Reduce The Impact of Shrimp Diseases On Productivity:

Anti-bacterial Warfare via the Feed

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Health management
The Key Bottleneck in aquaculture

- Disease outbreaks and parasitic infestations are a major treat for profitability of aquaculture
- Increasing limitations on antibiotics & chemicals (consumer demands, regulations)

- Increasing interest in disease prevention
- Natural solutions administered via the feed

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Identified diseases in penaeid shrimp

modified from Cuéllar-Anjel, J. (2009)

VIRUS
- White Spot Virus (WSSV)
- Taura Syndrome Virus (TSV)
- Lymphoid Organ Vacuolisacion Virus (LOVV)
- Infectious Hipodermic & Hematopoetic Necrosis Virus (IHHNV)
- Baculovirus Penaei (BP)
- Yellow Head Virus (YHV)
- Hepatopancreatic type Parvo Virus (HPV)
- Infectious Myonecrosis Virus (IMNV)
- Penaeus vannamei Noda Virus (PvNV)

INTRACELLULAR BACTERIA
- Rickettsias
- Alfa Proteobacteria (NHP)
- Clamidias (ZII-S)

EXTRACELLULAR BACTERIA
- Flavobacterium spp.
- Pseudomonas spp.
- Aeromonas spp.
- Plesiomonas sp.
- Vibrio spp.
- Filamentosas (Leucothrix sp.)

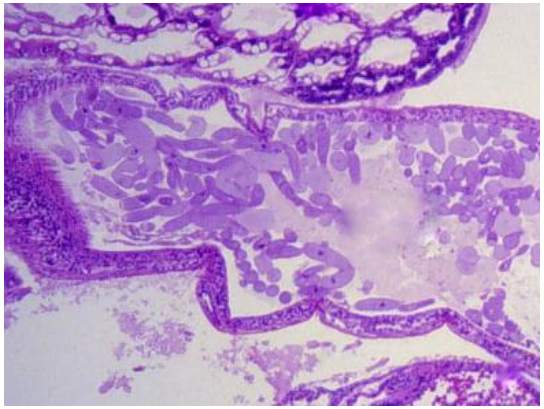
FUNGI
- Lagenidium spp. (micosis larval)
- Sirolpidium spp. (micosis larval)
- Fusarium solani y F. moniliforme (fusariosis)
- Phytium spp.
- Leptolegnia marina
- Haliphthoros milfordensis
- Atkinsiella dubia
- Enterocytozoon hepatopenaei (EHZ)

PROTOZOA
- Gregarinas (Nematopsis sp., Paraophioidina sp., Cephalolobus sp.)
- Zoothamnium sp.
- Epistylis sp.
- Vorticella sp.
- Acineta sp.

IMNV

WSSV

gregarinas



EMS/AHPND

Vibrio parahaemolyticus was identified as the causative agent of AHPND in 2013.

Vol. 105: 45–55, 2013
doi: 10.3354/dao02621

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published July 9

Determination of the infectious nature of the agent of acute hepatopancreatic necrosis syndrome affecting penaeid shrimp

Loc Tran[1,2], Linda Nunan[1], Rita M. Redman[1], Leone L. Mohney[1], Carlos R. Pantoja[1], Kevin Fitzsimmons[2], Donald V. Lightner[1,*]

Juvenile *Penaeus vannamei* from Vietnam.
Left with EMS; right appears normal.

Source: Dr. Loc, Vietnam

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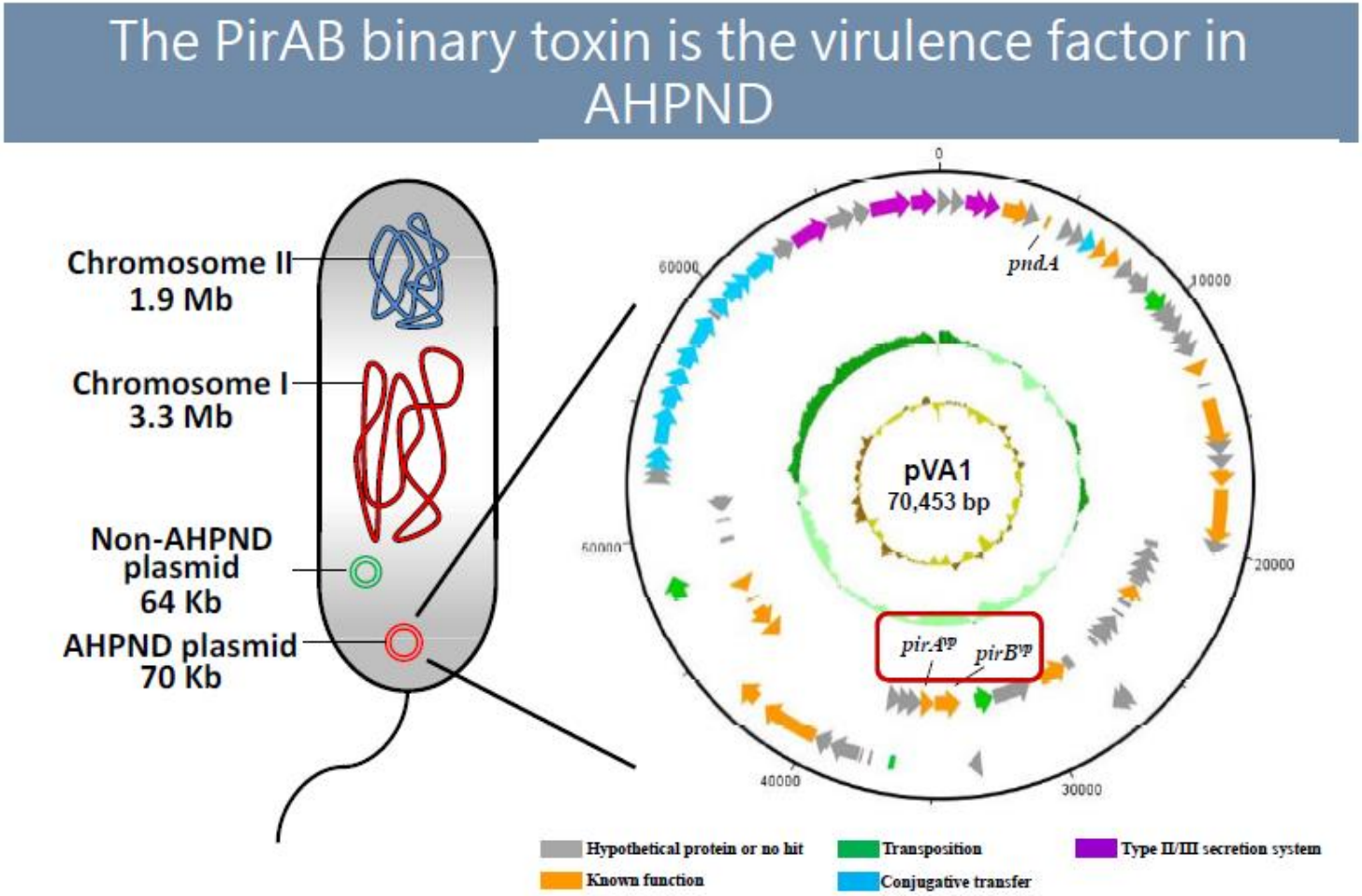
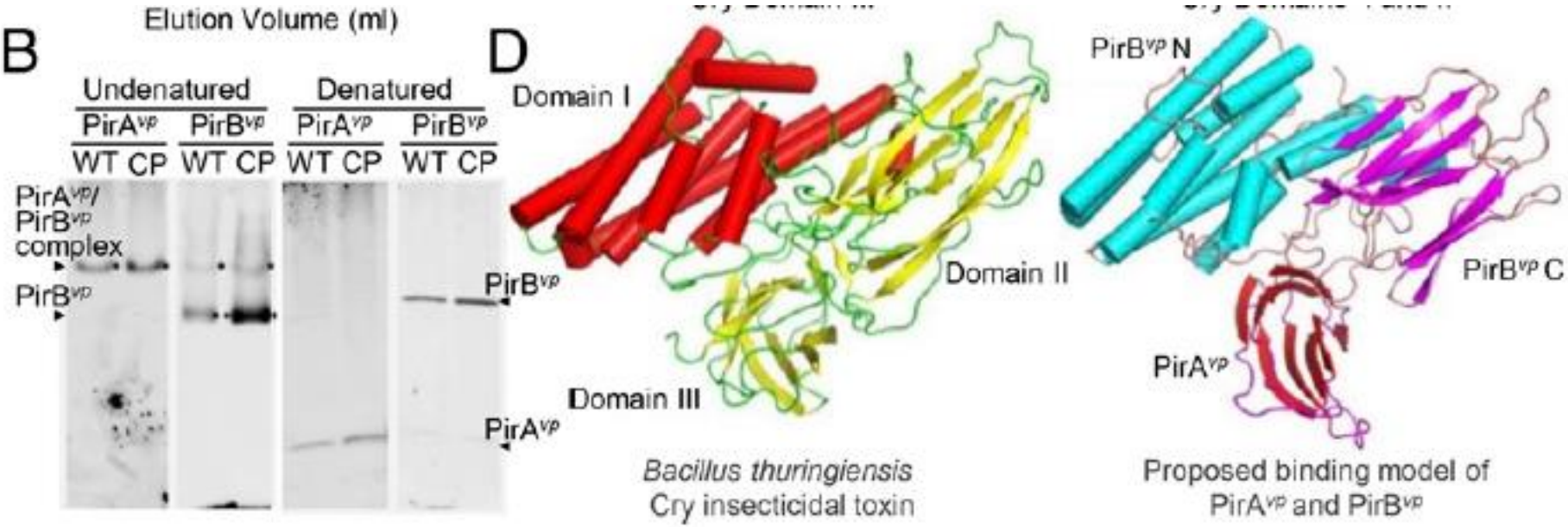
EMS/AHPND



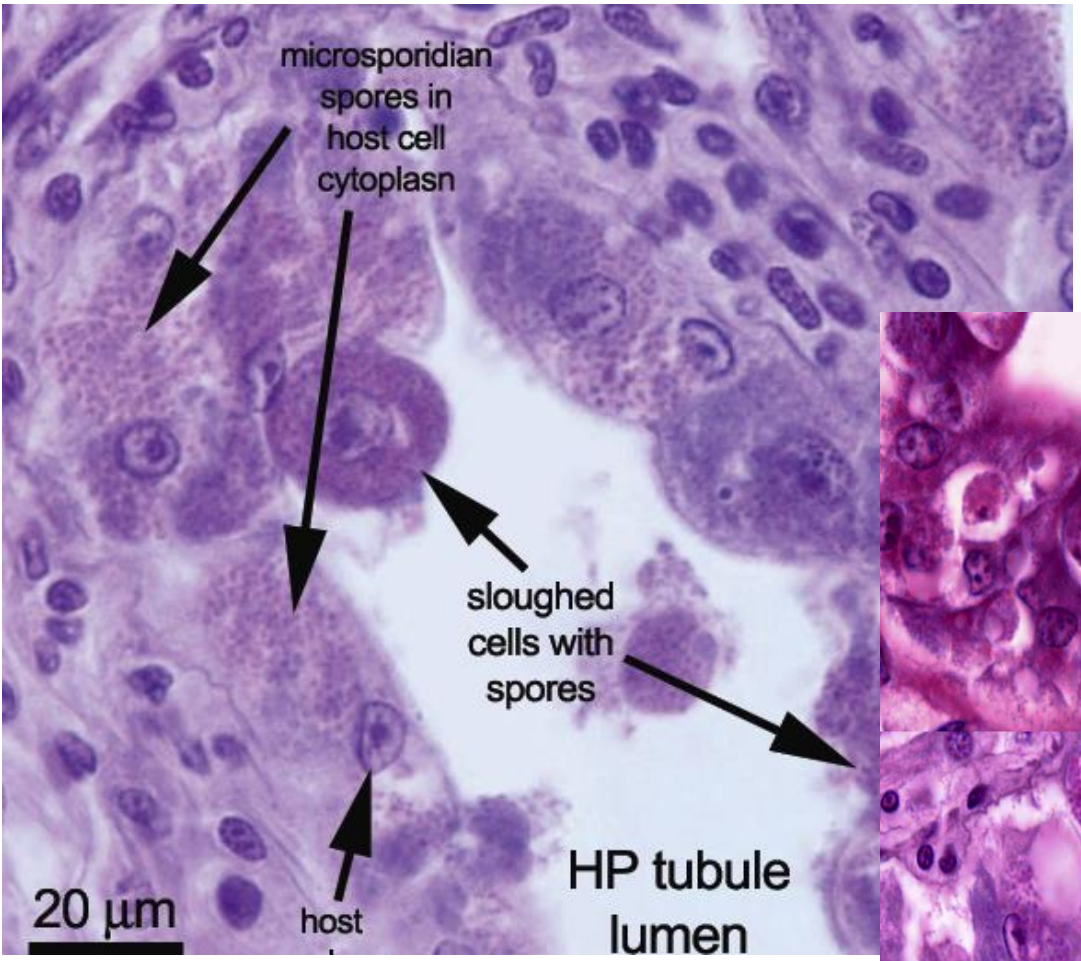
Source: Prof. Dr. Chu-Fang Lo & Dr. C.T. Lee (2014)
National Cheng Kung University, Tainan, Taiwan

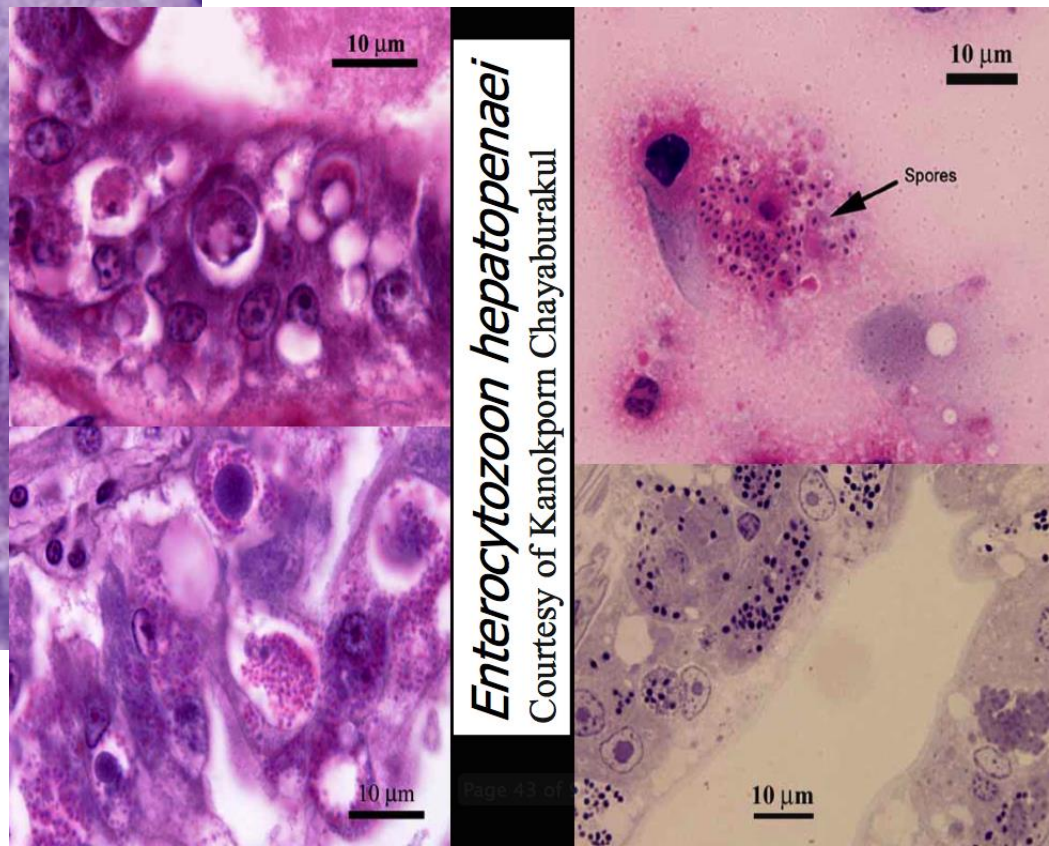
EMS/AHPND



Source: C.T. Lee et al. (2015)

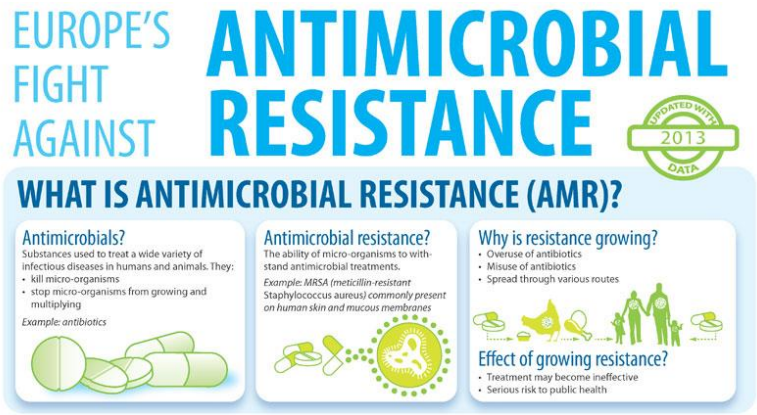
The new bad bug in shrimp: *Enterocytozoon hepatopenaei* (EHP)



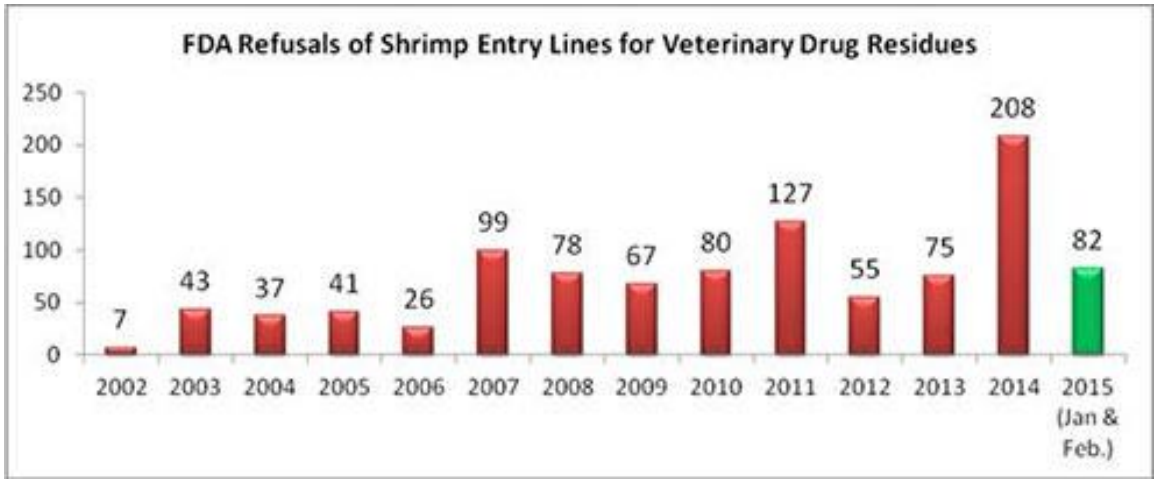


Antibiotics to combat shrimp disease : economic risks

- ***Zero tolerance policy*** by Food and Drug Administration (FDA) and European Commission (EC) on unauthorized antibiotics



- FDA refusals of shrimp entry for banned drug residues peak since 2014



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Antibiotics to combat shrimp disease : economic risks

March 6, 2015

Crackdown on shrimp imports contaminated with banned antibiotics

Noticias del día | 06 de marzo de 2015

The Southern Shrimp Alliance has compiled refusal information for shrimp products since 2002 available from the FDA.

USA: New data released by the U.S. Food and Drug Administration (FDA) (FDA) indicates that of the 114 entry lines of seafood refused in January, 24 were of imported shrimp for reasons related to veterinary drug residues. Nineteen of the entry lines refused for banned antibiotics were of shrimp shipped from Malaysia, four for shrimp shipped from India, and one for shrimp shipped from China, reports the Southern Shrimp Alliance.

These refusals involved a total of seven companies:

- Oce… elev… com… for s…
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- Pen… Mar…
- Ria… Mar…
- Xia… com…
- Sha… liste…
- For… liste…

USA: New data released by the U.S. Food and Drug Administration (FDA) (FDA) indicates that of the 114 entry lines of seafood refused in January, 24 were of imported shrimp for reasons related to veterinary drug residues. Nineteen of the entry lines refused for banned antibiotics were of shrimp shipped from Malaysia, four for shrimp shipped from India, and one for shrimp shipped from China, reports the Southern Shrimp Alliance.

April 24, 2015

Food Safety News

Breaking news for everyone's consumption

Consumer Reports: Tests Find 60 Percent of Frozen Shrimp Contaminated With Bacteria

Raw, wild-caught shrimp from U.S., Argentina had lowest bacteria levels of all samples tested

BY CATHY SIEGNER | APRIL 24, 2015

A new Consumer Reports (CR) study released Friday found that 60 percent of 342 samples of frozen shrimp it tested contained *Salmonella*, *Vibrio*, *Listeria*, or *E. coli*, and 2 percent tested positive for the superbug MRSA (Methicillin-resistant *Staphylococcus aureus*). For its new report, "How Safe is Your Shrimp?," CR researchers bought 284 raw and 51 cooked shrimp samples for testing last March in 27 cities across the country from retailers such as Walmart, Kroger, Albertsons, Costco, Fry's Marketplace, Hy-Vee and Sprouts Farmers Market. CR didn't test fresh, never-frozen shrimp since U.S. consumers don't buy much of that.

Results from testing for bacterial and drug residues showed that 16 percent of cooked, ready-to-eat shrimp contained several bacteria, including *Vibrio* and *E. coli*. Antibiotics were found in 11 samples of raw, imported, farmed shrimp, and MRSA was detected in 7 raw shrimp samples.

Nearly all (94 percent) of the raw shrimp available in the U.S. are farmed in Asian countries, including Thailand, Vietnam, India and Indonesia. Because of the crowded and polluted conditions that typically exist in fish-farming ponds or tanks, the shrimp are often given antibiotics such as tetracyclines, which is illegal in shrimp imported to the U.S.

"of 205 raw farmed *imported* shrimp samples, 11 samples from Vietnam, Thailand, and Bangladesh tested positive for one or more antibiotics: Nine tested positive for oxytetracycline, three contained enrofloxacin, and two contained sulfa antibiotics."

Vibrio sp. are experts in acquiring antibiotic resistance

Iranian Journal of Fisheries Sciences 11(3)618-626 2012

Antibiotic resistance pattern of some *Vibrio* strains isolated from seafood

Raissy M.[1, 3*]; Mo

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Abstract

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International Food Research Journal 16: 53-58 (2009)

Antibiotic resistance and plasmid profiling of *Vibrio parahaemolyticus* isolated from cockles in Padang, Indonesia

[1]Zulkifli, Y., [1]*Alitheen, N.B., [1]Raha, A.R., [1]Yeap, S. K., [4]Marlina,

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Hindawi

Research Article

Antibiotic-Resistant Vibrios in Farmed Shrimp

Renata Albuquerque Costa,[1,2] Rayza Lima Araújo,[1,2] Oscarina Viana Souza,[1,2] and Regine Helena Silva dos Fernandes Vieira[1,2]

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Vibrio species due
32 isolates of *V.*
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Antibiotic resistance and EMS/AHPND (Loc et al., 2013)

ANTIBIOTICS USE INTENDED TO REDUCE LOSSES DUE TO THE EARLY MORTALITY SYNDROME (EMS/AHPNS) IN SHRIMP FARMS IN VIETNAM

Loc H. Tran*, Melba Reantaso, Kevin M. Fitzsimmons, Donald V. Lightner, Phuc Nhu Hoang

- EMS/AHPNS affected area in Soc Trang province of Vietnam
- Farmers' interviews :
  - Use antibiotics to reduce losses due to EMS/AHPNS since 2012
  - Oxytetracycline (OTC) most common
  - antibiotic treatment quickly lost its effectiveness

- Isolates of both bacteria causing EMS/AHPNS (pathogenic) and non-pathogenic *Vibrio parahaemolyticus* (Vp)
  - 2011/2012 : 100% of the isolates are sensitive to OTC (both pathogenic and non-pathogenic Vp)
  - 2013 : isolates are highly resistant to OTC (**100%** non-pathogenic isolates and **85.7%** of pathogenic isolates)

- Conclusion: *Vibrio parahaemolyticus* causing EMS/AHPNS
  - can develop resistance to OTC in a short period of time
  - may have capacity to transfer antibiotic resistance via mobile genetic elements among bacterial strains

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Antibiotic resistance plasmid mediated (Han et al., 2015)

tetracycline resistance in Vibrio parahaemolyticus, HPND strain Mexico

Aquaculture Reports 2 (2015) 17–21

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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

Table 3
Pathogenic *V. parahaemolyticus* isolates used in this study, antibiotic disc test and resistance genes screening.

Isolates	Species	Disease	Origin	Disc test					PCR
				Amp	FFC	OTC	TE	NA	*tetA* to *E*
13-511/A1[a]	*V. parahaemolyticus*	AHPND	Mexico	R	S	R	R	S	*tetB*
13-306D/4[a]	*V. parahaemolyticus*	AHPND	Mexico	R	S	R	R	S	*tetB*
12-194/g	*V. parahaemolyticus*	AHPND	Vietnam	R	S	S	S	S	–
13-028/A3[b]	*V. parahaemolyticus*	AHPND	Vietnam	R	S	S	S	S	–
14-188/1	*V. parahaemolyticus*	AHPND	Vietnam	R	S	S	S	S	–
14-188/2	*V. parahaemolyticus*	AHPND	Vietnam	R	S	S	S	S	–
14-188/3	*V. parahaemolyticus*	AHPND	Vietnam	R	S	S	S	S	–
14-188/4	*V. parahaemolyticus*	AHPND	Vietnam	R	S	S	S	S	–
14-188/5	*V. parahaemolyticus*	AHPND	Vietnam	R	S	S	S	S	–

[a] Nunan et al. (2014). Isolates collected from Mexico.
[b] Tran et al. (2013). Isolates collected from Vietnam.

Functional feeds

- provide benefits other than just nutritional
- support health and reducing the risk of disease


Juvenile *Penaeus vannamei* from Vietnam.
Left with EMS; right appears normal.

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Functional feed additives to improve productivity in shrimp

Promoting healthy gut microflora

Benefits:

- Healthy Gut = functional gut for digestion (growth, FCR)
- Healthy Gut = barrier for entry of infections (survival)

Modes of action:

1. Modulating healthy gut flora :
   - Inhibiting growth of pathogenic bacteria (parasites)
   - Promoting beneficial bacteria (probiotics)
2. Reducing pathogenicity (Quorum Sensing Inhibition)

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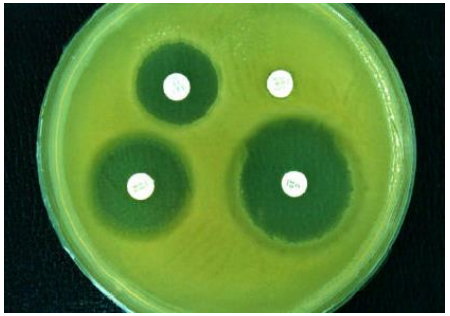
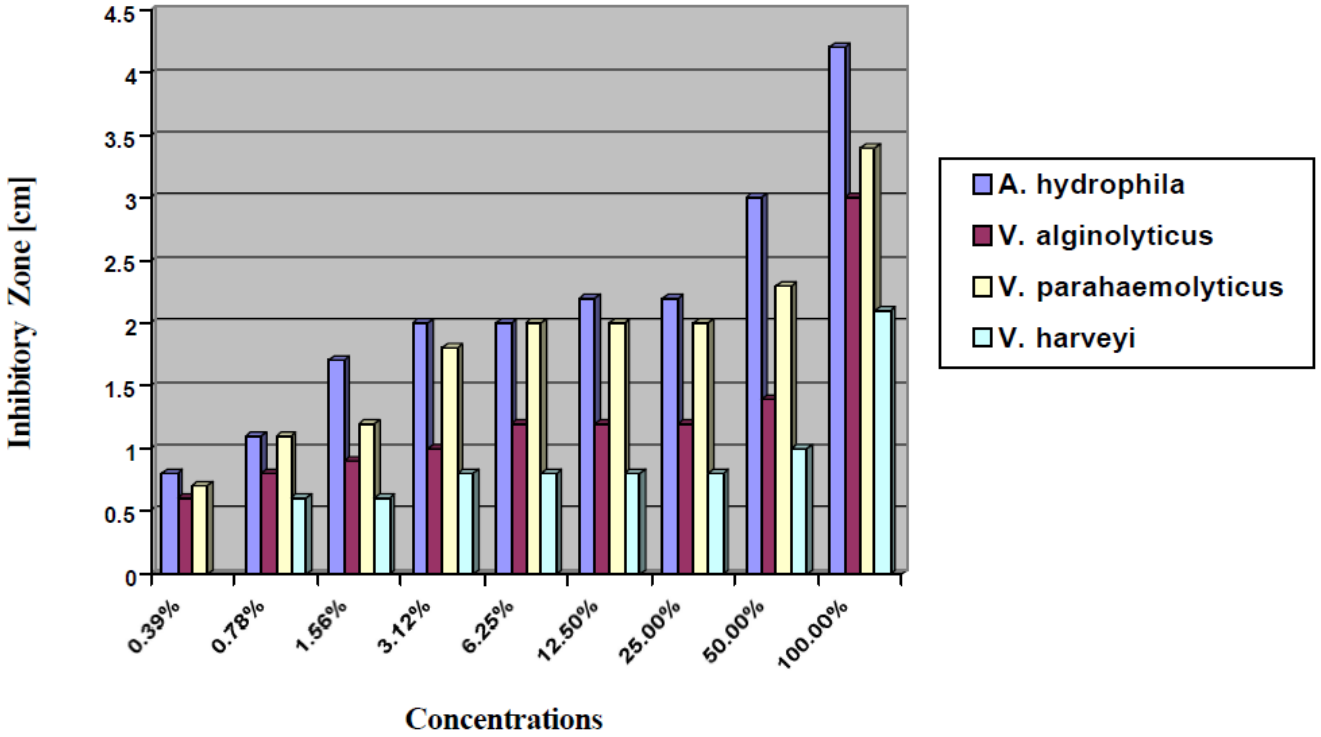
MOA 1: Gut Modulation = Inhibiting pathogenic + promoting beneficial bacteria





MOA 2: Reducing pathogenicity … Investigating a novel mode of action

For the development of SANACORE®GM
we explored a new line of research:

Quorum Sensing

QS TECHNOLOGY

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QS systems of different aquatic pathogens

The quorum sensing systems of different aquatic pathogens and the link between quorum sensing and virulence factor expression and/or virulence as such

Species	Signal	Quorum sensing-regulated virulence (factors)	References
Aeromonas hydrophila	BHL[a], HHL[b]	biofilm formation, exoprotease production	Swift et al. (1997), Swift et al. (1999), Lynch et al. (2002)
Aeromonas salmonicida	BHL[a], HHL[b]	serine protease production	Swift et al. (1997)
Vibrio anguillarum	ODHL[c]	unknown	Milton et al. (1997)
Vibrio harveyi	OHBHL[d], AI-2	siderophore production, production of type III secretion system components, extracellular toxin production	Bassler et al. (1993), Lilley and Bassler (2000), Manefield et al. (2000), Mok et al. (2003)
Vibrio parahaemolyticus	unknown	opacity	McCarter (1998)
Vibrio vulnificus	AI-2	protease and haemolysin production, lethality to mice	McDougald et al. (2000), Kim et al. (2003)
Yersinia ruckeri	unidentified AHL	unknown	Temperano et al. (2001)

[a] BHL: *N*-butanoyl-L-homoserine lactone.
[b] HHL: *N*-hexanoyl-L-homoserine lactone.
[c] ODHL: *N*-(3-oxodecanoyl)-L-homoserine lactone.
[d] OHBHL: *N*-(3-hydroxybutanoyl)-L-homoserine lactone.

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QS system in *V. parahaemolyticus*

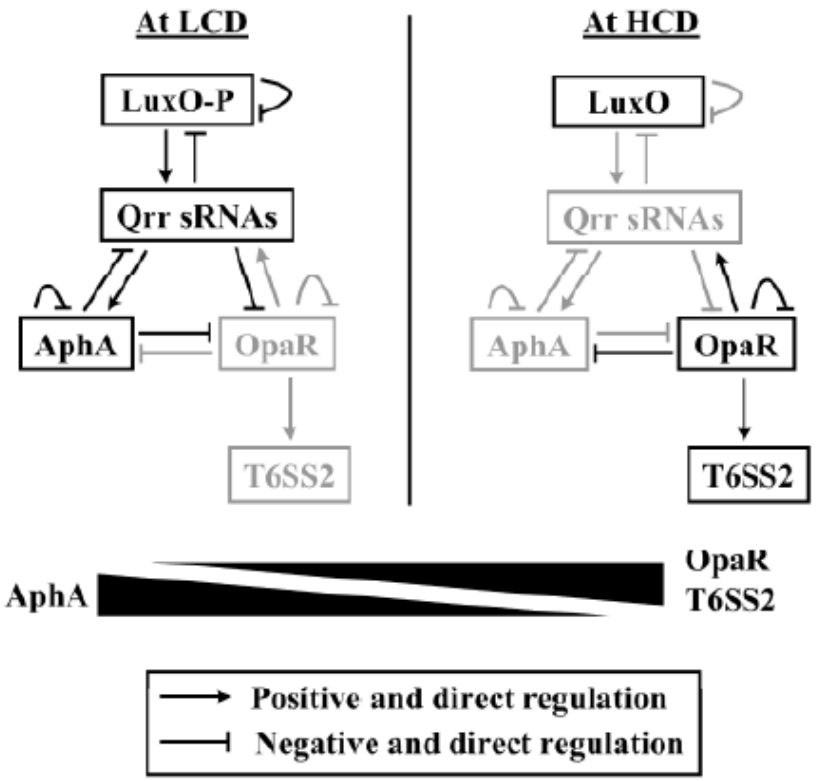


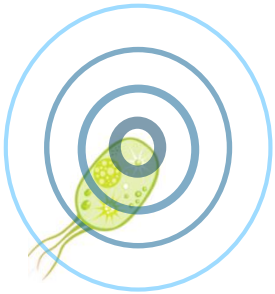
Figure 1. Action of *V. parahaemolyticus* QS systems.

L. Wang *et al.*, *PLOS ONE*, **8**, e73363 (2011)

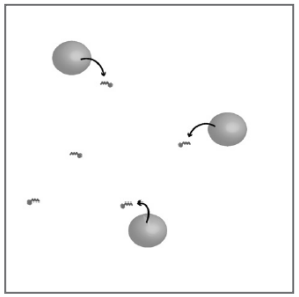
Quorum Sensing

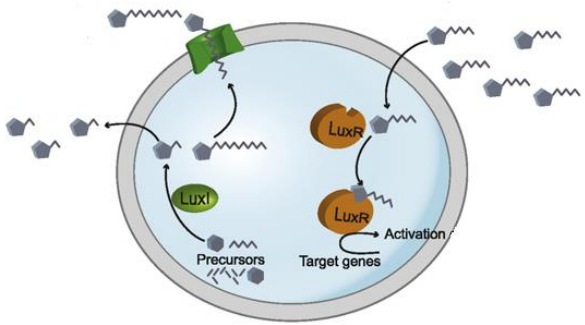
What is QS?

A form of bacterial communication



Bacteria send and receive signals
(secreted molecules / receptors)





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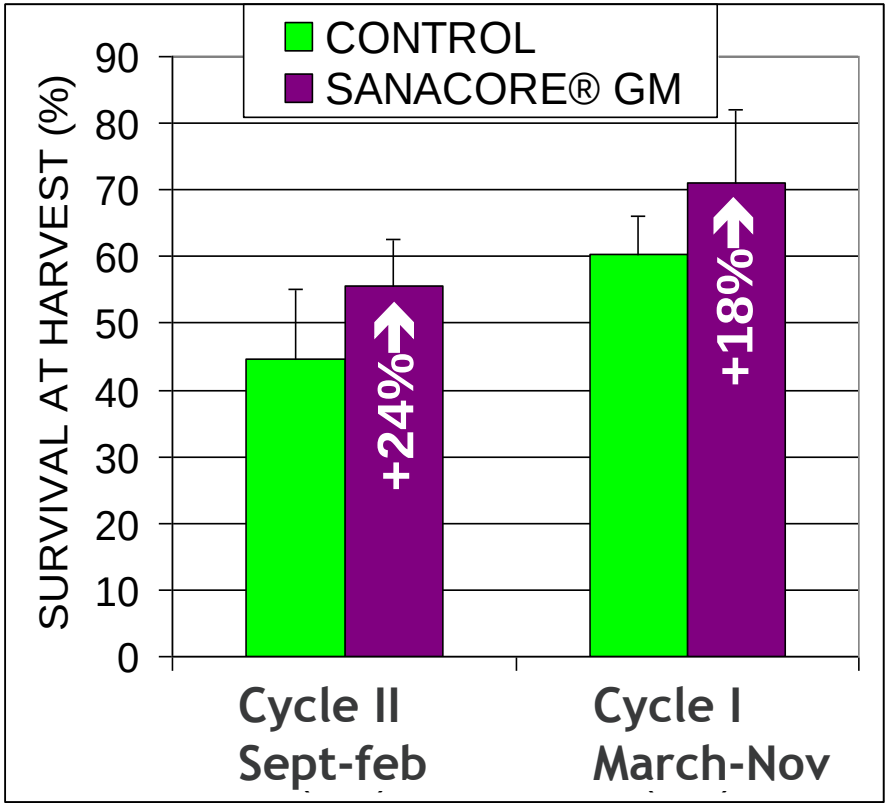
Improving survival in production of *Litopenaeus vannamei* in Panama exposed to disease pressure

- Semi-intensive production (8/m^2)
- 5-8 replicate ponds of 3 ha
- Disease challenges:
  - -White spot virus (WSSV)
  - -Necrotising hepatopancreatitis (NHP)
  - -Vibriosis



Source: Cuellar J. et al. (2010)

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Reducing inter-pond variability in production of *Litopenaeus vannamei* in Panama exposed to disease pressure

Coefficient of variation (cv) for production parameters from 8 replicate ponds (3 ha) at harvest (141 days of culture)

Treatment n=8	Survival (%)	Shrimp size (g)	Crop Yield (kg/ha)	Feed 3ha)	FCR	Weekly Growth (g/wk)	Average all parameters
SANACORE® GM	13%	9%	11%	8%	12%	9%	10%
CONTROL	24%	18%	16%	11%	18%	18%	18%

Reduction of the coefficient of variation among ponds with 40%

Source: Cuellar J. et al. (2010)

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ECUADOR 2015

production

Natural Feed Additive Improves Shrimp Productivity In Ecuador Demonstration

The inclusion of multi-action feed additives to shrimp diets can improve survival and overall crop yield.

ods in large ponds do not allow effective

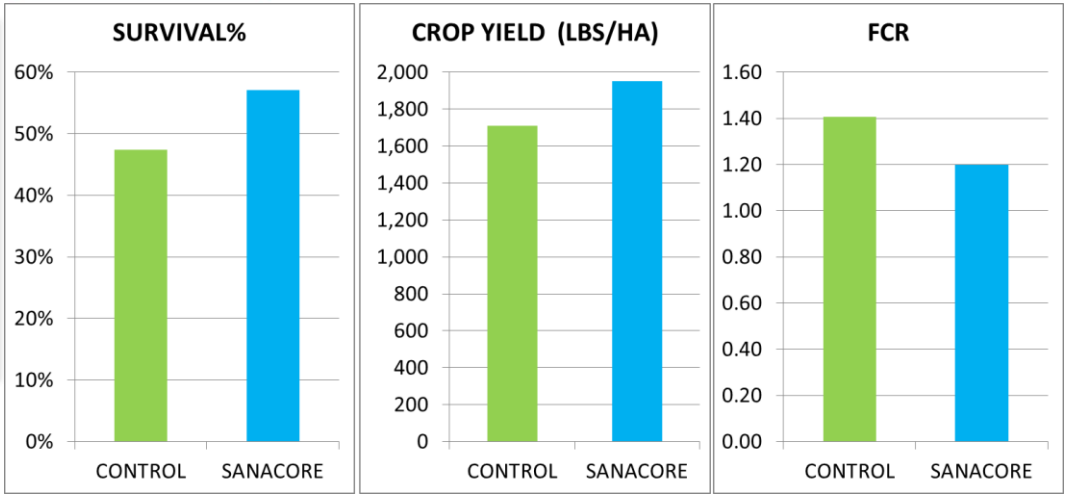

Table 1. Results at harvest for control ponds and treated ponds after 78 days of culture.

Treatment	Survival (%)	Shrimp Size (g)	Crop Yield (kg/ha)	Feed-Conversion Ratio	Weekly Growth (g)
Phytobiotic	57.4 ± 10.8	15.6 ± 1.0	885 ± 149	1.20 ± 0.20	1.40 ± 0.09
Control	47.3 ± 3.1	16.4 ± 0.7	776 ± 83	1.41 ± 0.14	1.47 ± 0.06
Change	20.5%	– 4.7%	14.1%	–14.9%	– 4.7%

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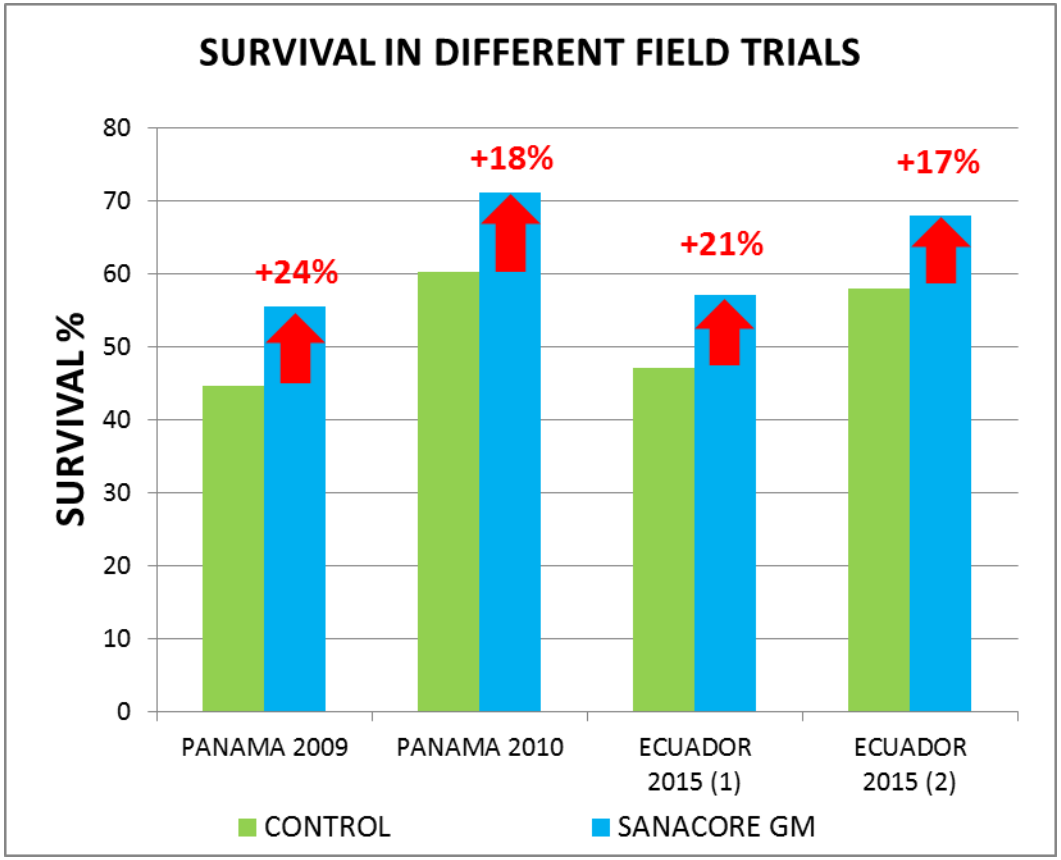
Source: Valle JC et al. (2015)

Comparing Ecuador vs Panama - effect on survival

- Panama: 5-8 replicate ponds of 3 ha at 8/m2
- Ecuador: 3-5 replicate ponds of 170 m2 at 10 per m2

- Disease challenges:
  -White spot virus (WSSV)
  -Necrotising hepatopancreatitis (NHP)
  -Vibriosis



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Functional feeds against EMS

Loc Tran et al. (2015) - Aquaculture Asia Pacific may-june

FOCUS

Feeding for health

Feed Additives Can Reduce the Impact of EMS/AHPND: evidence from an experimental challenge with *Penaeus vannamei*

Loc H. Tran (1), Phuc Nhu Hoang (1), Oanh Hoang Bui (1), Trang Dai Nguyen (1),

Allen Ming-Hsun Wu (2), Sam Ceulemans (2), Peter Coutteau (2)

1) Minh Phu AquaMekong Shrimp Vet Laboratory, HCMC, Vietnam

2) Nutriad International, Dendermonde, Belgium

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Objective

- Compare the efficacy of different health additives to reduce the impact from an experimental EMS/AHPND infection in *P. vannamei*

- 3 types of feed additives
  1. SANACORE : SANACORE GM @ 5 kg/MT of feed
  2. PHYTO: blend of phytobiotic products @ 3 kg/MT of feed
  3. OAC: blend of organic acids @ 5 kg/MT of feed

- Included in the feed by grinding/re-pelleting a commercial feed

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Challenge Methodology

- *P. vannamei* Specific Pathogen Free (SPF) from SIS origin (SPF confirmed by histopathology and PCR); pregrown from PL12 to 1-2g at AQUAMEKONG
- Acclimatization on experimental feeds in 90L tanks (30 shrimp/tank) during 21 days
- Challenge with *Vibrio parahaemolyticus* (LA37; virulent strain causing EMS/AHPND; *Loc et al. In prep),* tryptic soy broth +2% NaCl (TSB+) - 18h incubation
  1. Immersion Challenge : add bacterial culture to the culture water @ 3.10^5 cells/ml
  2. Per os Challenge (single meal) : mix bacterial culture with the feed @ 20% v/v ratio, air-dried 15 min
- Neg control receiving sterile TSB+
- Follow up (15 days post challenge, feeding experimental feeds continued)

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Challenge : treatment overview

Treatment	Feed	Challenge
NEG CONTROL	Commercial diet	None
POS CONTROL	Commercial diet	Immersion/Per os
SANACORE	Com. Diet + Sanacore GM 5 kg/MT	Immersion/Per os
PHYTO	Com. Diet + Phytobiotics mix 3 kg/MT	Immersion/Per os
OAC	Com. Diet + Org. Acid mix 5 kg/MT	Immersion/Per os

- Triplicate tanks per treatment and per challenge type
- Plating digestive system on TCBS and TSB+ medium prior to the challenge and 5-10-15d post challenge

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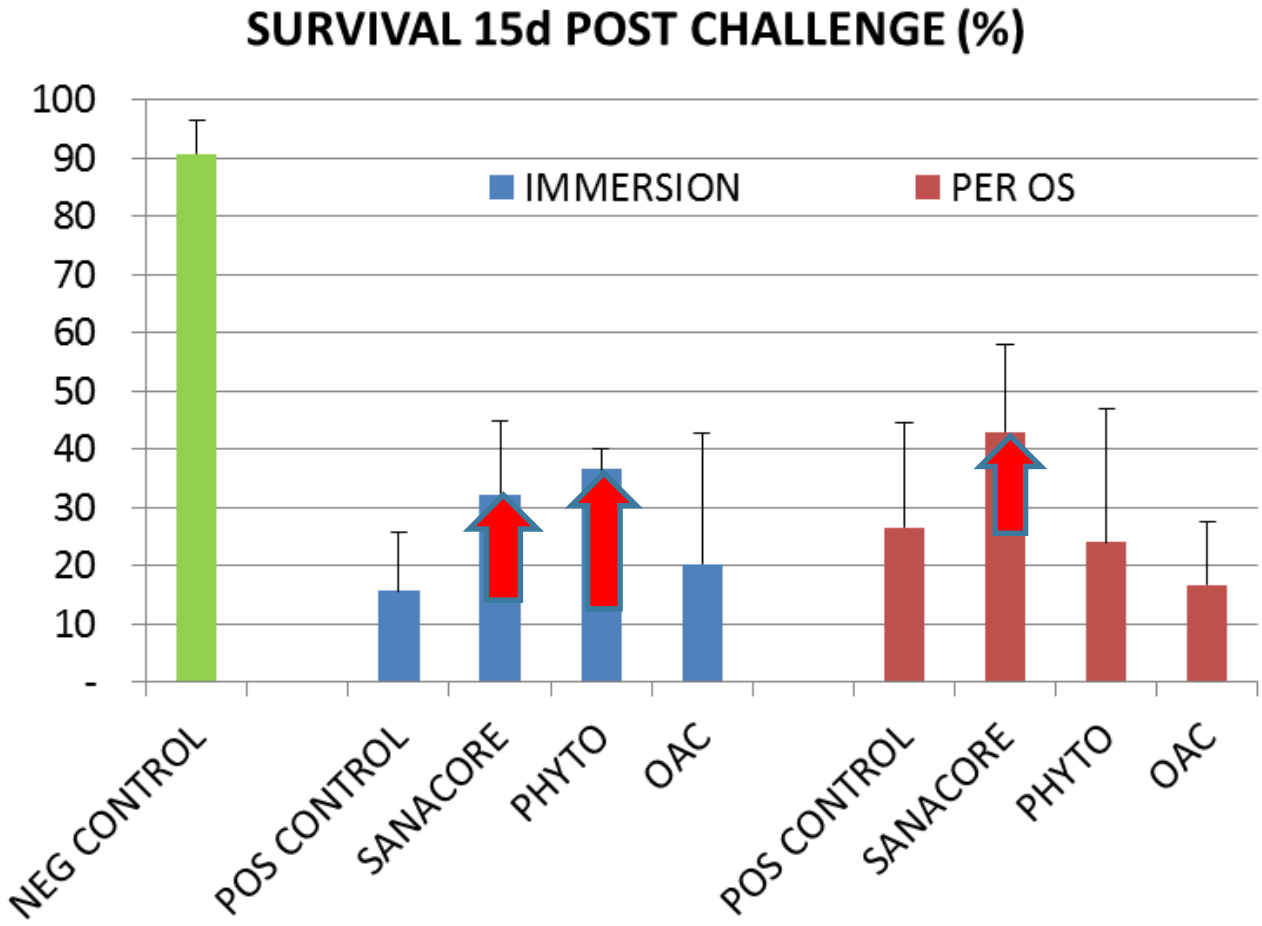
Survival rates following challenge



Statistically no significant differences ($P<0.05$)

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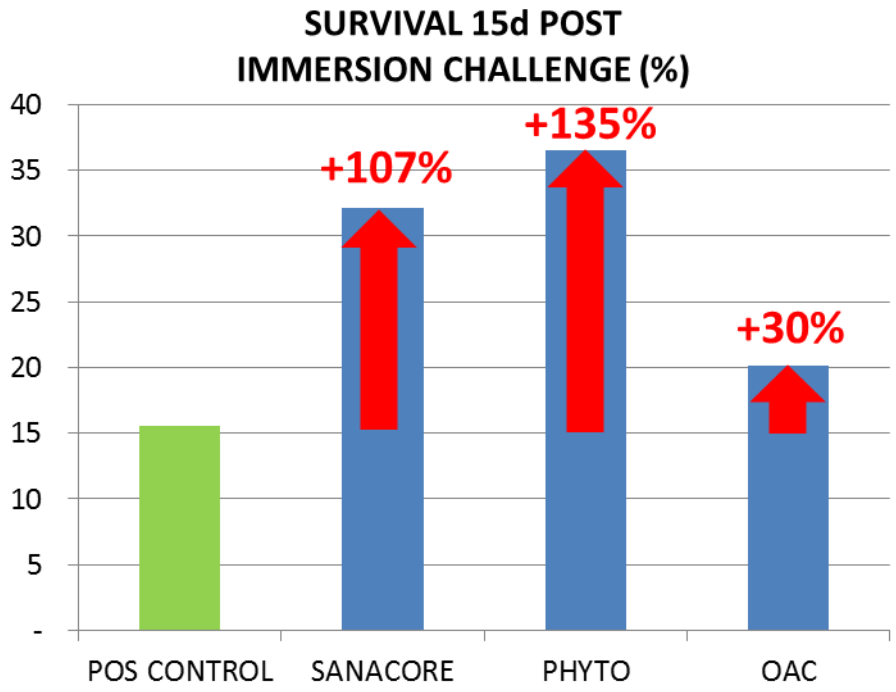
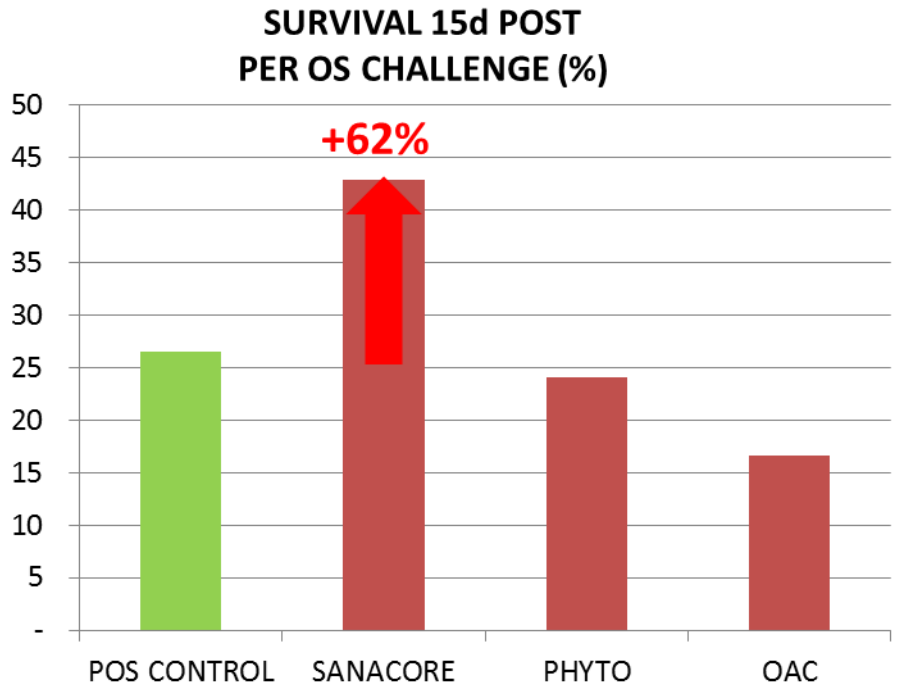
Survival rates following challenge - producer perspective





- Immersion challenge results in more mortality then *per os* challenge for positive control
- Treatment Sanacore shows effect in both types of challenge
- Treatment Phyto only shows effect in immersion challenge
- Treatment OAC did not show any effect in any of the challenges

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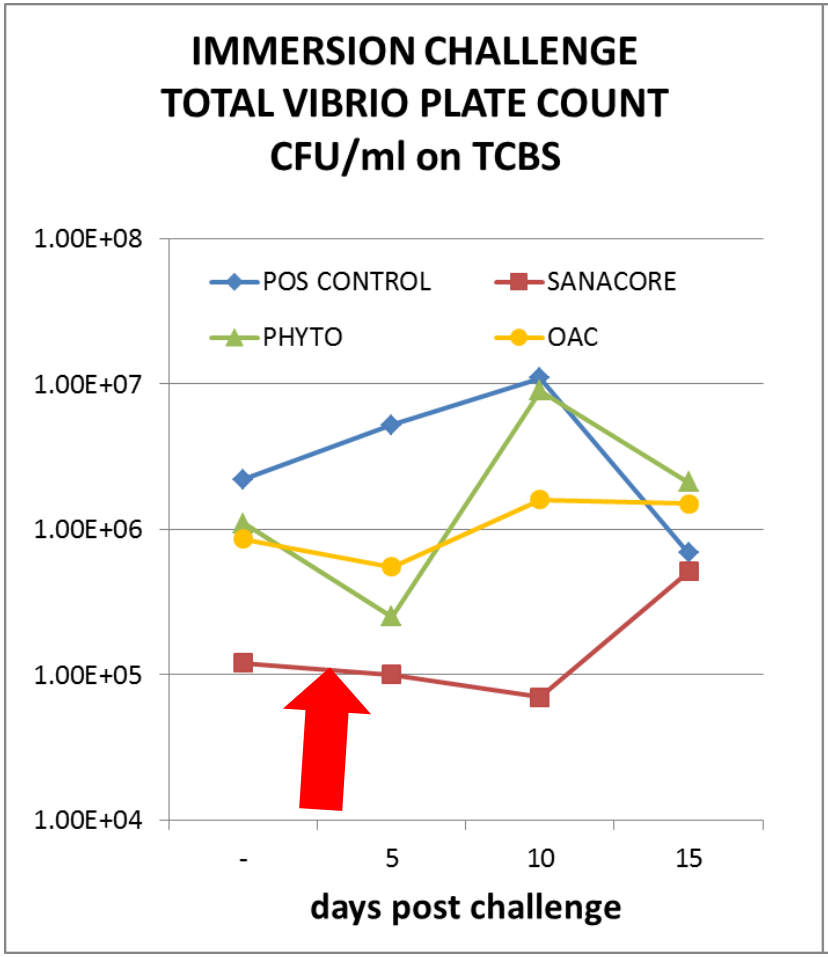
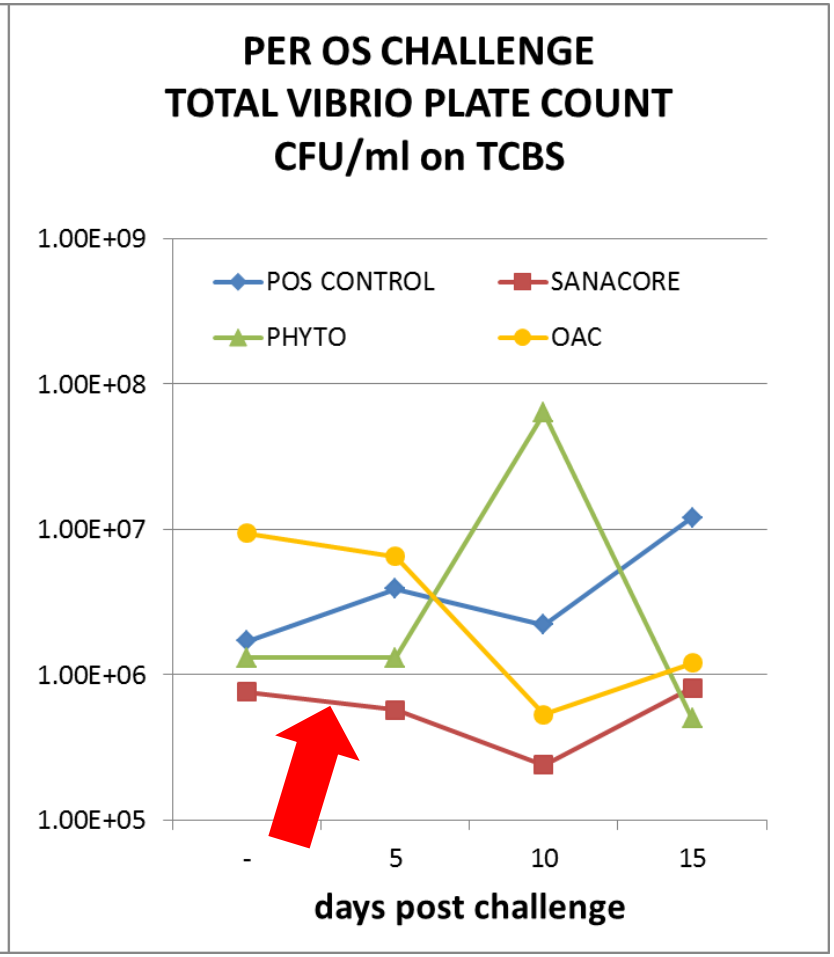
Total *Vibrio* plate count in the shrimp digestive system prior and following challenge





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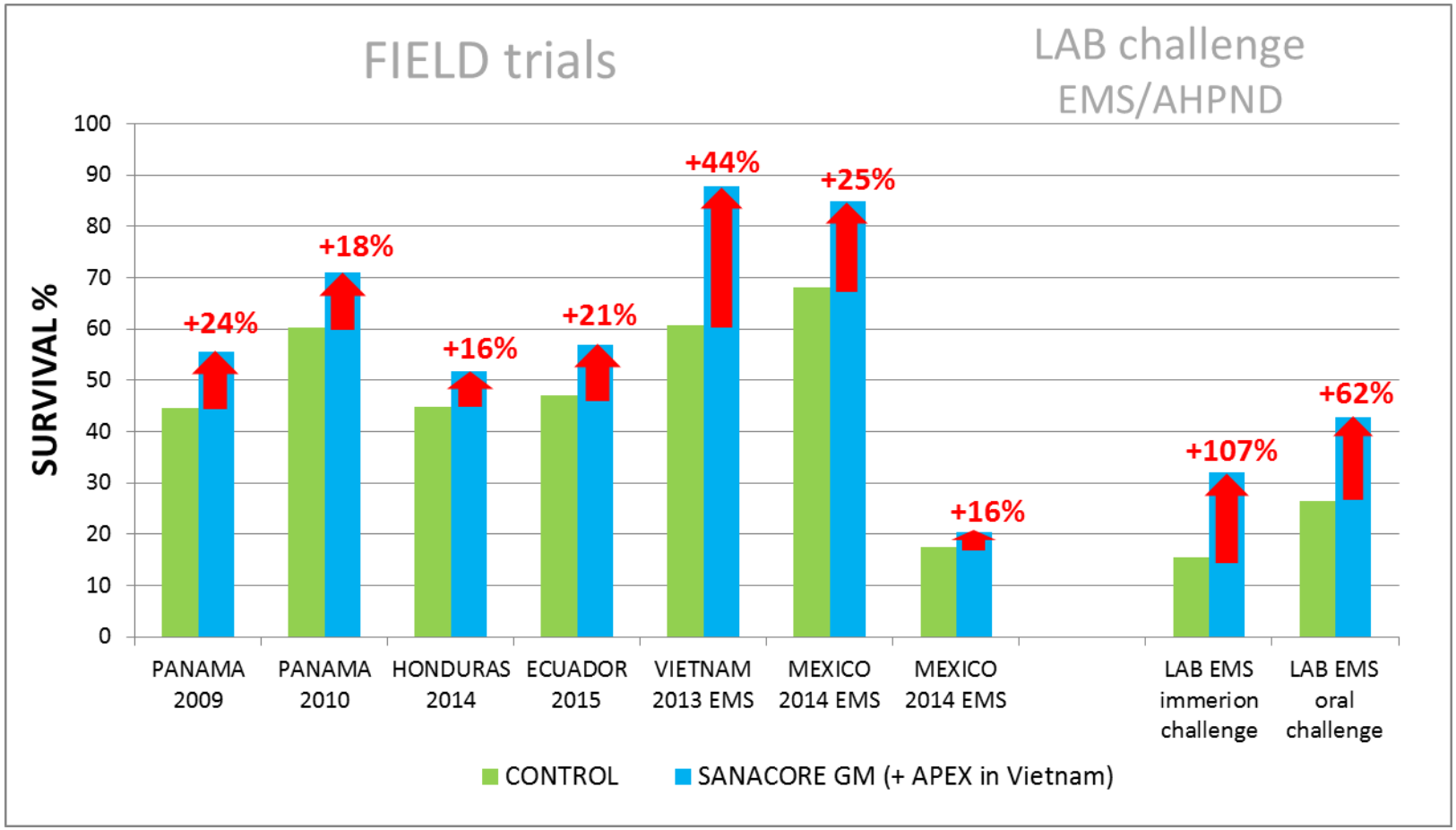
Effect of Nutriad's health strategy on survival of *P. vannamei* Field and lab experience in EMS and non-EMS countries



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Take-home message

- Functional feed additives designed to have multiple modes of action:
  1. Selective bacteriostatic action (@ MIC concentration) against a broad range of pathogenic bacteria, including Vibrio parahaemolyticus
  2. QS inhibiting activity (@ concencentrations below MIC) against Vibrio and other sp.
  3. Anti-parasitic action against gut parasites, e.g. gregarines

- Results :
  - reduce mortality due to bacterial (co-)infections
  - Control growth of bacterial pathogens & gut parasites
  - Reduce/delay onset of EMS toxin production
  - Work synergistically with probiotics

reduce the impact
from bacterial diseases on productivity

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