World Organisation for Animal Health

Overview of Diseases and Health Management Issues Related to Farmed Shrimp

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USMSFP

Main viral pandemics since1986

- 1986-87 - Taiwan's collapse due to MBV or YHV?
- 1991 - YHV in Thailand
- 1991/92 - TSV in Ecuador
- 1992/94 - WSSV Asian pandemic
- 1999 - WSSV in Central America starts Latin America pandemic
- 1999 – TSV in Asia
- 2004 - IMNV in Brazil

These viral diseases have caused major socioeconomic losses to the aquaculture community

ESTIMATED ECONOMIC LOSSES FROM DISCOVERY TO 2006

VIRUS	SINCE/YEAR	PRODUCT LOSS
IHHNV- Americas*	**1981**	**$ 0.5-1 billion**
YHV - Asia	**1991**	**$ 0.5 billion**
TSV-Americas	**1991/92**	**$ 1-2 billion**
TSV-Asia	**1999**	**$ 0.5-1 billion**
WSSV - Asia	**1992/93**	**$ > 6 billion**
WSSV - Americas	**1999**	**$ 1-2 billion**
IMNV – Americas	**2004**	**$ 100-200 million**
IMNV – Asia	**2006**	**??**

* Includes Gulf of California fishery 1989-1994

Main viral pandemics since1986

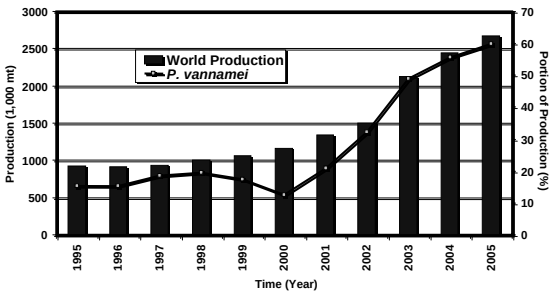
- These viral diseases can only be controlled by avoidance.
- The shrimp virus pandemics have changed the way shrimp are farmed.
- The requirement for clean shrimp stocks set in motion the industry switch to domesticated, SPF *Penaeus vannamei*.*

* Shrimp taxonomy according to Holthuis LB (1980) FAO Species catalog, Vol. 1. Shrimp and prawns of the world. FAO Fish Synop 125:46.

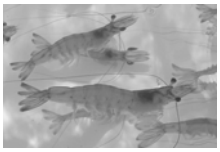
In approximately 5-6 years (2002 to 2006):

- Domesticated stocks of the Pacific white shrimp, *Penaeus vannamei*, surpassed all other penaeid shrimp as the dominant farmed species globally.
- In Asia where *P. vannamei* is an introduced species, its production now even exceeds that of the Americas where the species is native.

Global Farmed Shrimp Production



Change in Dominant Species



P. vannamei

P. monodon

Year	*P. vannamei*	*P. monodon*
2000	145,387 MT	630,984 MT
2005	1,599,423 MT	723,172 MT
% increase	**1,000%**	**15%**

FAO 2006

Why and how did this occur?

- Following viral pandemics of the 1990's, wild postlarvae & broodstock were increasingly found to carry many of these diseases (e.g. WSSV, MSGS, IHHNV and others).
- Domesticated SPF P*. vannamei* stocks became available following the viral pandemics.
- Trials in affected farming regions with SPF P*. vannamei* were successful.
- The switch to domesticated SPF P*. vannamei* was underway…

New Developments in Shrimp Diseases of Concern to SE Asia

- TSV:
  - ✓ TSV – significant in China, Taiwan, Thailand, Malaysia, Indonesia & others that grow *P. vannamei*
  - ✓ TSV – found in *P. monodon* in Thailand
  - ✓ New TSV strains emerging?
- WSSV – continuing problem
- HPV and MBV – associated with runting in *P. monodon*
- MSGV (Mondon slow growth virus) – newly recognized virus; could it be the cause of domestication failures with *P. monodon*?
- IHHNV – very high prevalence in *P. monodon*
- IMNV – Made its way into SE Asia.

New Developments in Shrimp Diseases of Concern to SE Asia

- Bacterial:
  - ✓ Vibriosis – continuing problem; antibiotic residues
  - ✓ Rickettsia – an overlooked problem?
- Parasitic:
  - ✓ "New" HP microsporidia – contributing to poor growth in *P. mondon*?

New Developments in Shrimp Diseases of Concern to the Americas

- Viral:
  - ✓ Recurring WSSV & TSV outbreaks
  - ✓ New TSV strains with increased virulence
  - ✓ IMNV – appears to be confined to Brazil; major threat to shrimp farming industry if it spreads
  - ✓ PvNV – Newly discovered. Appears to be confined to Belize. Effect(s) on farmed shrimp has not been fully evaluated & is not clear at present.

New Developments in Shrimp Diseases of Concern to the Americas

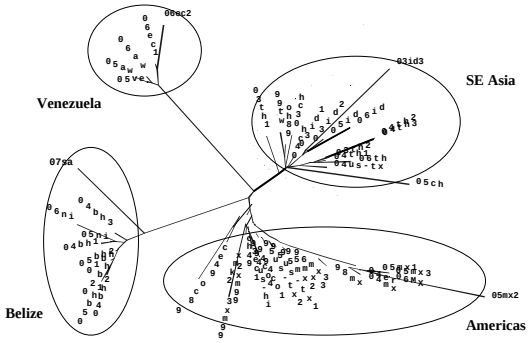
- Bacterial:
  - ✓ Vibriosis – recurring problem; antibiotic residues; antibiotic resistance
  - ✓ NHP – incidence increasing in semi-arid locations
  - ✓ Spiroplasmosis – Appears to be confined to Colombia. Potential to cause important losses
- Parasitic:
  - ✓ Haplosporidiosis

Genotypes & Biotypes of TSV

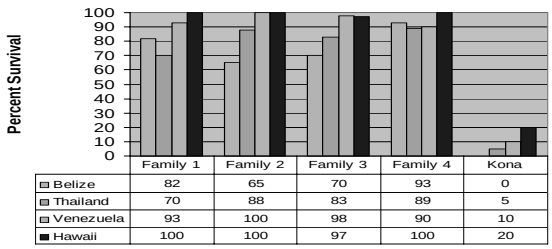
Since TSV emerged in Ecuador 1991/92:

- 4 distinct genetic lineages have emerged
- 2 serotypes have been documented
- Differences in virulence according to genotype & serotype documented
  - ✓Belize strain virulence > all other lineages

Un-rooted phylogenetic tree of 54 TSV isolates collected between 1993 and 2007



Survival Comparison of 4 TSV Resistant Families from 1 U.S. Company and SPF Kona Stock to 4 TSV Isolates



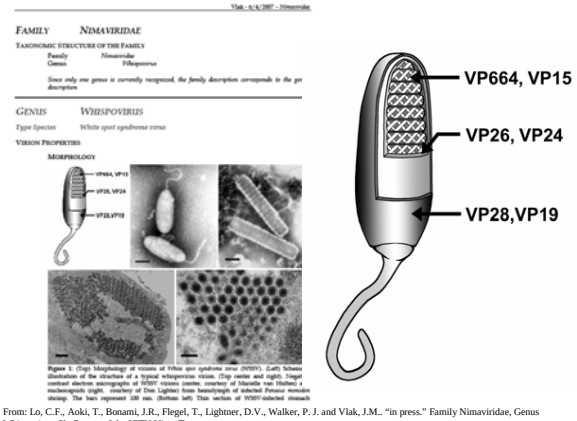
	Family 1	Family 2	Family 3	Family 4	Kona
Belize	82	65	70	93	0
Thailand	70	88	83	89	5
Venezuela	93	100	98	90	10
Hawaii	100	100	97	100	20

Chronic Phase TSV Study Comparing Standard PCR, Real-Time PCR & Sample Type

- Standard RT-PCR can give false negative results for TSV in shrimp with chronic phase infection.
- Best sample for TSV detection in chronic phase by RT-PCR is hemolymph.

Chronic Phase TSV A life-long infection?

- Adults 8 to 12 months P.I. remain TSV +
- TSV + cells present in LO spheroids
- Hemolymph RT-PCR & bioassay TSV +
- TSV infection cycle in LO spheroids maintains life-long persistent infection



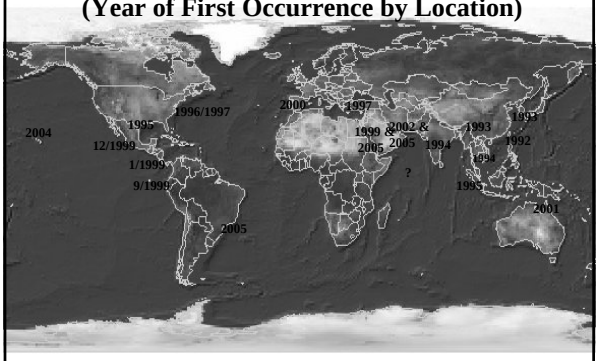
From: Lo, C.F., Aoki, T., Bonami, J.R., Flegel, T., Lightner, D.V., Walker, P. J. and Vlak, J.M.. "in press." Family Nimaviridae, Genus Whispovirus. 8ht Report of the ICTV-Virus Taxonomy.

WSSV- multiple strains?

- In terms of virulence, one major WSSV strain appears to have caused the global shrimp farm pandemic.
- Strain(s) with lower virulence recently reported, but not confirmed.
- Genome sequencing information shows that there are numerous, apparently minor, genetic variants.

WSD in *Penaeus monodon* – Malaysia 2005
photo courtesy of George Chamberlain

THE WHITE SPOT VIRUS PANDEMIC (Year of First Occurrence by Location)



How has WSSV spread around the world?

- Inadvertent introduction of live shrimp (all stages) asymptomatically infected
- Possible pathways from reprocessing plants to wild shrimp & other decapods?
  - ✓ Bait shrimp pathway is direct, but volume of use is relatively small.
  - ✓ Packing wastes from shrimp reprocessing (shells, heads, rinse water, etc.) often discharged directly into coastal bays and estuaries (= nursery grounds for shrimp & crabs) & can be hundreds of kg/per day.

WSSV Natural and Experimental Hosts

List of known hosts for WSSV is > 50:

- Penaeid shrimps & prawns
- Freshwater prawns
- Crabs - several genera
- Spiny lobsters
- Freshwater crayfish susceptible genera:
  - ✓ North American
  - ✓ European
  - ✓ Australian

Some strategies for management of WSSV

- Increased biosecurity
  - ✓ Filtration of water to exclude vectors from the wild
  - ✓ Reduced water exchange and lower densities
  - ✓ Complete dry out during cold season
- Use of SPF shrimp (WSSV free, at least)
- Hiperthermia
  - ✓ Reduced culture activity during the cold season
  - ✓ Use of green house systems

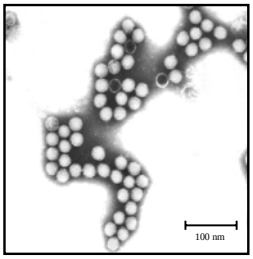
Main disease outbreaks in Sonora since 2005 (Number of farms)

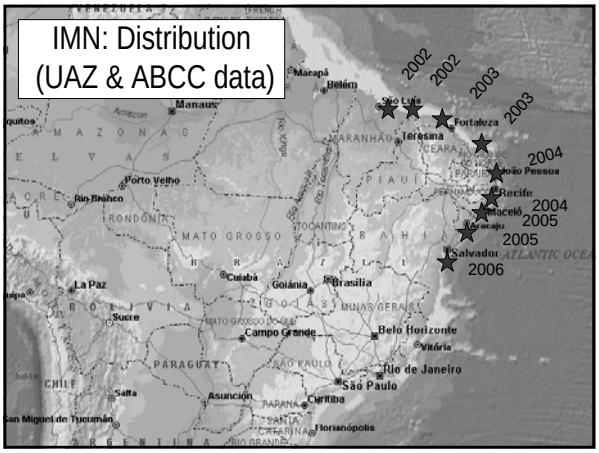
Pathogen	2005		2006		2007	
	Total/ Affected	%	Total/ Affected	%	Total/ Affected	%
NHP	128/73	57	126/32	25	112/40	36
WSSV	**128/96**	**75**	**126/25**	**20**	**112/3**	**3**
TSV	128/8	6	126/15	12	112/13	12

(www.cosaes.com)

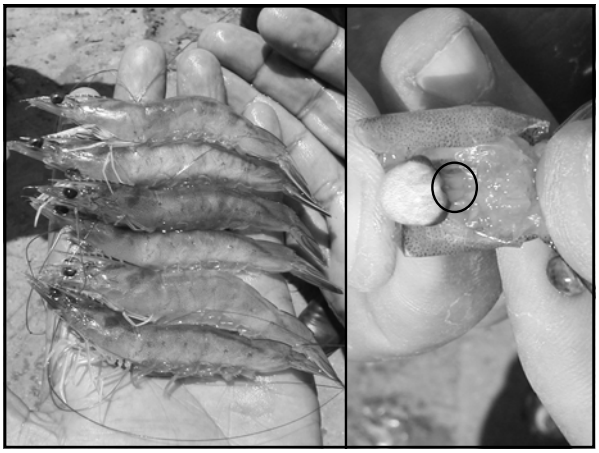
Infectious Myonecrosis – IMNV
Family *Totiviridae*

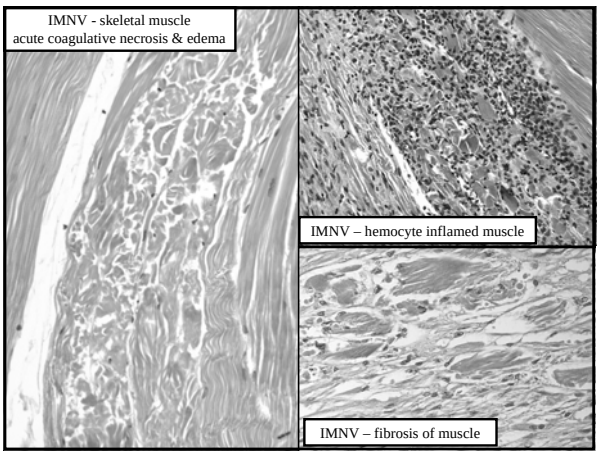
- Size: ~40 nm, unenveloped, icosahedron.
- Density (CsCl):1.369 g/ml
- Polypeptides: 1 major (approx. 106 kDa)
- Genome: dsRNA,~7.7 Kb
- Hosts: P. *vannamei*; chronic with with high mortalities

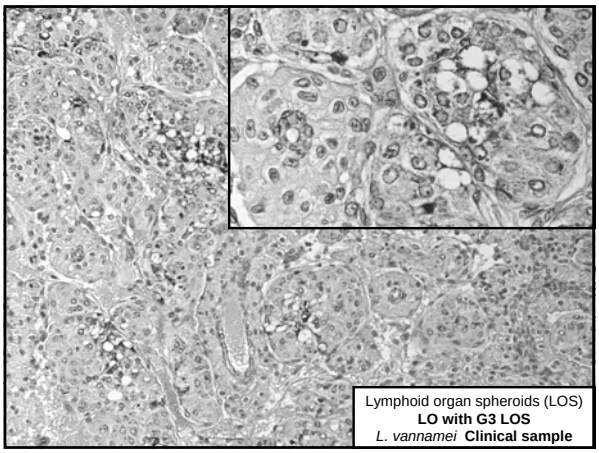


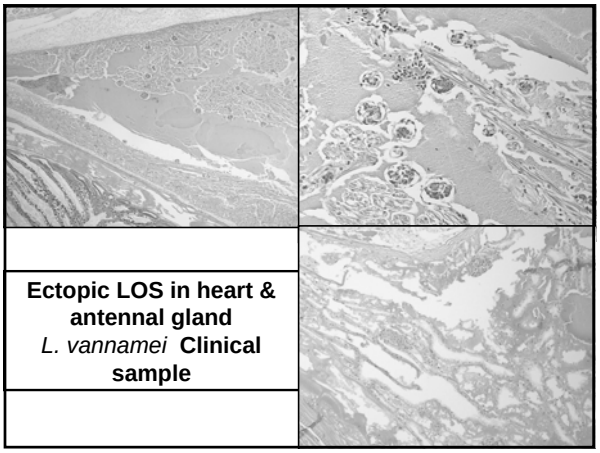


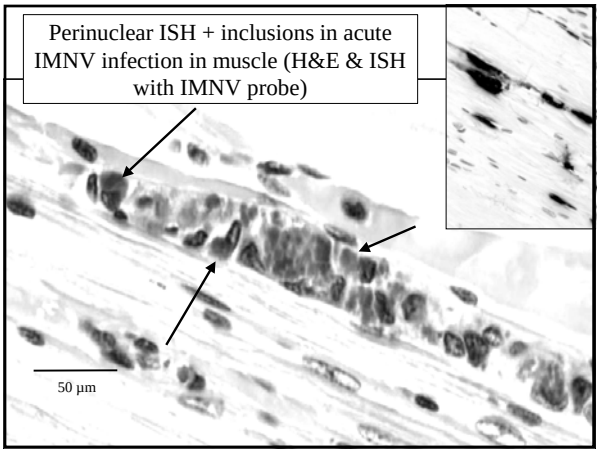
IMN in young juvenile *L. vannamei*

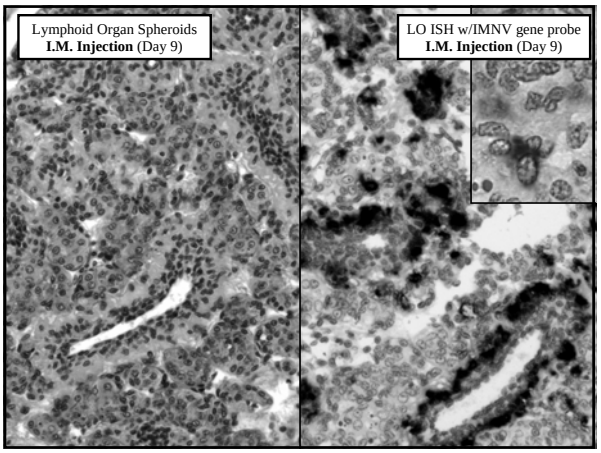




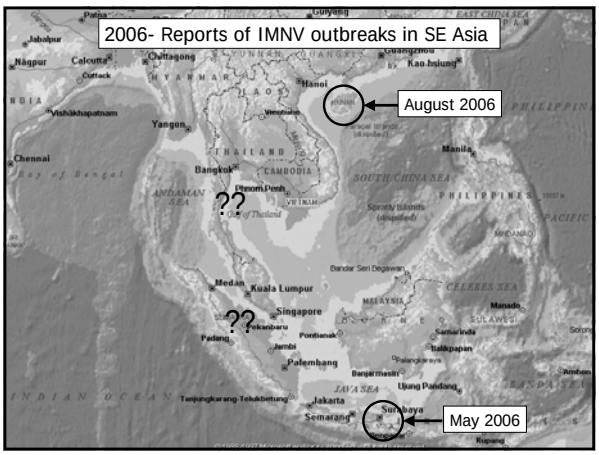

Lymphoid organ spheroids (LOS)
LO with G3 LOS
L. vannamei Clinical sample


Ectopic LOS in heart & antennal gland
L. vannamei Clinical sample


Perinuclear ISH + inclusions in acute IMNV infection in muscle (H&E & ISH with IMNV probe)
50 µm


Lymphoid Organ Spheroids
I.M. Injection (Day 9)
LO ISH w/IMNV gene probe
I.M. Injection (Day 9)


IMNV
Smart Detection for Better Production
IQ2000
IMNV
IQ2000™ IMNV Detection and Prevention System
Infectious Myonecrosis Virus Detection and Prevention for Shrimp Farming


2006- Reports of IMNV outbreaks in SE Asia
August 2006
May 2006
??
??
Hanoi
Yangon
Chennai
Bangkok
Phnom Penh
Manila
Medan
Kuala Lumpur
Singapore
Jakarta
Semarang
Surabaya
Palembang
Pekanbaru
Samarinda
Balikpapan
Manado
Ujung Pandang
Kao-hsiung
Guangzhou
Calcutta
Nagpur
Jabalpur
Vishakhapatnam
Cuttack
Chittagong
Tanjungkarang-Telukbetung

IMNV Economic Impact since Disease Emerged in 2002

- Brazil:
  - ✓ 2004 losses ~$20 million (Nunes 2004)
  - ✓ lost production since IMN disease emerged may be ~$200 million in NE Brazil.
- Indonesia & Hainan, China:
  - ✓ value of production losses?
  - ✓ potential for spread throughout region?

Penaeus vannamei specimens from Belize in 2005 with IMN-like pathology

- Significant mortalities noticed.
- Affected shrimp presented opaque muscle.
- Histopathology consistent with IMNV infection:
  - ✓ Acute, subacute & resolving myonecrosis.
  - ✓ Significant formation of lymphoid organ spheroids.
- However, RT-PCR tests & ISH for IMNV were negative.

Experimental transmission of the Belize agent

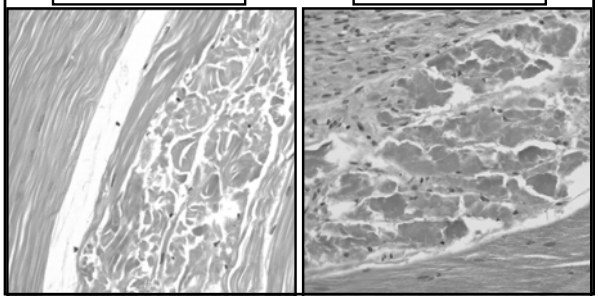
- Frozen P*. vannamei* from Belize presenting presumptive IMN-like gross signs were used.
- SPF Kona-line P*. vannamei* fed test shrimp in challenge bioassay.
- Challenged Kona shrimp developed IMN-like gross signs & pathology, but RT-PCR & ISH results remained negative for IMNV.
- A new nodavirus was isolated from challenged shrimp. PvNV.

Gross Signs – experimental infection PvNV

Muscle – acute phase of infection

IMNV - skeletal muscle acute necrosis

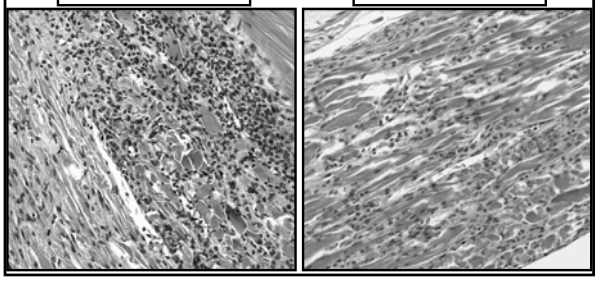
PvNV - skeletal muscle acute necrosis



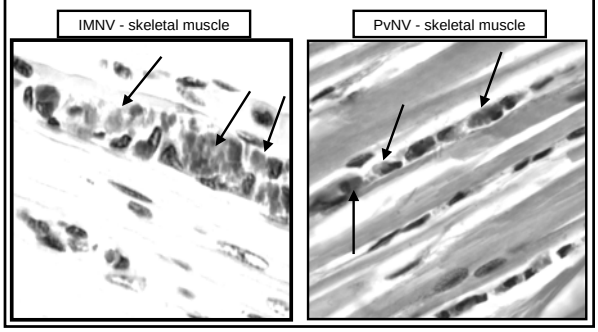
Muscle – inflammatory phase of infection

IMNV - skeletal muscle

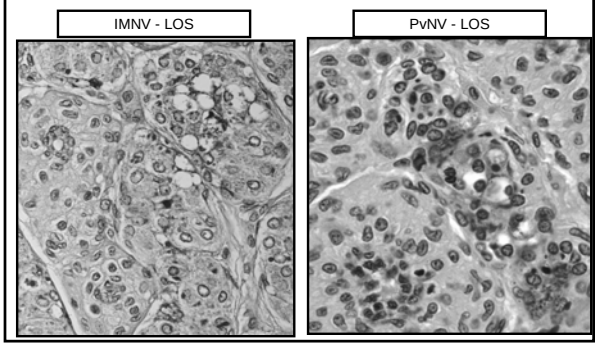
PvNV - skeletal muscle



Basophilic cytoplasmic inclusion bodies

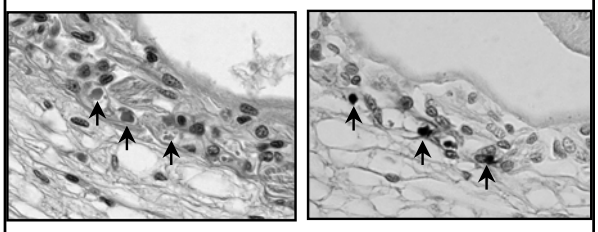


Lymphoid organ spheroids (LOS)



In situ HYBRIDIZATION ASSAY

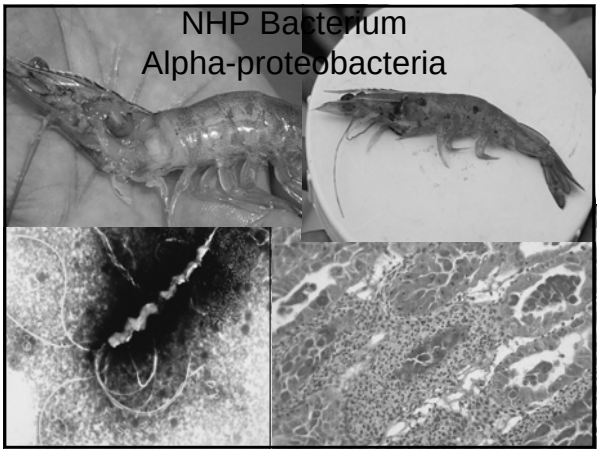
Gene probe prepared from 928 bp insert in clone PvNV-4



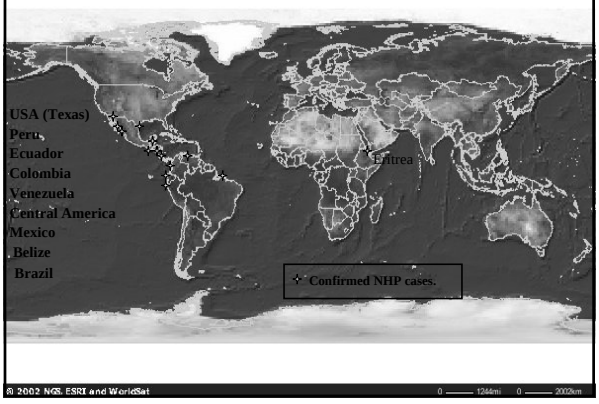
Comparison of IMNV and PvNV

Characteristic	IMNV (Totiviridae)	PvNV (Nodaviridae)
Gross signs	Muscle necrosis	Muscle necrosis
Particle shape, size	Icosahedral, 40 nm	Icosahedral, 30 nm
Nucleic acid	ds RNA-7560 bp	ss RNA-2 molecules-4328 bp
Genome organization	5'ORF=capsid protein 3'ORF=RNA polymerase	RNA 1=RNA polymerase RNA 2=capsid protein
Buoyant density	1.366 g/cm^3 in CsCl	Not Determined
Capsid protein	106 kDa	Tentative 67-79 kDa
Host Range (Experimental)	*P. vannamei* *(P. stylirostris, P. monodon)*	*P. vannamei* *(P. monodon)*
Molecular tests (ISH and RT-PCR)	No cross-reaction with PvNv	No cross-reaction with IMNV

NHP Bacterium
Alpha-proteobacteria

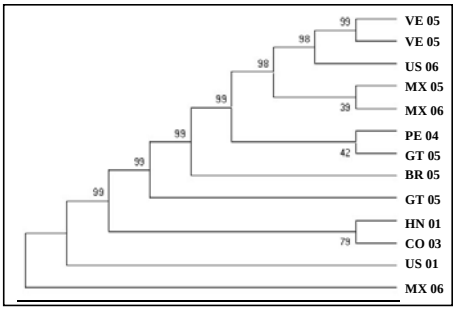


NHP GEOGRAPHIC DISTRIBUTION



NHP Geographic Isolates: Only 1 Strain?

Bootstrap Test of Phylogeny: Consensus Tree



(Sequences based on gryB gene region: updated 06-15-06)

NHP Disease Management

- Farm designed with deeper ponds to mitigate high temperatures.
- Water exchange to reduce salinity.
- Metaphylactic use of medicated feeds (OTC at 1.5 to 4 kg/t feed for 10-14 days).
  - ✓ anticipate from farm history when NHP likely to occur.
  - ✓ have medicated feed available on short notice
  - ✓ monitor stocks & begin treatment at first sign of disease.
  - ✓ Withdrawal for OTC is 2 days (FDA/INAD), but $\geq$ 7 days is advisable.
- Florfenicol recently developed for NHP in Mexico.

VIBRIOSIS

Primary and Secondary Infection by *Vibrio* spp.

- **Systemic infections.**
- **Enteric & oral region infections.**
- **Focal appendage necrosis.**
- **Wound infections.**
- **Shell disease.**

VIBRIO - Agents

Characteristics:

- Gram negative, pleomorphic, curved rods.
- Halophilic, require salt ($\geq$ 10 ‰ = $\geq$1%) for growth.
- Common in marine environments.
- Opportunistic or primary pathogens.
- Many species are in shrimp's normal microflora.

Antibiotic Resistance – An Emerging Problem?

- *Vibrio parahaemolyticus* – most strains sensitive to OTC & Romet.
- Resistant strains documented:
  - ✓Texas - resistant to >100 µl OTC/ml OTC.
  - ✓Sonora - strains resistant to OTC & Romet.
- Possible consequence of using these compounds to manage NHP w/o rotation?

METHODS FOR DISEASE MANAGEMENT IN PENAEID SHRIMP AQUACULTURE

- Maintain adequate water quality.
- Improve culture techniques & farm design to reduce stress, minimize handling.
- Sanitation among culture units & between crops
- Adequate feeds.
- Chemotherapy & antibiotics when necessary.
- Probiotics, immunostimulants.
- Use resistant stocks where pathogen enzootic.
- Avoidance where pathogen can be excluded.
  - ✓ use stocks that test negative for significant pathogens.
  - ✓ use SPF stocks when available.

Main viral pandemics since1986

- These viral diseases can only be controlled by avoidance.
- The shrimp virus pandemics have changed the way shrimp are farmed.
- The requirement for clean shrimp stocks set in motion the industry switch to domesticated, SPF *P. vannamei.*

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