Biosecurity & Health in US Indoor Shrimp Farming

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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
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
Two Campuses, One Lab

• Main Campus

• West Campus
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*
BIOSECURITY: Excluding Shrimp Pathogens in the Mixed of Microbial Milieu

GENETICS:
Genetically superior SPF/SPR stock

NUTRITION:
High Quality Balanced Diet

Healthy Harvest

Biosecurity:
Farm Management
Pathogen Exclusion = No Disease

Host (e.g. *Penaeus vannamei*)

Optimum Environment Temperature/salinity

Pathogen in water, wild population (crustaceans, plankton etc.)
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**
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- MrNV

Bacterial:
  • Necrotizing hepatopancreatitis bacterium-NHP-B
  • Acute hepatopancreatic necrosis disease-AHPND (listed in 2016)

• Fungal:
  • Crayfish plague- Aphanomyces astaci
Shrimp Viruses
According to OIE Aquatic Manual: Main recommended method for shrimp pathogen detection is PCR

<table>
<thead>
<tr>
<th>PATHOGEN</th>
<th>TARGETED SURVEILLANCE</th>
<th>Presumptive diagnosis</th>
<th>Confirmatory diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Larvas</td>
<td>PLs</td>
<td>Juvenile</td>
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<tr>
<td>YHV</td>
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<tr>
<td>IHHNV</td>
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<td>IMNV</td>
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<td>AHPND</td>
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<tr>
<td>NHP</td>
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<td>a</td>
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</tr>
<tr>
<td>TSV</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>WSSV</td>
<td>d</td>
<td>b</td>
<td>a</td>
</tr>
<tr>
<td>MBV</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>BP</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
</tbody>
</table>

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.
• 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.
Virulence Factor in AHPND: Toxin Genes ($pirA$ & $pirB$) in the Plasmid

Modified from Aranguren et al., 2015
AHPND Geographic Distribution

**Eastern Hemisphere**
- 2009 = China
- 2010 = Vietnam
- 2011 = Malaysia
- 2012 = Thailand
- 2015 = Philippines, Myanmar, Bangladesh

**Western Hemisphere**
- 2013 = Mexico
- 2017 = Texas, USA
AHPND: Clinical Signs

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

EMS in Sonora, Mexico
Photo by Ms. Silvia Gomez

Infected
Healthy
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 conjunction with necrotic & sloughed HP tubule cells.*
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 | - | US-TX
7 | 13-431/1 | - | India

Han et al., 2015
**AHPND Detection: TaqMan & SYBR Green qPCR**

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

**TaqMan assay for AHPND pirB gene detection**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’ to 3’)</th>
<th>Amplicon size</th>
<th>Target</th>
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<tbody>
<tr>
<td>VpPirA-F</td>
<td>TTGGACTGTCGAACCAAACG</td>
<td>135-bp</td>
<td>Real time PCR</td>
</tr>
<tr>
<td>VpPirA-R</td>
<td>GCACCCCATTGTATTGAATG</td>
<td>135-bp</td>
<td></td>
</tr>
<tr>
<td>TaqMan probea</td>
<td>AGACAGCAACACATACCTAT</td>
<td>135-bp</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CATCCCGGA</td>
<td>3462 nt</td>
<td></td>
</tr>
</tbody>
</table>

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

**Cruz et al., 2018. Manuscript in prep.**

Han et al., 2015
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

Identification of *Vibrio sp.* in Texas samples

Genotyping Texas isolates of *V. parahaemolyticus*

Dhar et al., 2018. Manuscript submitted.
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.*
Novel strains of *V. parahaemolyticus* carrying toxin genes

### AHPND pathogenic strain 13-028/A3

- **17103**
- **17501**

- **Transposon**
- **pirA**
- **pirB**

### AHPND Non-pathogenic strain R-13

- **15100**
- **17103**
- **1798 bp deletion**

- **pirA**
- **pirB**

### AHPND Non-pathogenic strain R-14

- **15100**
- **17000**
- **3 bp insertion**

- **pirA**
- **pirB**

**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.*
White Spot Disease Pandemic
(Year of First Occurrence by Location)

Recent WSD Outbreaks

- 2016: Australia
- 2013: Saudi Arabia – in *P. indicus*
- 2010-2012: Mexico – in *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

WSD: Clinical Signs, Histopathology & WSSV Morphology
• 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.

WSSV surveillance is needed in determining the emergence of any virulent strain.
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).
EHP detection in Hp, Feces and Tank Water by using 18S rRNA PCR. 

EHP detection by Histology & PCR
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).

AHPND challenge test

Aranguren et al., 2018. 
Manuscript in preparation
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.**
Aquaculture Pathology Laboratory

Director and P.I. of the Lab- Dr. Arun K. Dhar

**Main Campus Aquaculture**

**Histopathology Team:**
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**Graduate Students**
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