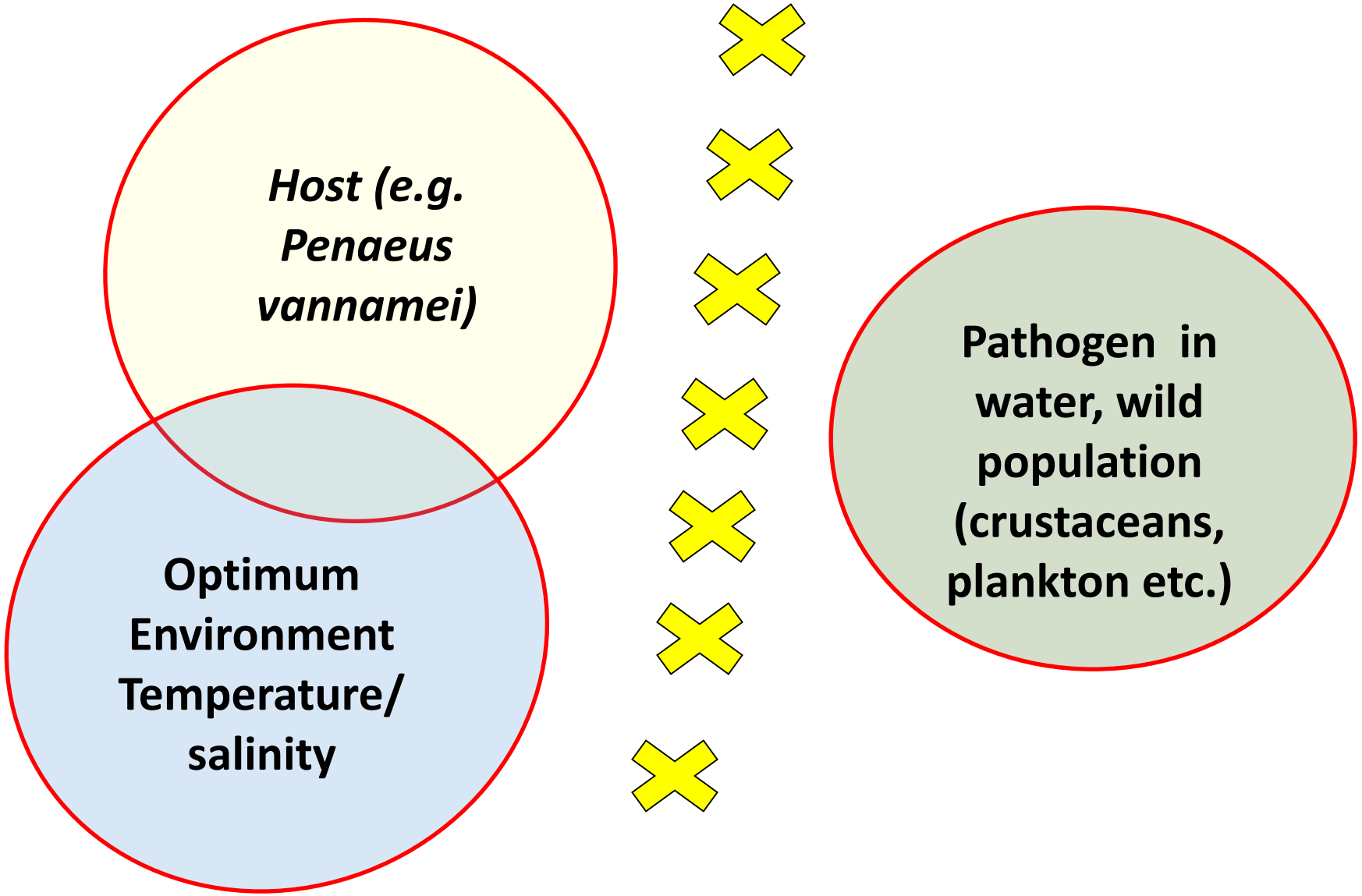
Pathogen Exclusion = No Disease



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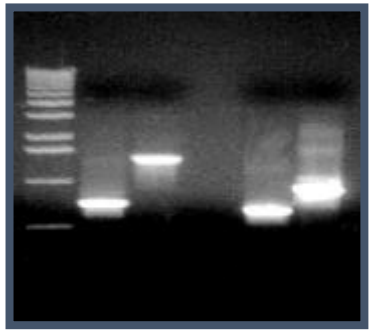
Components of Biosecurity

- **Knowledge of diseases of concern**
  - List of excludable diseases/pathogens.
- **Adequate diagnostic/detection methods.**
- **Use of "clean" shrimp stocks**
  - **Assurance/surveillance of cultured stocks.**
- **Disease containment, eradication & disinfection plans in place**

Correct diagnosis



Positive/Non detectable

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OIE-Listed Crustacean Diseases

- **There are ~25 diseases known in shrimp & 09 of them are included the OIE list.**

Viral:

- Taura syndrome – TSV
- White spot disease – WSSV
- Yellow head disease – YHV/GAV
- Infectious hypodermal & hematopoietic necrosis – (IHHNV)
- Infectious myonecrosis – IMNV
- White Tail Disease- *Mr*NV

Bacterial:

- Necrotizing hepatopancreatitis bacterium-NHP-B
- Acute hepatopacreatic necrosis disease-AHPND (listed in 2016)

Fungal:

- Crayfish plague- *Aphanomyces astaci*

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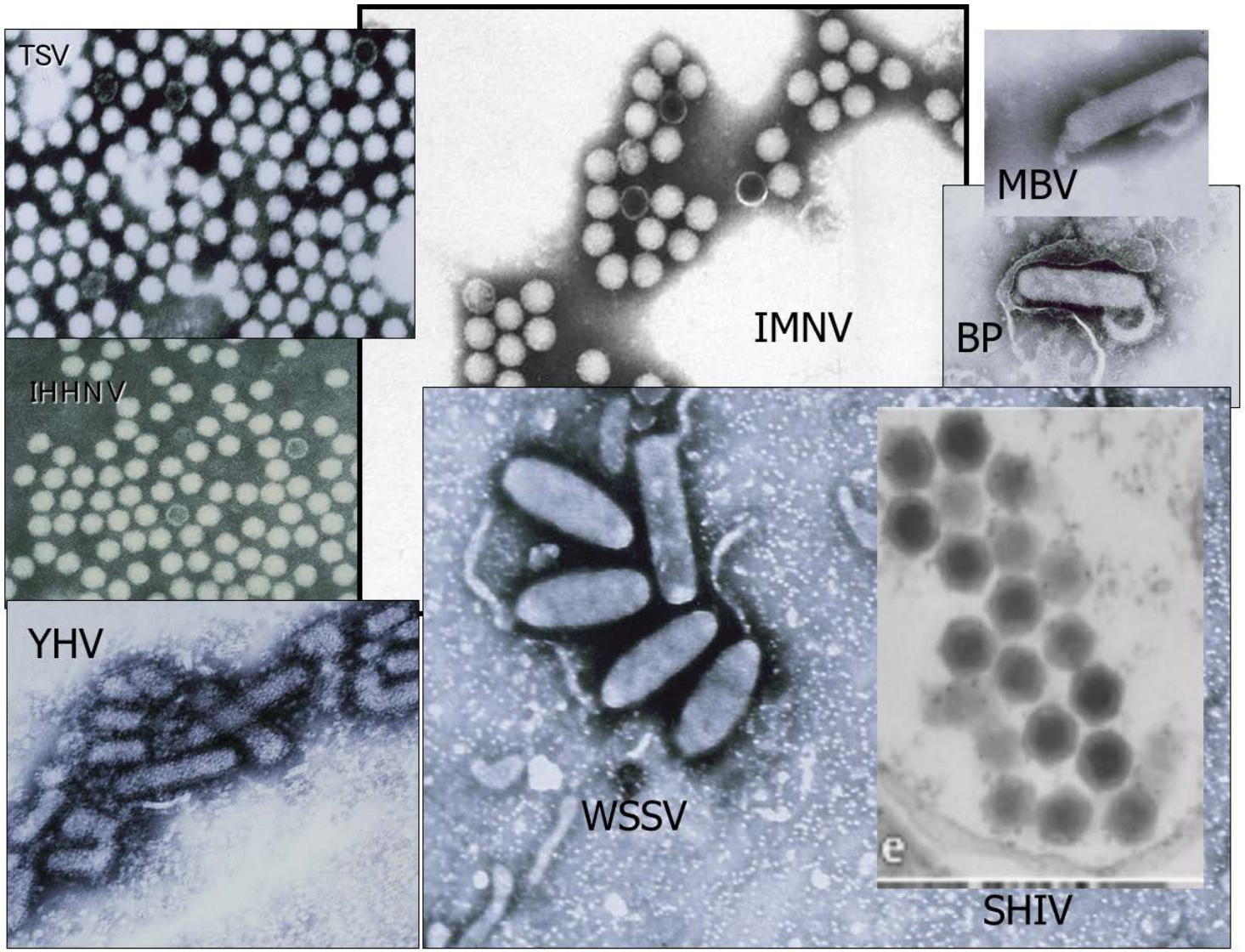
Shrimp Viruses



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According to OIE Aquatic Manual: Main recommended method for shrimp pathogen detection is PCR

PATHOGEN	TARGETED SURVEILLANCE				Presumptive diagnosis	Confirmatory diagnosis
	Larvas	PLs	Juvenile	Adult		
YHV	a	a	a	a	a	a
IHHNV	a	a	a	a	a	a
IMNV	a	a	a	a	a	a
AHPND	a	a	a	a	a	a
NHP	a	a	a	a	a	a
TSV	a	a	a	a	a	a
WSSV	d	b	a	a	a	a
MBV	a	a	a	a	a	a
BP	a	a	a	a	a	a

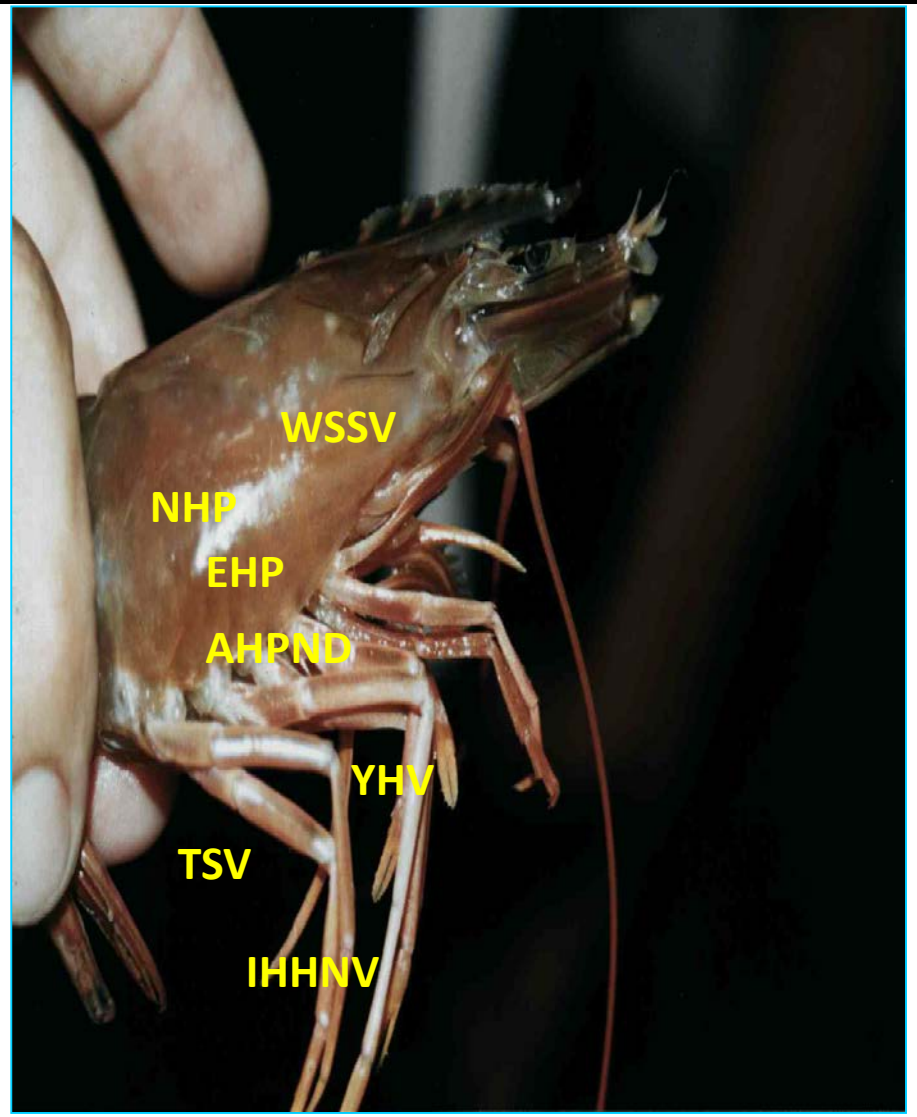
a= OIE recommended method for reasons of availability, utility, diagnostic specificity and sensitivity.

b= Standard method with good sensitivity & specificity.

c= Method with limited application due to costs or accuracy.

d= Method not recommended.

Exclusion of Pathogens in Shrimp



Challenges:

- **When pathogens are present at an undetectable level (technological limitation).**
- **Technical errors during pathogen detection.**

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PCR Results Interpretation

- Remember: we take only 1 sub-sample per shrimp for PCR analysis
- In shrimp chronically infected, a given pathogen is not necessarily present in one specific organ
- We sample 150 shrimp out of >100,000 : Assume a prevalence lower than 2%
- Efficiency of PCR processing is not a 100 %, e.g. DNA extraction, PCR amplification etc.

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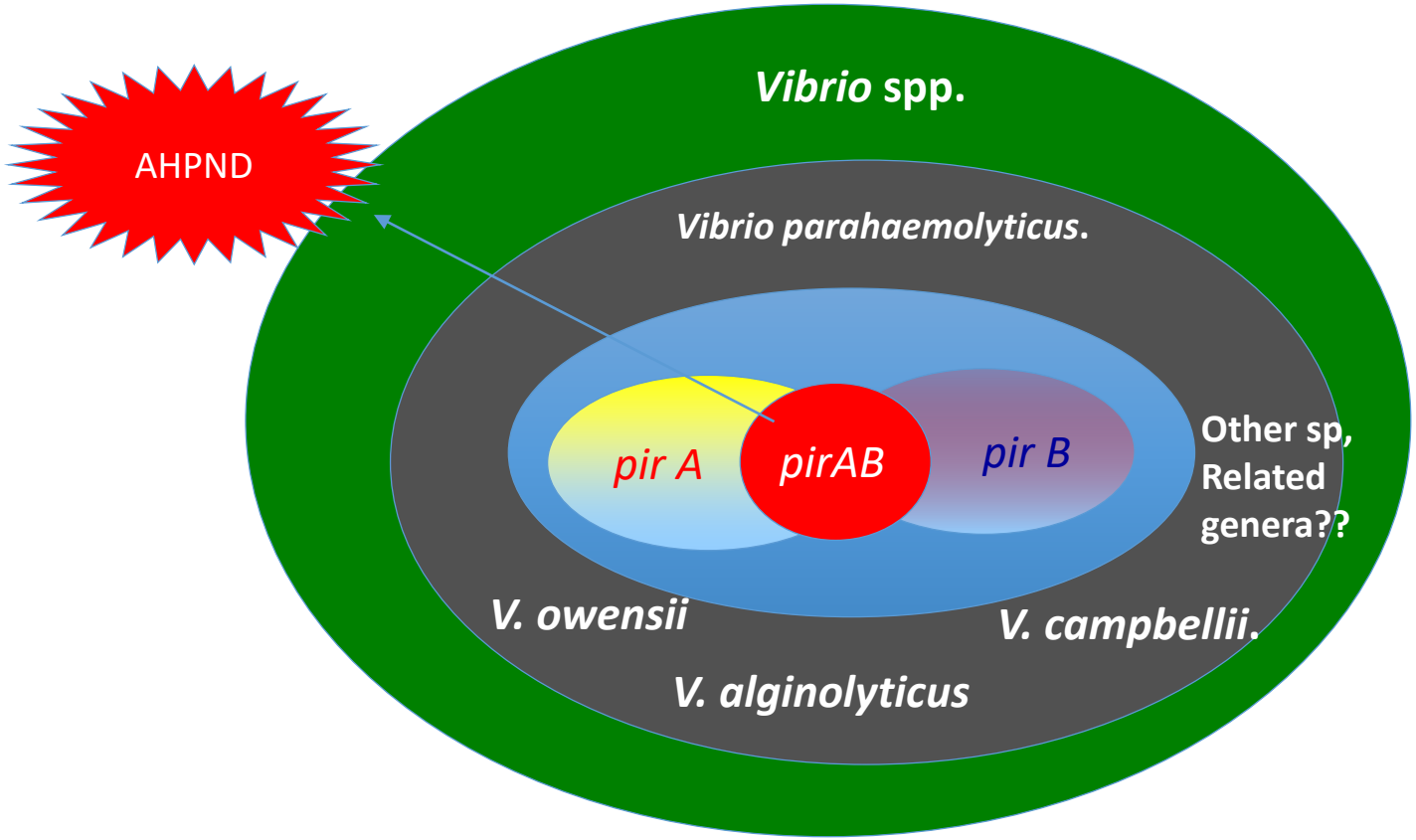
Virulence Factor in AHPND: Toxin Genes (*pirA* & *pirB*) in the Plasmid



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Modified from Aranguren et al., 2015

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AHPND Geographic Distribution

Eastern Hemisphere

2009= China
2010= Vietnam
2011= Malaysia
2012= Thailand
2015= Philippines,
2017= Myanmar, Bangladesh

Western Hemisphere

2013= Mexico
2017= Texas, USA

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AHPND: Clinical Signs

EMS in Sonora, Mexico

Photo by Ms. Silvia Gomez

- Pale & atrophied HP
- Sloughing of epithelium in HP tubule

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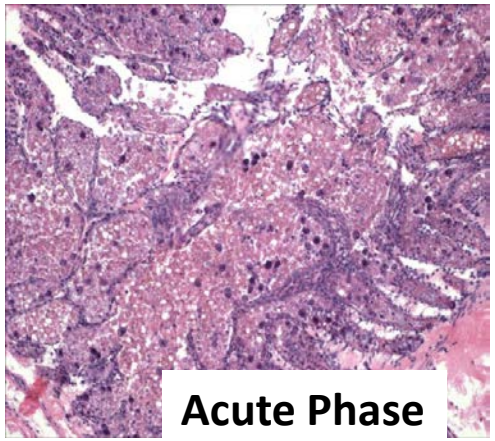
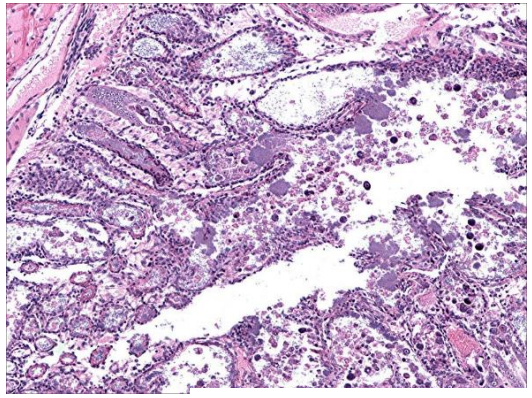
AHPND Histopathology: Susceptible Animals



Acute Phase



Terminal Phase

Acute phase

>Loss of function of HP tubule cells (R, B, F & later E-cells).

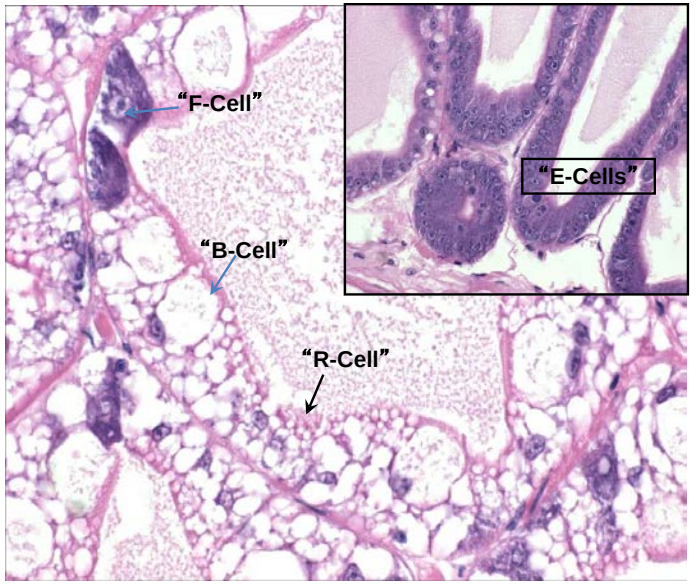
>Progressive degeneration of HP tubules from medial to distal with dysfunction of all HP cells, ***prominent necrosis & sloughing of these tubule epithelial cells***.

>***Bacteria are not easily demonstrated*** by *in situ* hybridization

Terminal phase:

>Marked inter- & intra-tubular hemocytic infiltration

>Development of ***massive secondary bacterial infections in conjuction with necrotic & sloughed HP tubule cells.***



Healthy

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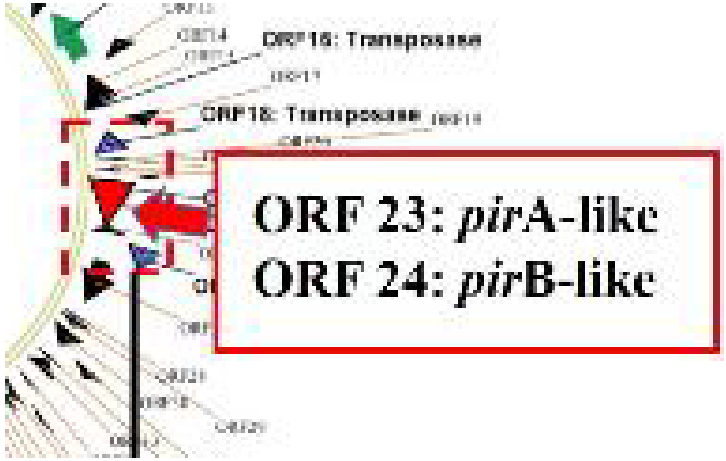
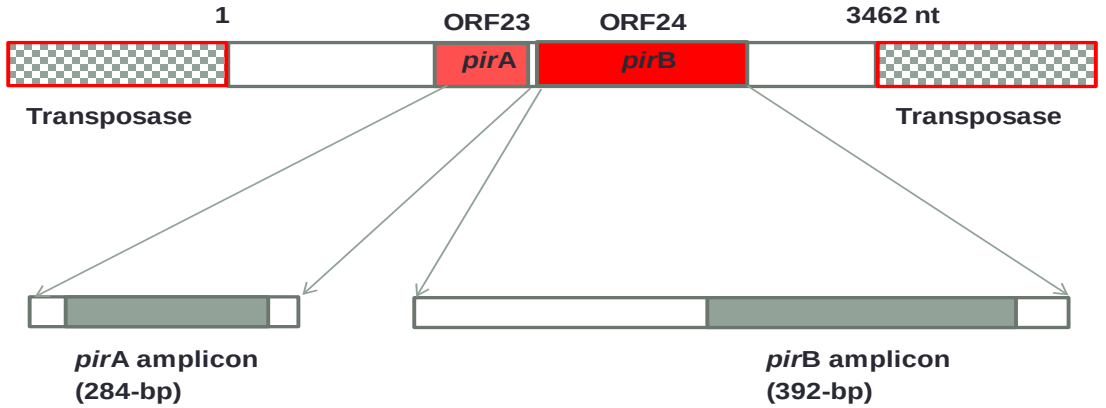
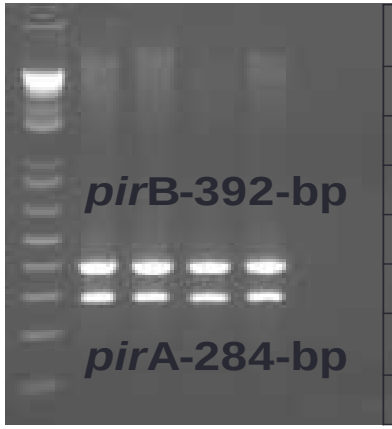
AHPND Detection- Duplex PCR Assay



70 kb Plasmid





Lane #	Strain	AHPND	Origin
1	13-511A/1	**Pos**	MX
2	A3	**Pos**	VN
3	13-306D/4	**Pos**	MX
4	12-194G	**Pos**	VN
5	A2	-	VN
6	13-488L	- (SHPN+)	India
7	13-431/1	-	US-TX

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Han et al., 2015

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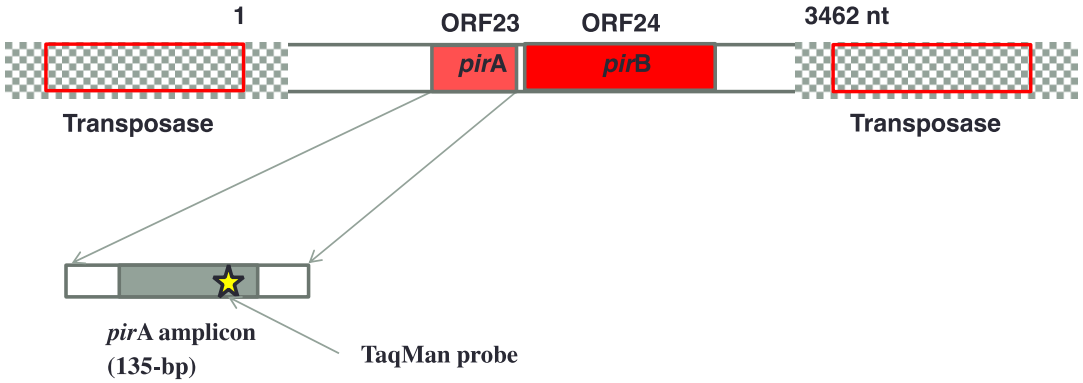
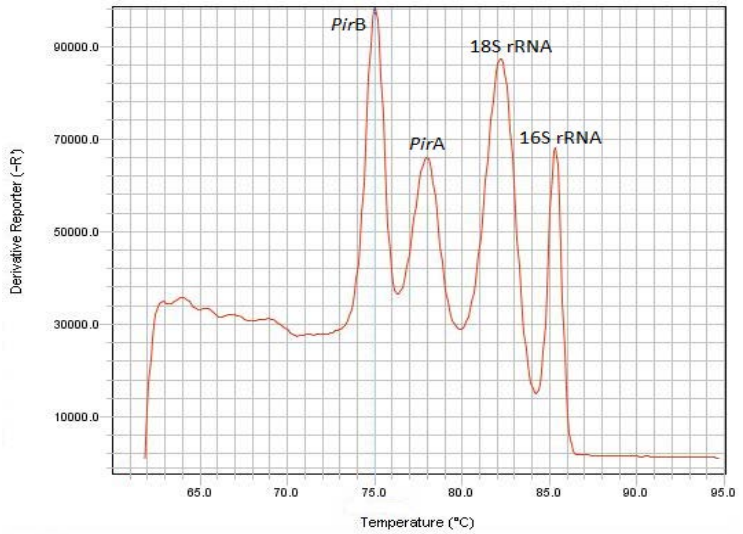
AHPND Detection: TaqMan & SYBR Green qPCR

TaqMan assay for AHPND pirB gene detection

- **Specific, Fast & Sensitive**
  - Within 30 min
  - Detection limit: <10 copies of virulence plasmid





Primer	Sequence (5' to 3')	Amplicon size	Target
VpPirA-F	TTGGACTGTCGAACCAAACG	135-bp	Real time PCR
VpPirA-R	GCACCCCATTGGTATTGAATG		
TaqMan probe[a]	AGACAGCAAACATACACCTAT CATCCCGGA		

Han et al., 2015

Multiples SYBR Green qPCR for the detection of pirA and pirB genes in AHPND causing Vibrio species.

Cruz et al., 2018. Manuscript in prep.

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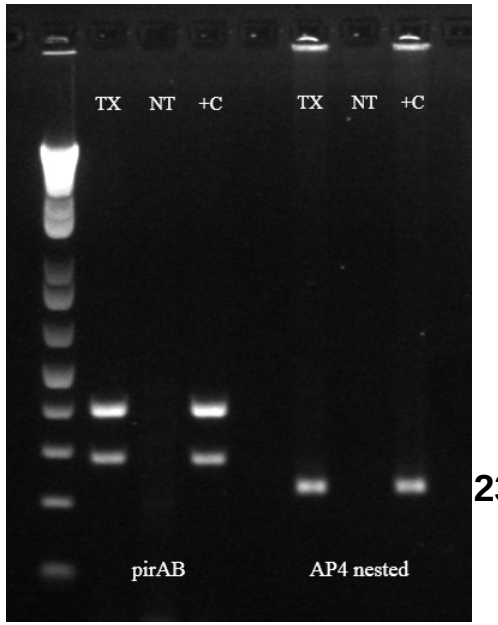
Detection of AHPND in Texas, USA

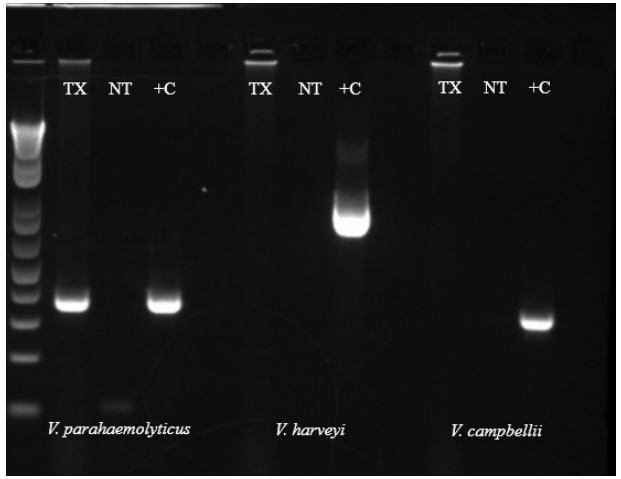
Unusual mortalities → Sample submission → PCR analysis for AHPND → Confirmatory tests for AHPND → Histopathology → Genotyping, Sequencing of Toxin Genes → Report submission

PCR Screening of TX-Samples



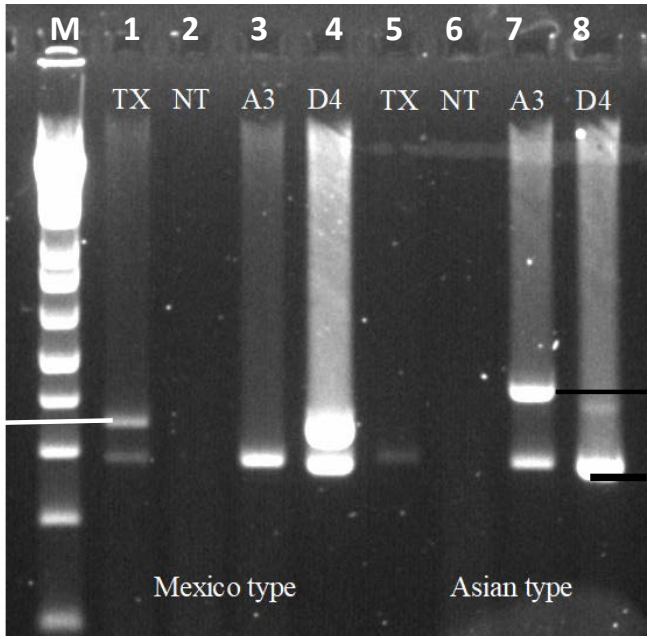
*pir*B 392 bp
*pir*A 284 bp
230 bp

Identification of *Vibrio sp.* *in* Texas samples



963 bp
368 bp

Genotyping Texas isolates of *V. parahaemolyticus*



345 bp
482 bp
*pir*A 284 bp

Dhar et al., 2018. Manuscript submitted.

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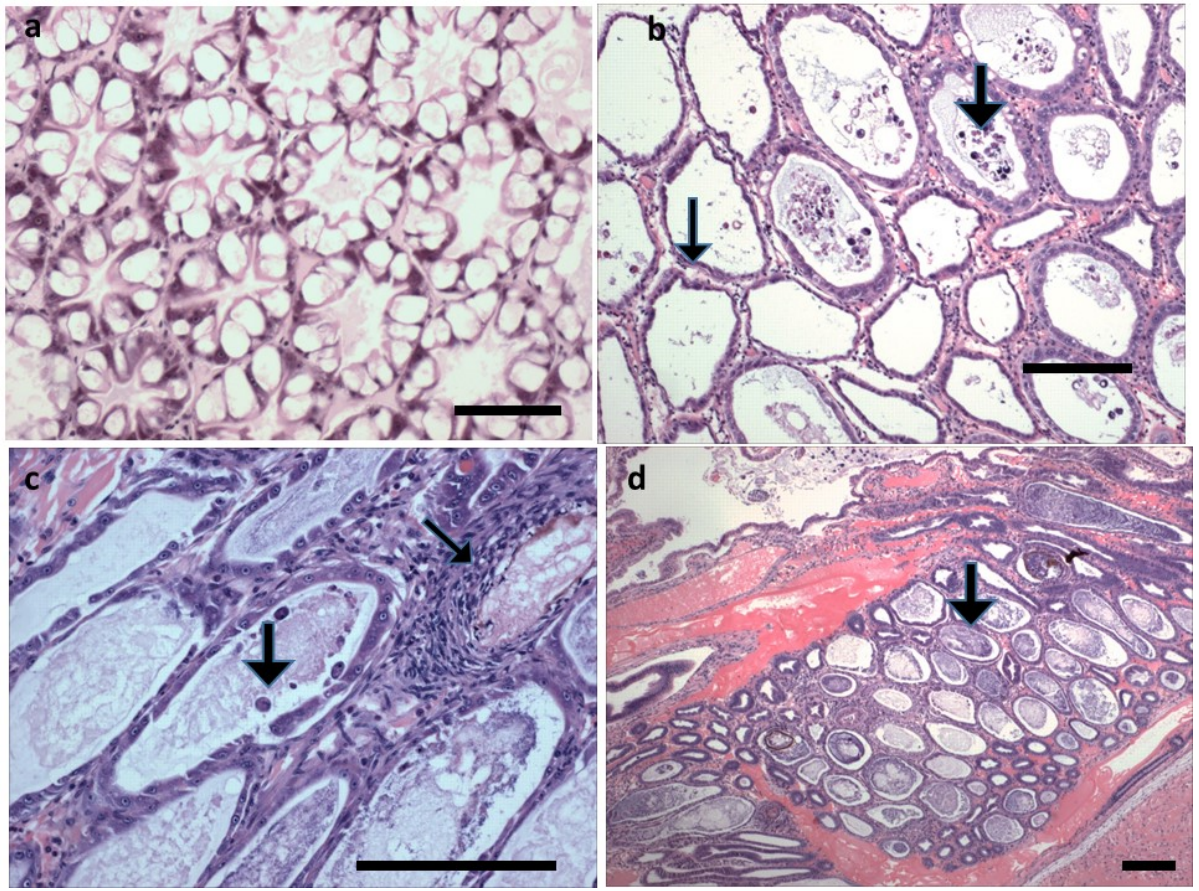
Histopathology of *P. vannamei*, Texas, USA samples



a) Cross section of a normal HP tubule epithelium.

(b) Acute sloughing of HP tubule epithelial cells (Large arrow). Atrophy of HP tubule is indicated (small arrow).

(c) Sloughing of HP tubules cells (Large arrow) and hemocytic inflammation (small arrow)

(d)AHPND terminal phase characterized by massive bacterial infection in association with the necrotic and sloughed tubule cells in the HP lumen (Arrow).

Scale bars= 100 µm.

Dhar et al., 2018. Manuscript submitted.

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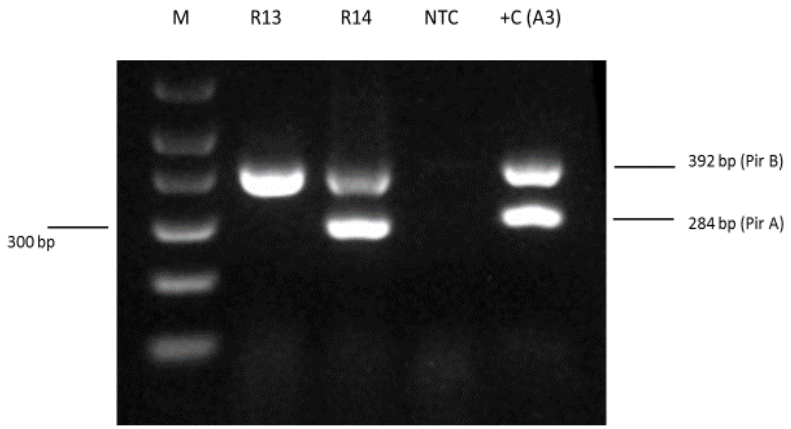
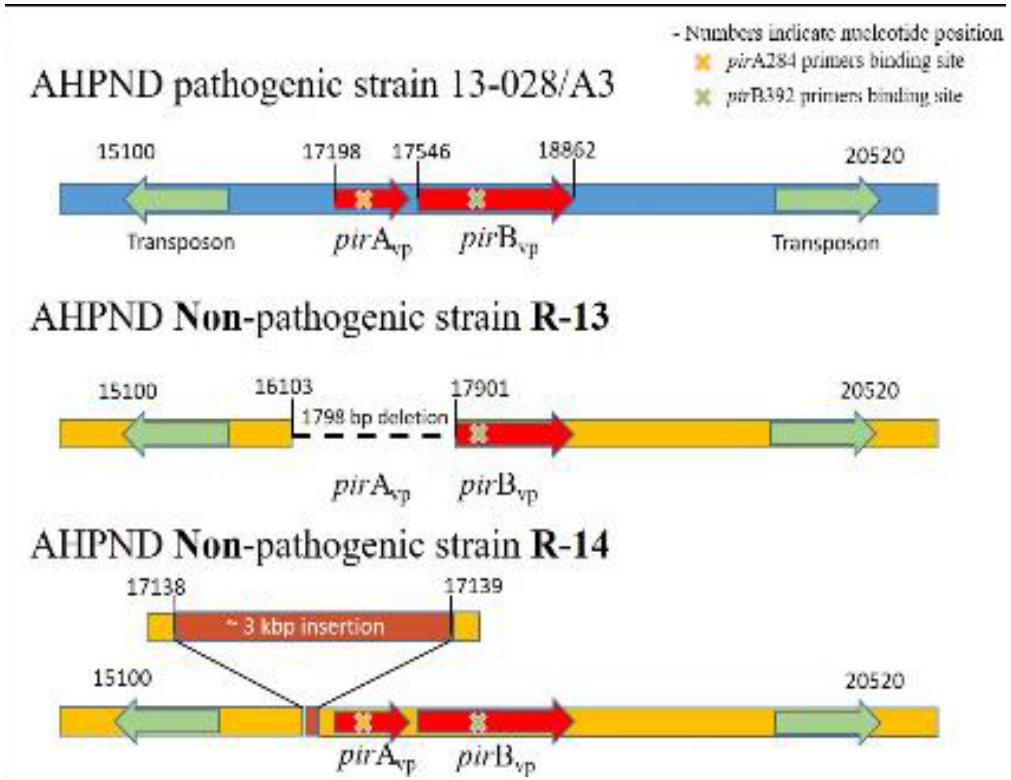
Novel strains of *V. parahaemolyticus* carrying toxin genes



A3: *pir A* (+)/ *pir B* (+)/ Pathogenic

R13: *pir A (-)/ pir B (+)/ Non-pathogenic*

R14: *pir A (+)/ pir B (+)/ Non-pathogenic*



- DNA-PCR alone can not confirm AHPND
- Confirm by Histopathology
- Bacterial Challenge Test

Kanrar & Dhar, 2018a, b. Genome Announcements
Aranguren et al., 2018. Manuscript In Prep.

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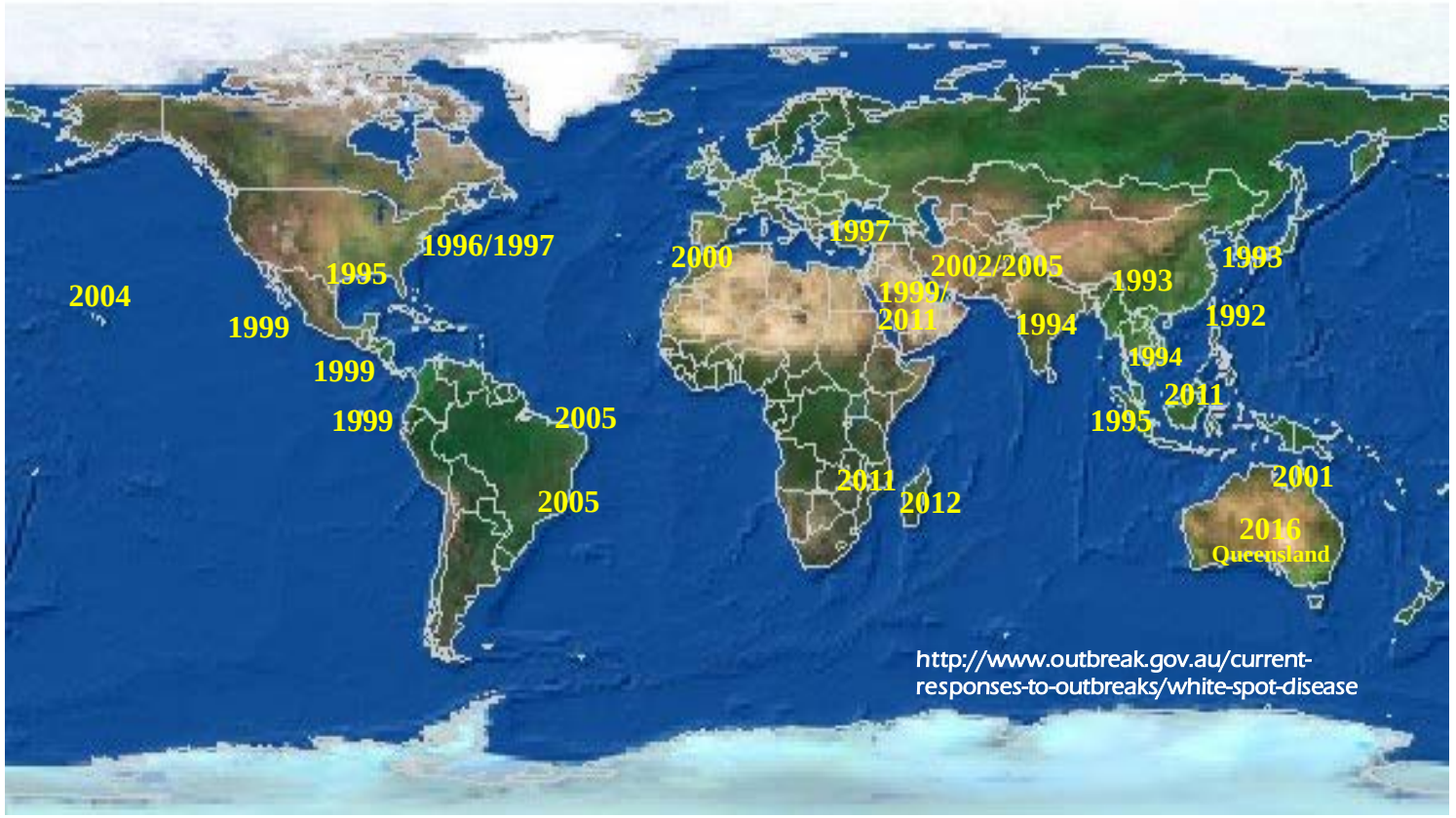
White Spot Disease Pandemic

(Year of First Occurrence by Location)



Recent WSD Outbreaks

- 2016: Australia
- 2013: Saudi Arabia- in *P. indicus*
- 2010-2012: Mexico- n *P. vannamei*
- 2012: Brunei– in *P. stylirostris*
- 2012: Madagascar – in *P. monodon*
- 2011: Saudi Arabia – in *P. indicus*
- 2011: Mozambique – in *P. monodon*

2018: WSD causing mortalities in Crayfish in Louisiana

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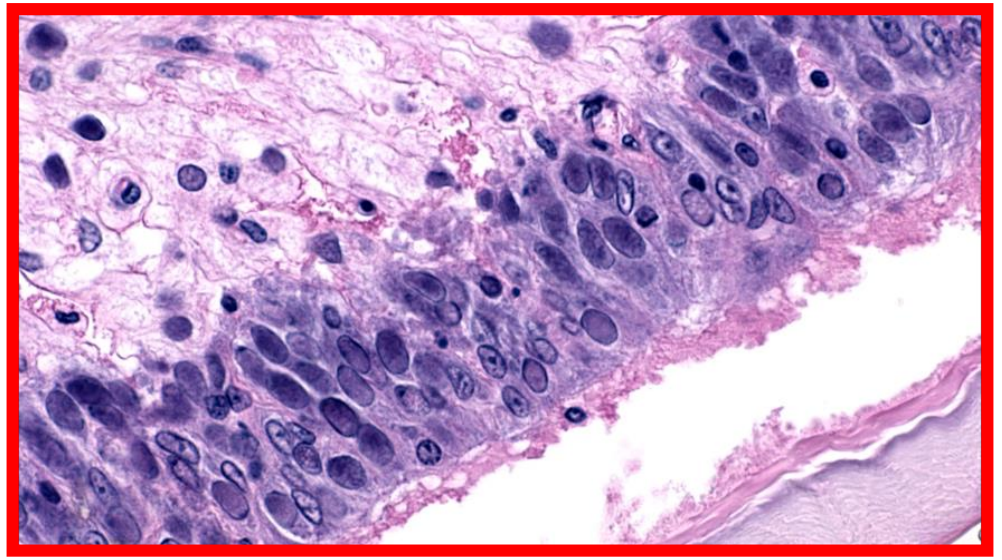
WSD: Clinical Signs, Histopathology & WSSV Morphology



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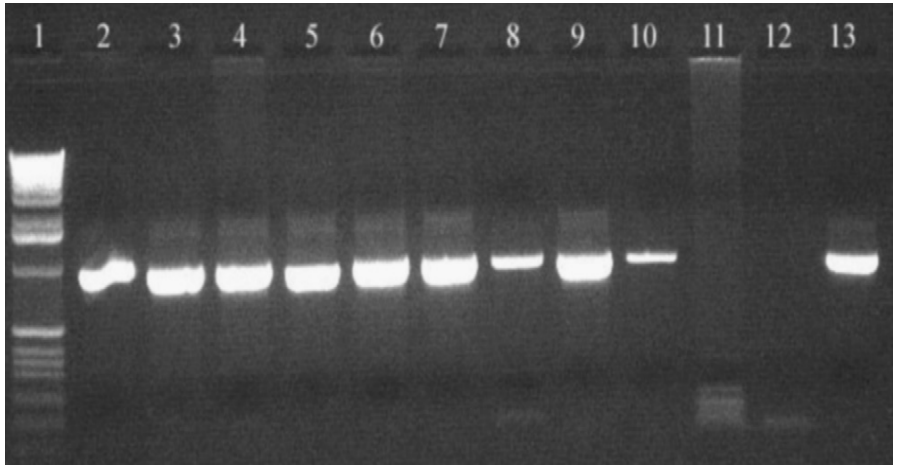
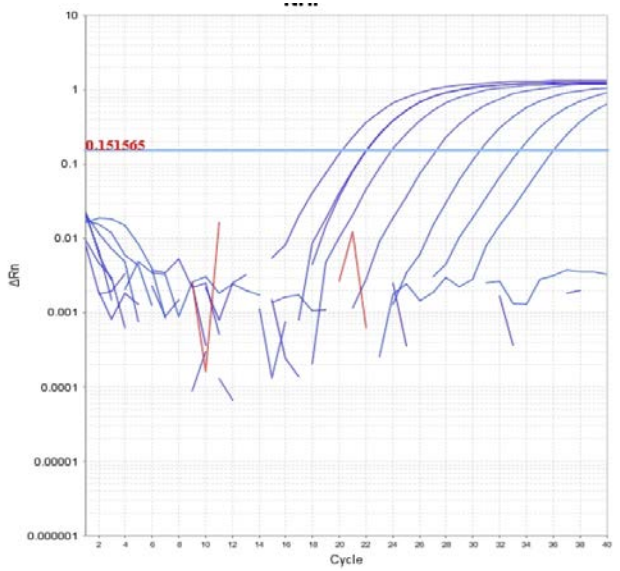
Molecular Detection & Genomic Properties of WSSV

- **WSSV Detection tools are available.**
- **WSSV has numerous hosts**
- **dsDNA circular genome, with 293-305 kb size.**
- **WSSV has number of genotypes but the relation between genotypes and virulence is not unequivocally established.**



942bp

WSSV Detection by PCR & qPCR



WSSV surveillance is needed in determining the emergence of any virulent strain.

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EHP: Clinical signs

- Severely retarded growth & causes "Size Variability".
- Enteric pathogen: Infects only the tubules of the hepatopancreas.
- Causes chronic mortality in severe cases.
- Transmitted by oral fecal route, contaminated water and cohabitation.
- EHP Infection increases susceptibility to AHPND and secondary vibriosis (Aranguren et al., 2016).

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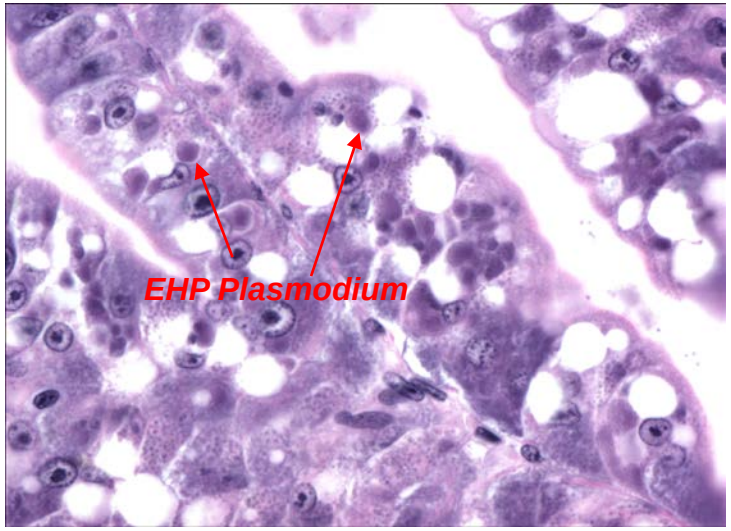
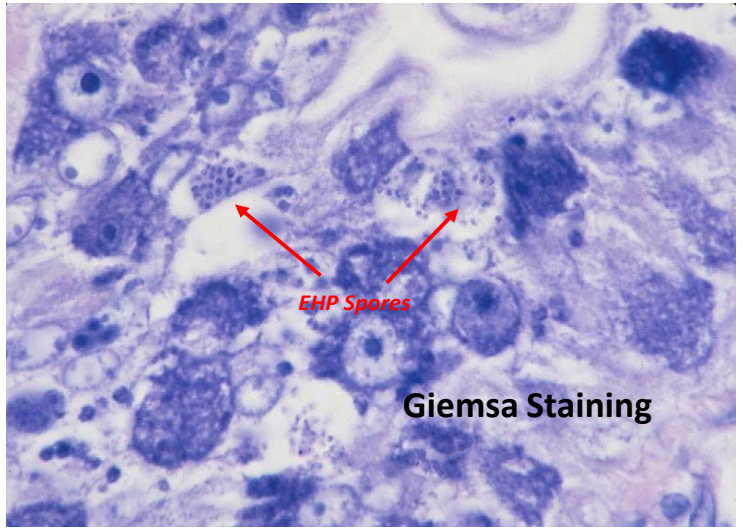
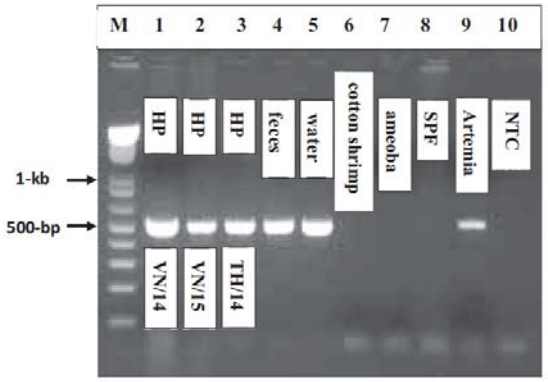
EHP Detection by Histology & PCR

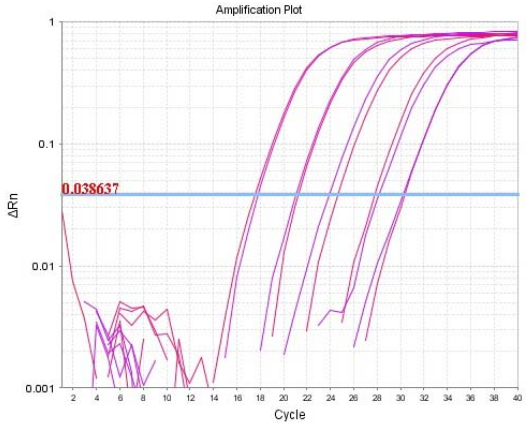
EHP detection By Histology







EHP detection in Hp, Feces and Tank Water by using 18S rRNA PCR.



EHP detection in Hp by qPCR

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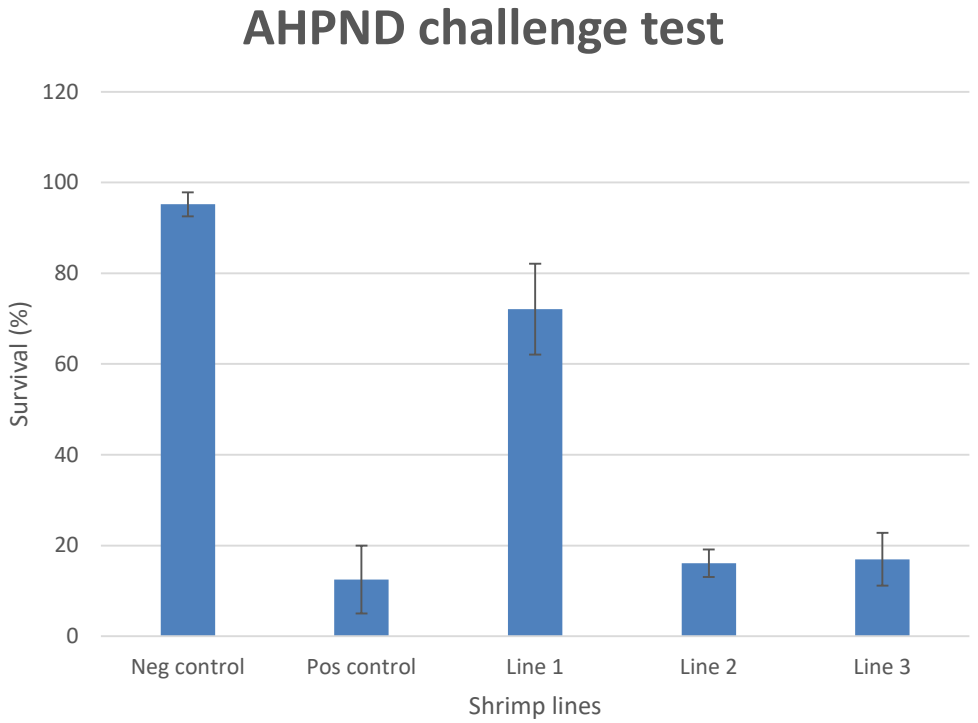
Perspectives in Disease Prevention & Management

- *Preventing pathogen entry in the culture system will continue to remain as a cornerstone in disease management.*
  - *This is critical for indoor shrimp farming.*
- *Use of SPF broodstock & Post-larvae*
- *Farming of disease resistant line e.g. AHPND-resistant line (when available).*



Aranguren et al., 2018.
Manuscript in preparation

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Perspectives in AHPND Management

- **Disease Prevention/ Management:**
  - Avoid high concentration of organic matter /sediment.
    - Water exchange to reduce organic matter.
  - Since biofloc that can competitively eliminate microbial pathogens, maintaining biofloc could be another avenue to managing the disease.

- **Probiotics, prebiotics, organic acids, immunostimulants & many other organic products can help to minimize the risk of pathogen introduction.**

- **Functional feed – containing disease therapeutics.**

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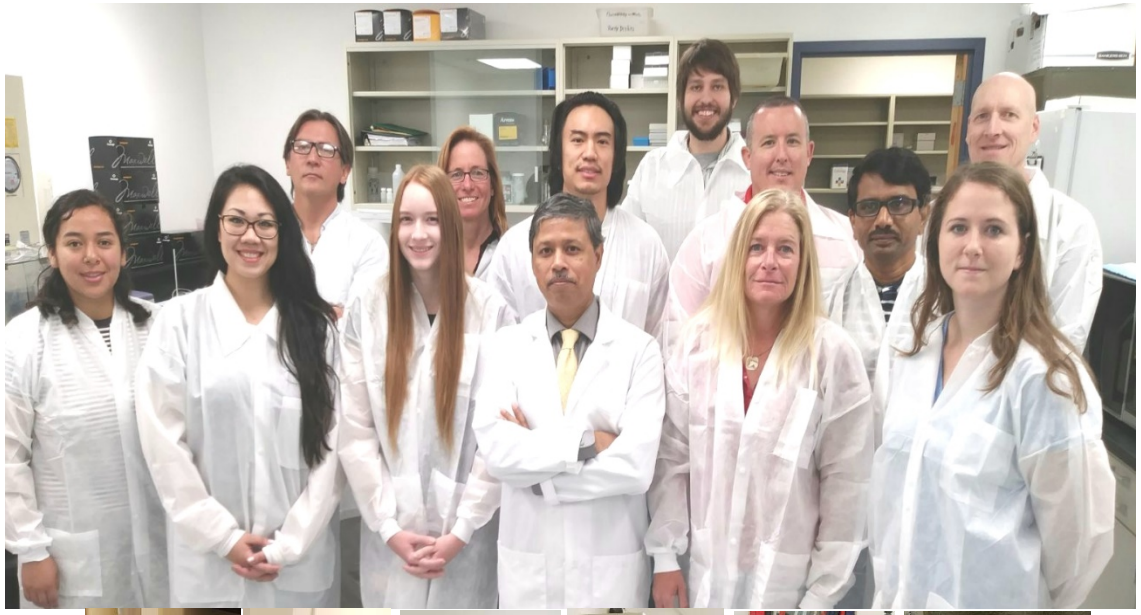

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Greg Lyons

Microbiology/Genomics Team:
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Graduate Students

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