DISEASE CONTROL THROUGH MANAGEMENT OF THE WHOLE PRODUCTION CYCLE

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Farm & Feedmill Product Manager
DISEASE CONTROL IS A FOCUS POINT

- Global production loss estimates:
  - Viruses ~65%
  - Bacteria ~20%
  - Viruses are the most important disease agents by almost 3:1 over bacterial diseases.

Since 2009, AHPND has resulted ~$1.0 billion losses in Asia.

Dhar, 2017
HOW TO HANDLE DISEASES?

- Know your enemy: example of EMS/AHPND
- Understand your enemy
- Know your enemy: example of EMS/AHPND
- Understand your enemy

<table>
<thead>
<tr>
<th>Species</th>
<th>Signals</th>
<th>Quorum sensing-regulated virulence factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aeromonas hydrophila</td>
<td>BHL, HHL</td>
<td>biofilm formation, exoprotease production</td>
</tr>
<tr>
<td>Edwardsiella tarda</td>
<td>probably HHL and HeHL</td>
<td>“virulent strain-specific protein” extracellular protease production, haemolytic activity and siderophore production, lethality to Pagrus major unknown</td>
</tr>
<tr>
<td>Vibrio alginolyticus</td>
<td>AI-2</td>
<td></td>
</tr>
<tr>
<td>Vibrio anguillarum</td>
<td>ODHL, AI-2 and at least 2 other AHLs</td>
<td></td>
</tr>
<tr>
<td>Vibrio campbellii</td>
<td>HAI-1, AI-2 and CAI-1</td>
<td>lethality to brine shrimp</td>
</tr>
<tr>
<td>Vibrio harveyi</td>
<td>HAI-1, AI-2 and CAI-1</td>
<td>siderophore production, production of type III secretion system components, extracellular toxin production, metalloprotease production, lethality to brine shrimp and rotifers</td>
</tr>
<tr>
<td>Vibrio mimicus</td>
<td>AI-2</td>
<td>protease</td>
</tr>
<tr>
<td>Vibrio parahaemolyticus</td>
<td>HAI-1, AI-2 and CAI-1</td>
<td>production of type III secretion system components, lethality to brine shrimp unknown</td>
</tr>
<tr>
<td>Vibrio scophthalmi</td>
<td>AI-2, OHdDHL</td>
<td></td>
</tr>
<tr>
<td>Vibrio vulnificus</td>
<td>AI-2, BHL</td>
<td></td>
</tr>
<tr>
<td>Yersinia ruckeri</td>
<td>OOHL, OHL</td>
<td></td>
</tr>
</tbody>
</table>
HOW TO HANDLE DISEASES?

- Know your enemy: example of EMS/AHPND
- Understand your enemy

Laboratory analysis of samples collected in 2014

<table>
<thead>
<tr>
<th>Types of sample</th>
<th>AHPND Plasmids</th>
<th>AHPND Toxin gene</th>
<th>WSSV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total sample</td>
<td>% positive</td>
<td>Total sample</td>
</tr>
<tr>
<td>Broodstock</td>
<td>73</td>
<td>0</td>
<td>73</td>
</tr>
<tr>
<td>Broodstock feces</td>
<td>27</td>
<td>19</td>
<td>27</td>
</tr>
<tr>
<td>Nauplii</td>
<td>53</td>
<td>0</td>
<td>53</td>
</tr>
<tr>
<td>Post larvae</td>
<td>2174</td>
<td>11</td>
<td>1363</td>
</tr>
<tr>
<td>Juvenile</td>
<td>1490</td>
<td>28</td>
<td>1261</td>
</tr>
<tr>
<td>Farm water</td>
<td>3116</td>
<td>29</td>
<td>2166</td>
</tr>
<tr>
<td>Hatchery water</td>
<td>542</td>
<td>19</td>
<td>23</td>
</tr>
<tr>
<td>Farm sediment</td>
<td>1614</td>
<td>36</td>
<td>1054</td>
</tr>
<tr>
<td>Others</td>
<td>33</td>
<td>21</td>
<td>15</td>
</tr>
</tbody>
</table>

ND = not detect.

Tim Flegel (2014)
Putth Songsangjinda (2016)
HOW TO HANDLE DISEASES?

- Know your enemy
- Understand your enemy
- Genes associated with toxin production jumping from 1 species to another
- Mechanisms of communication between Vibrio and other bacteria may interfere with virulence mechanisms
- Distribution/location in hatchery/farm
MANAGEMENT DURING CYCLE

- Identify points of entry (SWOT)
- Close point of entry or reduce dramatically
HOW TO HANDLE DISEASES?

- Establish prophylactic protocols to prevent/reduce disease
- Establish protocols to cure or reduce disease
- Establish protocol to control spread of disease
- Understand lack of efficacy and risks associated with past protocols

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Aquaculture Reports
journal homepage: www.elsevier.com/locate/aqrep

Short communication
Plasmid mediated tetracycline resistance of *Vibrio parahaemolyticus* associated with acute hepatopancreatic necrosis disease (AHPND) in shrimps
Jee Eun Han *, Leone L. Mohney, Kathy F.J. Tang, Carlos R. Pantoja, Donald V. Lightner
School of Animal and Comparative Biomedical Sciences, University of Arizona, Tucson, AZ, USA
MANAGEMENT DURING CYCLE

Broodstock:
- SPF animals (for existing pathogens including WSSV, AHPND and EHP)
- Disinfection of water and facilities, including low pH treatment (vs EHP) and efficient biocides (vs biofilm of Vibrio)
- Use of confirmed pathogen-free feed/food. Focus on certified/registered diet
- Water treatment to control accumulation of organic waste (vs Vibrio, including AHPND, and EHP)

Risk factors
The most important risk factors for the international spread of AHPND are:
- Movement of live shrimp from a geographic region where AHPND is prevalent to an unaffected region for aquaculture (AHPND is thought to have been transmitted to Mexico from Asia by this route).
- The importation of live animals (e.g. polychaetes, clams) as feeds for shrimp broodstock (polychaetes imported from P.R. China may have been the major route for introduction of AHPND to Thailand).
Hatchery – stocking of nauplii:
- To reduce Vibrio outbreak, disinfect water and recolonize prior to stocking
- To reduce EHP (possibly transferred from spawning tank), transfer with minimum amount of water from the spawning tank, as nauplii mouths are closed
Hatchery – during larval rearing:
- Use quality feed to improve growth and reduce the accumulation of organic waste
- Control the entry of bacterial pathogens via algae and artemia
- Use microbial products to degrade organic waste
- Use microbial product to compete with bacterial pathogens
MANAGEMENT DURING CYCLE

Control of vibrio during culture, using probiotic treatment

Control of Vibrio at harvest, using disinfectant or plant-based treatment

<table>
<thead>
<tr>
<th>Application</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nauplii disinfection</td>
<td>3 dips (max 45 seconds) in 40ppm Sanocare PUR bath, before stocking in larval tank</td>
</tr>
<tr>
<td>PL4 transfer</td>
<td>Dip PL4 for max 1 minute in 10-15ppm Sanocare PUR bath</td>
</tr>
<tr>
<td>PL10 harvest</td>
<td>Dip PL4 for max 1 minute in 10-15ppm Sanocare PUR bath</td>
</tr>
</tbody>
</table>
Control of Vibrio in harvested algae (30 min biocide treatment)

Control of Vibrio in Artemia (treatment during hatching)

Control of Vibrio in biofilm
MANAGEMENT DURING CYCLE

Hatchery – preparation for transfer to nursery:
- Use health booster to strengthen PLs before transfer
- “Colonize” the gut of PLs with good bacteria prior to transfer
- Reduce the Vibrio load before transfer

Sanocare FIT applied in the culture water during 24h PL10 transport.

- Drastic reduction of *Vibrio* in transport water and shrimp.
MANAGEMENT DURING CYCLE

Hatchery – preparation for transfer to nursery:
- Use health booster to strengthen PLs before transfer
- “Colonize” the gut of PLs with good bacteria prior to transfer
- Reduce the Vibrio load before transfer

More robust shrimp by stimulating protein chaperones

Loss of production due to bacterial and viral infections is a recurring problem in shrimp aquaculture. The fact that shrimp mortality is often perceived as uncontrollable, is mainly due to a lack of a holistic approach and tools in developing control strategies which assists in preventing diseases. Only focusing on the pathogens by biosecurity management does not suffice, monitoring and controlling stress is critical as well. As stressed animals are more susceptible to diseases, both the environment and the host’s defense have to be measurably improved.
PL quality ➔ effect on performance in grow-out
**MANAGEMENT DURING CYCLE**

Nursery
- Use quality diet to improve growth and reduce waste
- Apply microbial product to degrade wastes and control Vibrio
- Use health booster to strengthen PLs before transfer
MANAGEMENT DURING CYCLE

Nursery
- Use quality diet to improve growth and reduce waste
- Apply microbial product to degrade wastes and control Vibrio
- Use health booster to strengthen PLs before transfer and bacterial challenge

Disease challenge test in collaboration with Can Tho University (Vietnam)
MANAGEMENT DURING CYCLE

Nursery
- Use quality diet to improve growth and reduce waste
- Apply microbial product to degrade wastes and control Vibrio
- Use health booster to strengthen PLs before transfer and bacterial challenge

MANAGEMENT DURING CYCLE

Ongrowing
- Adapt farm design/management to conditions
Ongrowing
- Adapt nursery and ongrowing protocols
Ongrowing
- Establish most cost efficient and predictable protocols

(Poulain, 2017)
MANAGEMENT DURING CYCLE

Ongrowing
- Control organic waste, using microbial products alone or in combination with oxidizing agents

Biochemical Methane Production model at LabMET

(INVE Technologies and University of Ghent)
Ongrowing
- Control organic waste, using microbial products alone or in combination with oxidizing agents

(INVE Technologies and University of Ghent)
MANAGEMENT DURING CYCLE

Ongrowing
- Control organic waste, using microbial products alone or in combination with oxidizing agents
Ongrowing
- Control Vibrio load in the sediment/water, using probiotics that are and remain active in the farm conditions

<table>
<thead>
<tr>
<th>Species</th>
<th>Species code</th>
<th>Doubling time (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>INVE Bacillus 1</td>
<td>LMG S-26827</td>
<td>20.4</td>
</tr>
<tr>
<td>INVE Bacillus 2</td>
<td>LMG S-23090</td>
<td>35.3</td>
</tr>
<tr>
<td>INVE Bacillus s 2</td>
<td>LMG S-24845</td>
<td>43.1</td>
</tr>
<tr>
<td>Vibrio harveyi</td>
<td></td>
<td>38.4</td>
</tr>
<tr>
<td>Vibrio parahaemolyticus</td>
<td></td>
<td>27.5</td>
</tr>
</tbody>
</table>

→ Less Vibrio in the sediment

Santos et al. 2009.
MANAGEMENT DURING CYCLE

Ongrowing
- Control Vibrio load in the shrimp gut

Bacillus in feed

displace Vibrio from shrimp gut

➔ Improved survival with AHPND
MANAGEMENT DURING CYCLE

Ongrowing
- Reduced Vibrio and improved utilization of nutrients

➤ Better growth and FCR
MANAGEMENT DURING CYCLE

- Prevent pathogen transfer from one phase to the other
- Incorporate each improvement in the whole cycle
- Consider nutrition, health and environment
OBRIGADO