Evaluating the performance of a bacterin, AquaVac Vibromax in farmed shrimp-
Challenges and progress.

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MSD Animal Health Aquaculture

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- M Aguirre, J Gomez, R Zanolo, S Puckiao, P Kancham, A Madathen- SPAH

Abstract

- Evaluating the performance of a bacterin, AquaVac Vibromax in farmed shrimp-
  Challenges and progress.
  This short paper will present some of the research and development work that has been
  carried out by MSD Animal health and its partners around the world investigating the
  possibilities for harnessing the “immune system” of shrimp to help control or prevent
  Vibrosis caused by several species and strains of vibrio pathogens including V.
  parahaemolyticus.
- The talk will illustrate some of the challenges that confront the research work in creating
  and measuring an effective response in shrimp from both a laboratory and field trials
  perspective. The objective being to stimulate discussion on the power of immunity to
  help shrimp farmers control these ubiquitous disease challenges.

Vibriosis in Shrimp

- EMS- early Mortality Syndrome- V parahaemolyticus
- One month mortality syndrome- 4-6 weeks after stocking ponds- few specific symptoms but high mortality
- Black/Brown or Burn spot- lesions and erosion of cuticle
- Black splinter disease- black lesions in muscles
- Black gill disease
- Septic hepatopancreatic disease
- Luminescent vibrio disease- hatchery
- Bolitas- gut infection and damage
- Sea Gull syndrome-

Challenges to immunization:

- What antigens/ components and how to deliver them to shrimp larvae.
- What response provides protection
- What is the nature of the protection- specific or non specific.
- How to measure It-
  - Lab
  - Field
- Is the response economically valuable to the shrimp farmers?
Aquavac Vibromax Administration method

• Cultivate Artemia (34 hrs) Instar II
• Preparation of the bacterin
• Bio-encapsulation process (2 – 4 hours)
• Artemias enriched with Aquavac Vibromax
• Harvest the Artemia
• Feeding the PL’s to be “activated”

Evidence of specific immunity

• Field observations & Clinical trials
• Challenge and infection work conducted at Chulalonghorn University
• In vitro antibacterial assay developed at Swansea University

Efficacy study of AquaVac/Plus in post larval stage of Black tiger shrimp Penaeus monodon

Assoc. Prof. Dr. Janenuj Wongtavatchai
(E.V.M,MS.,PhD)
Department of Medicine, Faculty of Veterinary Science, Chulalonghorn University, Bangkok, Thailand November, 2006

Challenge and infection studies – Chulalonghorn University

• The study was designed to evaluate the performance of “activated PL’s” in the face of Vibrio challenge.
• Activated and non Activated PL’s of Black Tiger and White prawns were exposed to Vibrio challenge cultures 1 and 7 days after treatment.
• Mortality and infection rates were monitored and compared in controls and activated PL’s.

\[
\text{Survival Rate of PL's Black Tiger Shrimp after challenge with } L.\text{-}a-xD^{5} CFU/ml. \text{ Vibrio parahaemolyticus on day 1 and 7 after feeding with AquaVac Vibromax-enriched } \text{artemia}
\]

• Survival of PL’s are higher in group fed with AquaVac Plus – enriched Artemia than group fed with control Artemia, observed within the parallel treatments (α = 0.05).
Efficacy study in post larval stage of Black Tiger Shrimp *Penaeus monodon* (Thailand Prof Janenuj)

Total Vibrio count (cfu per gram of Black Tiger PL) after challenge

<table>
<thead>
<tr>
<th></th>
<th>Challenge: 1 day post-vaccination (PL15)</th>
<th>Challenge: 7 days post-vaccination (PL21)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AquaVac Vibromax activated PL's</strong></td>
<td>38.3</td>
<td>101.6</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td>49.0</td>
<td>101.1</td>
</tr>
</tbody>
</table>

Total Green colony count (cfu per gram of Black Tiger PL) after challenge

<table>
<thead>
<tr>
<th></th>
<th>Challenge: 1 day post-vaccination (PL15)</th>
<th>Challenge: 7 days post-vaccination (PL21)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AquaVac Vibromax activated PL's</strong></td>
<td>6.2</td>
<td>43.3</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td>10.3</td>
<td>24.3</td>
</tr>
</tbody>
</table>

Antibacterial Assay:
In Vitro evaluation of response in White shrimp

- Swansea: Professor Rowley’s group specializes in invertebrate immunology and comparative immunology.
- Investigating the specific elements that could account for the observed effects of Aquavac Vibromax
- Shrimp homogenates were prepared from Activated PL's
- Incubated with Vibrio cultures and bacterial growth was studied.
- Similar to a serum killing Assay

Efficacy study in White shrimp

- Growth of *Vibrio anguillarum* incubated with shrimp homogenates

<table>
<thead>
<tr>
<th></th>
<th>Absorbance (bacterial growth)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Untreated PL's</strong></td>
<td></td>
</tr>
<tr>
<td><strong>AquaVac Vibromax Activated PL's</strong></td>
<td></td>
</tr>
</tbody>
</table>

Size Distribution of Harvest Weight

<table>
<thead>
<tr>
<th></th>
<th>Normal Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (kg)</td>
<td>10.9367</td>
</tr>
<tr>
<td>Std Dev (sigma)</td>
<td>0.5306</td>
</tr>
</tbody>
</table>

Key Benefits.

- Increase in PL quality: weight/length and survival
- Increased rates of growth
- Increase in survival depending upon the sanitary conditions.
- Improved feed conversion ratios
- More uniform growth
Conclusions

- The knowledge does exist to develop and use vaccines in shrimp
- AquaVac Vibromax is the first widely tested product that shows the benefits of vaccinating shrimp
- The specific mechanisms are becoming understood—but there is more science to be done!

Additional info:

- Controlled Field study conducted at the CENAIM research laboratory in Ecuador
  - PL’s fed with Aquavac Vibromax enriched Artemia
  - Transferred to triplicate rearing ponds and monitored on a daily basis.
  - Data relating to survival, growth feed conversion and size distribution were collected.

Harvested biomass

<table>
<thead>
<tr>
<th>Harvest Biomass per Hectare</th>
<th>AquaVac Vibromax activated PLs</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Biomass lbs/ha</td>
<td>1714.25</td>
<td>1388.75</td>
</tr>
<tr>
<td>Standard deviation (n=4)</td>
<td>190.62</td>
<td>152.77</td>
</tr>
<tr>
<td>Difference</td>
<td>325 lbs/ha</td>
<td>23%*</td>
</tr>
</tbody>
</table>

*P=0.0375 t-test result considered statistically significant

Average size

<table>
<thead>
<tr>
<th>Average individual weight</th>
<th>AquaVac Vibromax activated PLs</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean weight (grams)</td>
<td>12.1150 g</td>
<td>10.6325 g</td>
</tr>
<tr>
<td>Standard deviation (n=4)</td>
<td>0.3820</td>
<td>0.5351</td>
</tr>
<tr>
<td>Difference</td>
<td>1.525g or 15%*</td>
<td></td>
</tr>
</tbody>
</table>

*P=0.00030 t-test result considered very statistically significant

Survival

<table>
<thead>
<tr>
<th>Average Survival</th>
<th>AquaVac Vibromax activated PLs</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival %</td>
<td>64%</td>
<td>60%</td>
</tr>
<tr>
<td>Standard deviation (n=4)</td>
<td>6.99</td>
<td>7.62</td>
</tr>
<tr>
<td>Difference</td>
<td>4%*</td>
<td></td>
</tr>
</tbody>
</table>

*P=0.4425 which is not statistically significant
Feed Conversion

<table>
<thead>
<tr>
<th>Feed Conversion Rates</th>
<th>AquaVac Vibromax activated PLs</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCR</td>
<td>1.26</td>
<td>1.445</td>
</tr>
<tr>
<td>Standard deviation (n=4)</td>
<td>0.1577</td>
<td>0.1777</td>
</tr>
<tr>
<td>Difference</td>
<td>0.185</td>
<td>(12.8%) *</td>
</tr>
</tbody>
</table>

*p=0.1704 which is not considered highly significant.

Conclusions

- Vibromax improves the response against Vibrio species in shrimp and, more specifically, against the V. parahaemolyticus strain used in the experimental challenge.
- Shrimp homogenates showed natural antibacterial activity against Vibrio anguillarum.
- Vibromax increased the antibacterial activity toward some pathogenic bacteria.
- AquaVac Vibromax activated PL’s are able to resist the negative effects of Vibrio infections on growth, FCR resulting in improved production.

History of Shrimp Vaccination

- Many reports with bacterins and protein antigens
- Specific mechanisms
- Duration of protection?
- Memory?
- Evolutionary status of Shrimp means a limited immune system.