Early Mortality Syndrome
(AHPNS/AHPND)

Part I
Aquapro product line
Part II

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Nasty disease with dire consequences

Note discolored HP
Early Mortality Syndrome/AHPNS

Strains of *Vibrio parahaemolyticus* are responsible

Green colonies on TCBS

Virulence may be plasmid mediated with possibly more than one plasmid involved. Why is this important?
Microbiology 101-agar media

Bacteriologists use media that is selective to make it easier to find and identify bacteria that are of interest.

- Sometimes the difference in the ability to grow is based on some metabolic difference.

A good example is TCBS (thiosulfate-citrate-bile salts-sucrose agar). This media is green and when vibrios grow on it they either excrete an organic acid that is a metabolite of sucrose that causes the colony and the surrounding area to turn yellow or they do not and they remain green.

There are many species of bacteria that grow on this media, not just vibrios.
Microbiology 101-agar media

Color difference is because of the ability to use one sugar, sucrose. This is not related to virulence in any way.

Yellow vibrios can cause nasty diseases V. cholerae, V. alginolyticus, V. harveyi,

Many greens cause no disease.
Microbiology 101  What is a plasmid?

Can code for toxins, virulence mechanisms, biofilm formation, antibiotic resistance, etc.

A piece of circular DNA that is not part of the main genome.

– Easily transmitted in whole or in part between bacterial species. Plasmid DNA is promiscuous.

– Unique to individual species typically
Microbiology 101-plasmids

- Bacterial DNA
- Plasmids
- Cell replication
- Integrated plasmid
- Plasmid integration
- Cell replication
Microbiology 101- vibrio background

• > 100 vibrio strains characterized so far
  – They inhabit principally aquatic ecosystems
• \textit{Vibrio parahaemolyticus} is a common inhabitant of marine ecosystems
• Most strains are benign
Microbiology 101 - vibrio background

Very small percentage cause illness

Vibrios affect many different species.

They are the major cause of food poisoning from eating wild caught contaminated seafood

Some species can infect and kill humans (cholera).
Acute hepatopancreatic necrosis syndrome (AHPNS)

• More widely known as Early Mortality Syndrome (EMS) although many things can cause early mortality

• Does not appear to be highly virulent in non-stressed healthy animals under controlled conditions

• Huge impact to date with no sign of improvement on the horizon
AHPNS first reported in 2009 in China

Probably in India as well, although still not 100% sure

Mexico affected with bacteria spreading south by currents.

No reported disease though yet. Do not expect anybody to be forthcoming.

Possible disease has occurred elsewhere before 2009
Symptoms are obvious

Note discolored HP
Bacteria colonizes the stomach and excretes a toxin that destroys the HP.
Biofilm on chitinous surface internal
What have we learned about EMS from field observations

*Disease has not been reported or observed in broodstock.* There are reports that it has been found in broodstock although this is likely fecal contamination which is a common route of egg and nauplii contamination.

*Disease has not been reported in the hatchery,* although the pathogen has been found in the hatchery-typically requires enrichment as PCR does not detect it.

- Very low level of infection
- Some isolates have been reported to be PCR positive but not virulent
Some additional interesting observations

- It can infect and kill most life stages in bioassays.
- Disease does not appear to occur at very low salinities. Below 5 it is less common and below 3 rare if it occurs at all. This could change.
- Prefers warmer water temperatures.
- When animals are held in nursery tanks prior to stocking they do not develop the disease. Immediately post stocking they do. They can die in three to eight days.
- When animals are ill and they are taken off food the mortality slows down and stops until they are put on feed again.
- The virulence of the bacteria seems to vary. It is stress related or even possibly there may be more than one bacterial isolate involved.
- Using well water seems to prevent the problem.
Even more interesting observations

• There are instances where VP is present and animals are not ill
• Role of nutrients in the growth of the bacteria remains to be determined but there appears to be a link between highly nutrient enriched environments and the development of the disease.
• Strong indications that there may be concentrators operating at some level; bryozoans that concentrate the bacteria. When the shrimp eat carriers they can ingest large loads. We do not know much about the where of this in farmed ponds.
What can Ecuador do?

• Screen and confirm virulence if PCR shows positives. Use all the primers that are available for screening.

• Enact strict biosecurity measures at maturation facilities and in the hatcheries. PLs not a major source of infection. Be vigilant.

• Consider modifying existing paradigm
  – Tilapia in reservoirs
  – Closed systems
  – Better filtration of water
Production paradigm shift(s)

Stock shrimp in raceways and stock on farm as juveniles

Another possible paradigm shift

Switch species as *P. monodon* is refractory to EMS. *P. monodon* (SPF) are well established in the Gulf of Mexico and off the east coast of the US south into Brazil.
Other possible paradigm shifts

Biofloc based culture systems that exclude the bacteria (not a consistent effect)

Co-culturing with tilapia QSI affect, not consistent
Super high density indoor production systems with minimal exposure to the external influences of the environment

Small (1000 meter square) stocked at super high densities 500 plus Post larvae per square meter. Yields are equivalent to 60 to 70 MTs per ha.

Could offer best long term strategy
Aquapro product line for water quality improvement and stress reduction of farmed shrimp and fish

Stephen G. Newman Ph.D.
President and CEO
Aquaintech Inc.
Water Quality Improvement

• Aquapro water quality management tools for bioremediation and bioaugmentation of shrimp and fish production environments
• Critical for sustainability
• Lessen stress
• Improve animal resistance to disease
• Provide a cleaner healthier environment
Stress what is it?

• Production of animals in environments that impact the animals physiology in a manner that is inconsistent with optimum growth, feed conversions and sustainable animal health

• Goal of any shrimp farmer should be to minimize stress on shrimp
• LOW stress = HIGHER productivity

• HIGHER productivity = HIGHER profits

• HIGHER profits = Happier shrimp farmers
Many tools

• Management of stress requires the use of many different tools
• Most important is aeration. Inadequate oxygen level is the largest stressor of shrimp.
• Clean environment free of metabolites is second. Shrimp produce metabolites such as ammonia, feces (organic material) and molts
Aquaintech with a history of providing Tools for sustainable aquaculture based on biotechnology

- PenStim, DS-1999, Mega X  Single cell protein that benefited shrimp. Inexpensive and field proven in many billions of PLs.
- Cell wall extracts to increase stress tolerance such as LPS, glucans, SCP, etc.
- Aquapro B  only patented product; in use since the 1980’s
How our products can help

Cleaner Pond Bottoms

= Complete reduction of ammonia
  Shorter time between cycles
  Less lime needed to oxidize organic material
  Lower levels of hydrogen sulfide
  Less water exchange required

Increased Survivals
Less stress
Lower levels of vibrios
Less blue green algae
Eliminate antibiotics

Better Growth

Excellent cost benefit due to reduced costs of production as a result of improved quality of environment
Aquapro B product line

• History
  – In the late 1980’s we started working with various microbial preparations designed to clean up a variety of environments
  – We conducted testing of a series of these products in catfish farms (patented) and shrimp farms
  – Only patented product
Four primary Products

All Bacillus based

Why Bacillus?

Long term shelf stability due to spore formation. No refrigeration is required

Broad range of enzymatic and metabolic activities

Naturally occurring and safe to use

Proprietary strains with proven track record for improving water quality and increasing productivity

Compete against other bacteria including vibrios and blue green algae
Microbiology 101
why bacillus species?

Bacillus refers to a specific cell shape. The genus refers to a large group of spore forming bacteria that prefer aerobic environments.

Lactobacillus is also a rod shaped bacteria. They are not members of the genus Bacillus. They do not form spores and they die rapidly at room temperatures and above. They are only suitable for feed applications if the product is held cool before use and used immediately upon application. Some companies add high levels to products to compensate for this even though viable numbers are not known at the time of product usage.
Spore forming Bacillus

Germination

Degradation of organic material
Nitrogen fixation
Water quality improvement
Aquapro B

- Powdered formulation
- Designed for activation
- Contains 2 strains at > 2 billion CFU per gram
- Field test results show significant benefits
Aquapro EZ

• Original formula in biodegradable bags
• Allows for easy distribution of product into problem areas
• Each bag averages > 1 trillion CFU total
Aquapro F

• Formulated for addition to the feed
• Contains six bacillus species, 14% MOS by weight (a prebiotic)
• Top dress feed with it or mill directly into the feed
PRO 4000X tablets

- Convenient no activation needed
- Drop directly into ponds into problem areas or spread around bottoms
- Approximately 16 grams each with > 64 billion CFU of spores per tablet
Testing

• Products are in use in many countries and have been extensively field tested and shown to be a cost effective tool for improving the culture environment

» Hatchery
» Farm
Florida Pond trials with AQPB

Duda & Sons Shrimp Operations, Rockledge, Florida 1999

Four ½ acre ponds, stocked at 50-55 animals per square meter.

The treated ponds produced a 40% greater harvest by weight.

The farm manager noted “the greatly reduced organic sludge build-up in the AQUAPRO-B treated ponds.” He estimated the sludge layer to be ½ inch or less. This meant that he did not have to dredge the treated ponds before re-stocking them. The untreated ponds built up organic sludge that must be removed annually.
Florida Pond Trial  1990’s

- AQPB Plus: 19607
- AQPB Control: 14029
Belize Field Trials

Royal Mayan Shrimp Farms

Started with AQPB and did tablet development trials here

Small farm in Belize

13 ponds, 1.6 to 10.3 ha in size

Max 70 animals per square meter, most at 35.
RMSF  Less fuel and better growth by using PRO4000X

Cleaner pond bottoms
India Field Trials

Hatchery Trial  India
Following two graphs show the reduction of green and yellow (TCBS) vibrio colonies in hatchery tank(s) treated with one tablet for each 5 MTs of water daily during the course of the production cycle. PRO4000X tablets substantially impacted vibrio loads in the production system.
PRO4000X reduces green vibrios in hatchery tanks (India)

TCBS green loads reduced to almost zero in hatchery tanks
PRO4000X reduces yellow vibrios in hatchery tanks. (India)

Use of Pro4000x tablets in hatchery tanks significantly reduces TCBS yellow colonies.
Farm trial India

Starting with 8 tablets added to problem areas 14 days post stocking and ending with 75 tablets at day 112 post stocking for a total of 497 tablets per pond per ha per cycle.

This is about 8 kgs of tablets total per pond. Tablets were added roughly every 10 days. Ponds averaged 1 ha in surface area and 1.4 m deep.

Stocked with SPF *P. vannamei* at 35/square meter. Using the tablets the farmer was able to run the cycle closed. For the control pond he could not.
Tablet application rates per ha per cycle

![Graph showing the increase in tablet application rates over time](chart.png)
Reduction of TCBS green in farms

Regular use of PRO4000x tablets reduced the load of TCBS green colonies in three closed ponds.

Days of culture

CFU/ml
Reduction of TCBS yellow vibrio loads over the course of the production cycle.

TCBS yellow vibrio loads were significantly lower in ponds treated with PRO4000x.

Days of culture

CFU/ml
Pro4000X tablets increased the growth rates of three experimental ponds.
Elimination of ammonia in closed ponds

Control pond even with exchange had ammonia accumulation. None of experimental ponds did.
Other clients in the hatchery and the farm report the same observation

• Successful control of common vibrio infections by the use of the tablets in conjunction with molasses and selective water exchange.
• Completely replaces use of antibiotics, sludge reduction, ammonia reduction, less stress

TAKE HOME MESSAGE: It is very important to bear in mind that bacteria need nutrients and that this is what limits product effectiveness. Our product work. How well they work depends on nutrient loads.
When we see dying animals often this is only the tip of the iceberg.
How our products can help

Cleaner Pond Bottoms

- Complete reduction of ammonia
- Shorter time between cycles
- Less lime needed to oxidize organic material
- Lower levels of hydrogen sulfide
- Less water exchange required

Increased Survivals

- Less stress
- Lower levels of vibrios
- Less blue green algae
- Eliminate antibiotics

Better Growth

- Excellent cost benefit due to reduced costs of production as a result of improved quality of environment
Conclusions

• AHPNS is a complex disease process that will not be easy to control (iceberg)
• It is likely here to stay and all efforts should be made to exclude it from farming areas
• Vibriosis can be controlled through ecological manipulation although the ecosystems resist anything but short-term change
• Our products are science based, field tested tools that can improve production and lessen risks associated with uncontrolled vibrio populations.
Gracias !