Crustacean diseases of concern in Africa:

White Spot Disease as a case study for exotic pathogens, & important endemic pathogens described in the region

OIE Regional Training Seminar :
OIE Focal points on aquatic animals

June 15 - 19, 2010 :
Swakopmund (NA)

Marc Le Groumellec, Aqualma, Madagascar

Southern Africa Shrimp Farm Model: a brief presentation

- Farm Siting: Salt flat only, with minimum mangrove clearing
- Farm design: semi-intensive, big farms (up to 800 Ha), big ponds (10 Ha); Latin american model
- Water use: separation of intake and discharge. Continuous water exchange
- Feed management: high quality feed, overall good SR, growth and low FCR.
- Aiming at big sizes, premium quality shrimp recognized by certifications (Label Rouge, ISO 9001, IFS/BRC, WWF private partnership

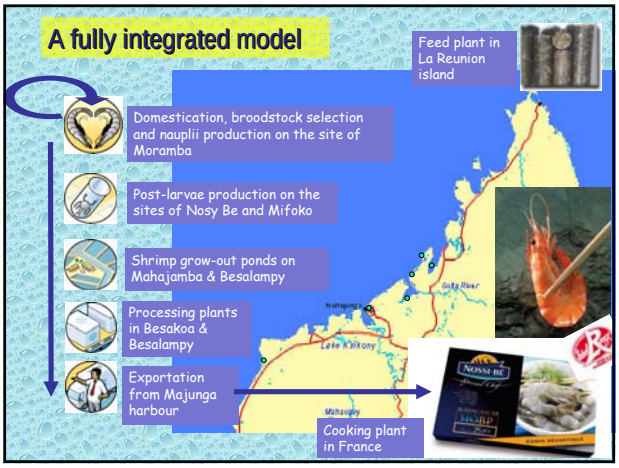
- Due to bad logistics, high energy costs, the breakeven is high and is only achieved through high performances of the farm and global organization.

Southern Africa Shrimp Farm Model: a brief presentation

- Environmental responsibility: ecological surveillance of the bay, mangrove plantation programs
- Social Responsibility: building hospitals, schools, electric power, water availability.
- Health management: st… filtration, surveillance prog… laboratory, pond disinfecti…
- Food safety: internal foo…

Conclusion: since 1991, the project respected recom… published by FAO in its "international principles for responsible shrimp farming", aiming a long term sustainable business.

A fully integrated model



Label Rouge: certification de la qualité supérieure du produit fini

- Objectifs:
  Mettre en évidence la qualité… produit fini.
- Besoins :
  Définir les caractéristiques … souhaité communiquer car … du milieu de gamme. Standa… originellement validés par d… scientifiques. Traçabilité complète, … semestriel.
- Bénéfices attendus :
  différenciation positive du produit et … du marché, en produisant constamment … qualité supérieure.

Thank you for your attention!

Examples of potential vectors / hosts

Big diversity of vectors, sometimes morphologically similar, but carrying different pathogens

These farms are highly submitted to a diversity of wild crustaceans, all of which are potential vectors for diseases

Disease diagnosis lab for shrimp & others

- Histopathology
- In Situ Hybridization
- Molecular Biology:
  - PCR
  - RT-PCR
- ISO Certified

Working with reference labs:

- UAZ TAA, since 1997
- Dr Lo, Taiwan
- French Universities

Updated on OIE listed and other exotic diseases

Research on new / emerging

Why other countries drastically changed their rearing methods ?

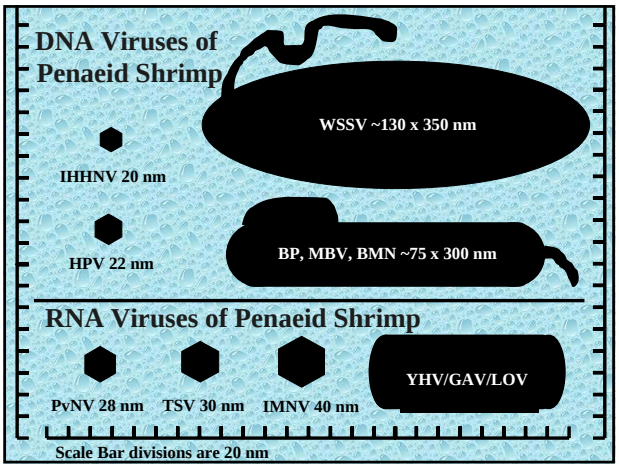
White Spot Disease case study: example of an exotic disease

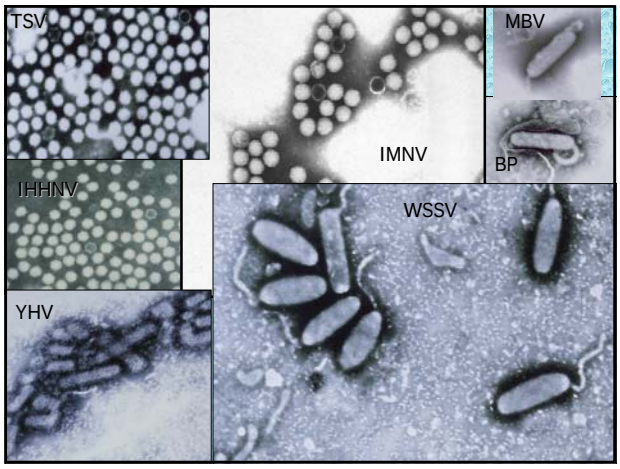
White Spot Syndrome Virus*

Family *Nimaviridae* n.f.; Genus *Whispovirus* ; Type Species: *WSSV*

Replication	**Nuclear**
Morphology	**enveloped, elliptical rod with prominent apical "tail"**
Size	**virion: 80-120 x 250-380 nm nucleocapsid: 58-67x230-350 nm**
Density	**1.18-1.25 g/ml**
Nuclei Acid	**dsDNA, circular, supercoiled, ~305 kb**
ORFs	**~531 ORFs for 181 functional proteins**

* Various publications 1995-2002

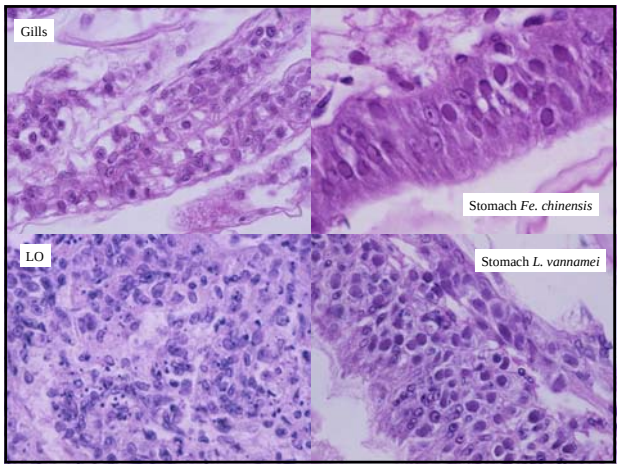


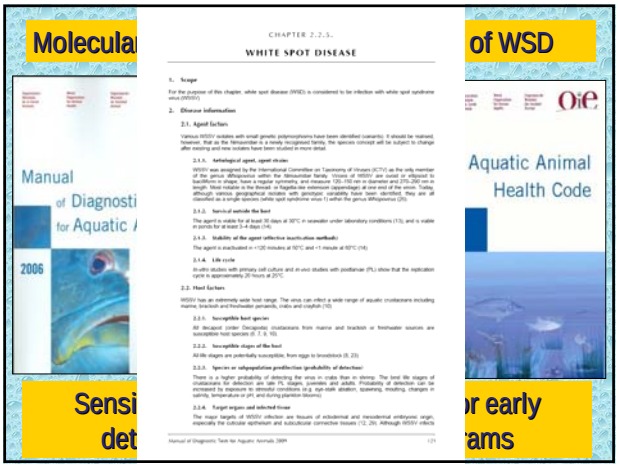


White Spot / Red Disease
Clinical Signs

- Sudden reduction in feeding, lethargic.
- Red discoloration in *P. monodon, L. vannamei, L. stylirostris*.
- Soft, loose shells
- White spots 0.5 to 2 mm under cuticle (less common in Western Hemisphere penaeids)
- Up to 100% mortality within 3 days of onset of disease signs.

Acute WSSV disease in adult Pacific white shrimp *L.vannamei*


Gills
Stomach *Fe. chinensis*
LO
Stomach *L. vannamei*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WHITE SPOT DISEASE
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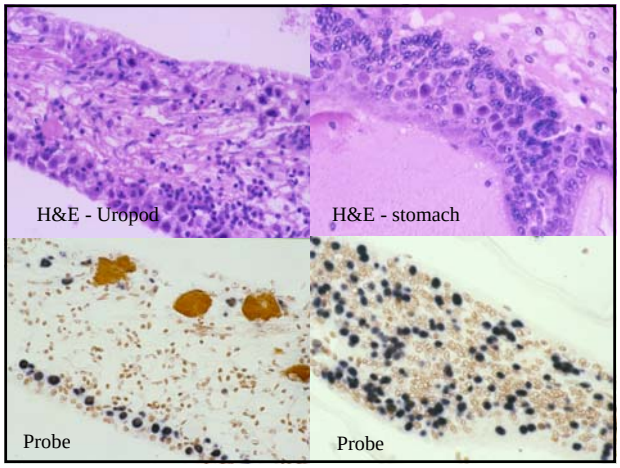
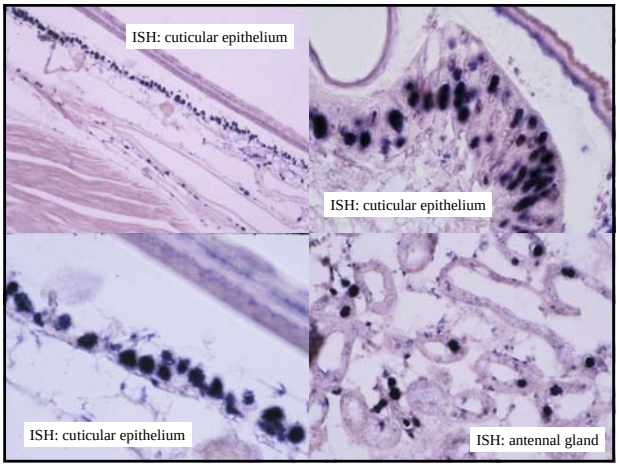
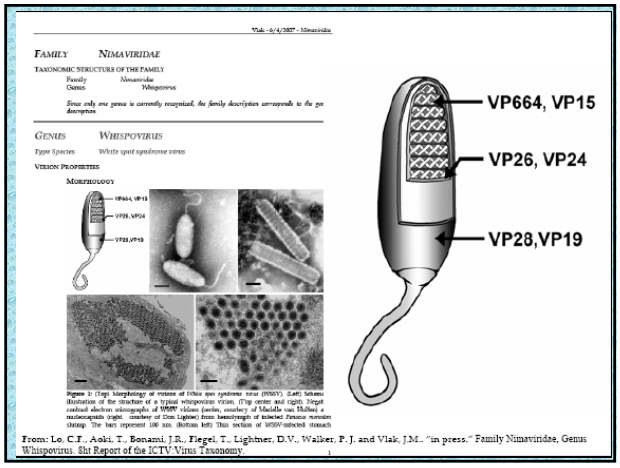
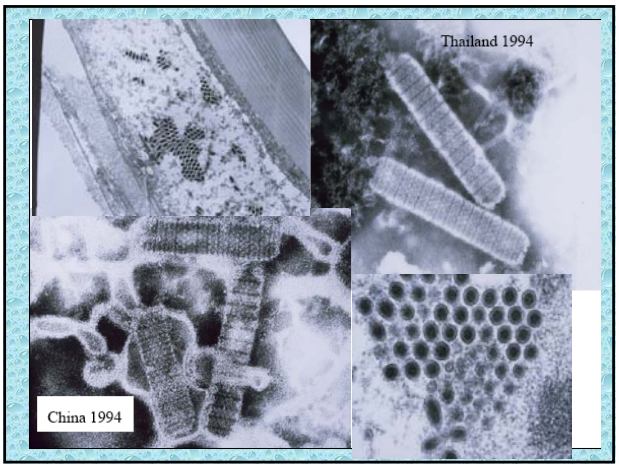

H&E - Uropod
H&E - stomach
Probe
Probe


ISH: cuticular epithelium
ISH: cuticular epithelium
ISH: cuticular epithelium
ISH: antennal gland


FAMILY NIMAVIRIDAE
GENUS WHISPOVIRUS
VP664, VP15
VP26, VP24
VP28,VP19
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.



White Spot Syndrome Virus

Family *Nimaviridae*

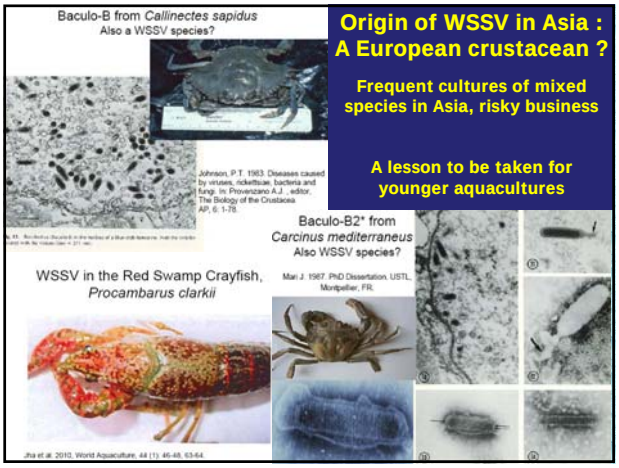
- **WSSV causes significant disease in:**
  - **All penaeids – acute disease & high mortalities**
  - Crabs - several genera
  - Freshwater prawns
  - Spiny lobsters
  - Freshwater crayfish
- **Known natural & experimental hosts is > 50 species.**

White Spot Syndrome Virus

Partial List of Natural & Experimental Hosts (Western Hemisphere*)

P. monodon
P. semisulcatus
Fe. merguiensis
Fe. indicus
Fe. chinensis
Fe. penicillatus
Ma. japonicus
**L. vannamei*
**L. stylirostris*
**L. setiferus*
**L. occidentalis*
**Fa. aztecus*
**Fa. duorarum*
**Fa. Californiensis*

Metapenaeus ensis
Trachypenaeus curvirostris
Exopalaemon orientalis
Macrobrachium rosenbergii
**Ocronectes punctimanus*
**Promcambarus clarkii*
Charybdis feriatus
Portunus pelagicus
Portunus sanguinoientus
Scylla serrata
Thalamita sp.
Panulirus versicolor
Panulirus penicillatus
Cherax quadricarinatus



Origin of WSSV in Asia : A European crustacean ?

Frequent cultures of mixed species in Asia, risky business

A lesson to be taken for younger aquacultures

White Spot / Red Disease

Epidemiological gross data

- Transmission modes :
  Horizontal : through cannibalism, predation on contaminated wild fauna. Free form in water. Remains infectious in the soil for several days. Birds are an important vector for long distances infections of a new zone
  Vertical : a female at low grade infection can produce carriers in offsprings.
- Temperature drops triggers the disease (below 30°C-28°C).
- Diffusion of the disease worldwide due to unregulated live and frozen animals transports

White Spot / Red Disease

Disease management

In a country free from the WS disease:

- Active and passive surveillance
- Regulations forbidding imports
- If not possible: quarantine
- If there is an outbreak: early detection. Eradication. Fallowing is the only solution. Continued surveillance.

In a country where WSD is endemic:

- Deeply modifies the rearing methods: water treatment is mandatory (filtration, disinfectants, pesticides ?), and consequently intensification plus strong aeration to reduce water exchange. Stock SPF post-larvae.
- National Focal Points: zoning or compartmentalization ?

White Spot Syndrome Virus (WSSV)

From Dr. P. Walker, CSIRO, Australia

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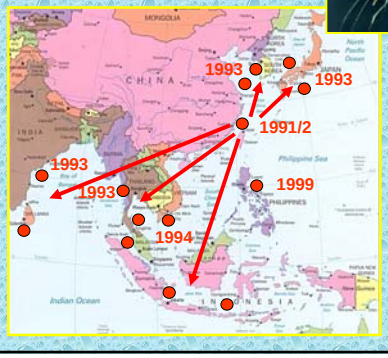
The Asian pandemic

From Dr. P. Walker, CSIRO, Australia



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

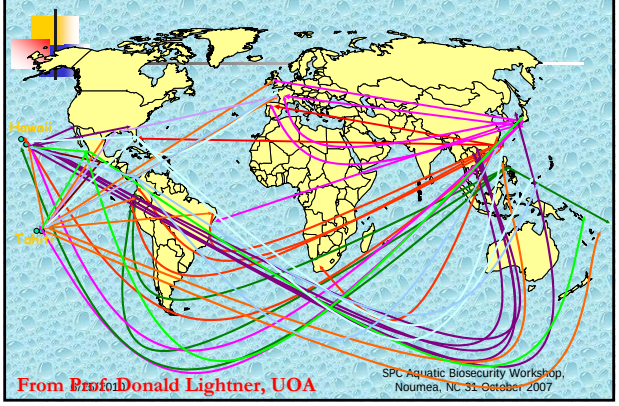
Introduction from Asia & Spread of WSSV in the Americas

(date) = eradicated from affected farms

Penaeus monodon in Southern Africa:

one of the only important regions remaining with a free status for the more nasty epidemic diseases

Global Transfers of Live Shrimp



From Prof. Donald Lightner, UOA

Recent movements of *Penaeus monodon* in the world

Why did Asia and Americas fail to stop this epizootics ?

Not enough knowledge on diseases shared by all stakeholders
Low involvement of the private sector
Nice regulations and technical guides, but smuggling and illegal imports almost everywhere

What could we learn from history ?

Capacity building of National Focal Points, including emerging diseases & early detection
Biosecurity implementation
Active surveillance programs (and passive through farmers)
Capacity building of all stakeholders, network including farmers
One good example for Africa: Australia
We will succeed or fail together, arrival of WSD means the end of this original model, and probably the collapse of this industry
Consider working regionally for more efficiency

YES WE CAN !

CCl: Central role and responsibility of OIE National Focal Points

Current regulations and strategy in Madagascar

Active regulations forbidding live or frozen crustacean imports (Décret n°2001/030)
Self declaration of freedom process, active surveillance program for WSD, TSD, YHD. Surveillance plan (active and passive) validated by an international epidemiologist expert.

Opportunity for Africa to stop exotic crustacean diseases

Opportunity to build a different approach, taking advantage of the recent development of crustacean aquaculture in Africa (not only shrimp, but crabs, lobsters, etc.). Learn for others' mistakes.
Regional organization similar to NACA required (Sarnissa, others ?)
Help from FAO, OIE needed
Share analytical and expertise resources for the region ?

CCl: crustaceans having nearly no defenses against these pathogens, the best solution is maximum prevention

Others aspects on National Focal points activities

Rickettsia Like bacteria and microsporidia case studies: examples of local disease of concern (both on Madagascar and mainland Africa)

Rickettsia Like Bacteria systemic

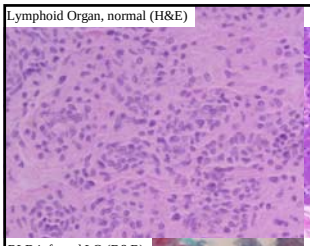
- Morphology: bacillary form, Gram negative.
- Dimensions: 0.45 µm x 1.5 µm.
- Replication: Cytoplasmic; not intravacuoles.
- Target tissues: connective tissues, lymphoid organ , cuticular epithelial cells, hematopoietic cells & hemocytes.
- Taxonomy: Proteobacteria, probably a gamma-proteobacteria, while NHP is an alpha-proteobacteria and Rickettsiales is a separate order. No relations with human rickettsias transmitted through arthropods (Ixodes, etc.)

Rickettsia Like Bacteria "Milky" Disease
Clinical Signs

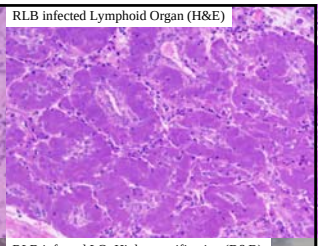
- **Lethargic shrimp at the edge of the pond, presence of birds.**
- **No obvious impact on feed consumption or growth**
- **No discoloration in *P. monodon*. Dirty gills.**
- **Hepatopancreas pale. HP and lymphoid organ hypertrophied**
- **Hemolymph is white, looking like milk. Absence of clotting**

Rickettsia Like Bacteria Disease
Clinical Signs and wet mount observations

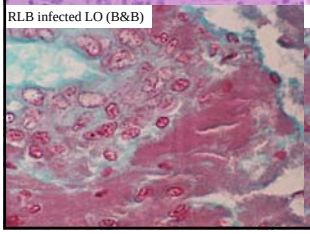
Lymphoid Organ, normal (H&E)



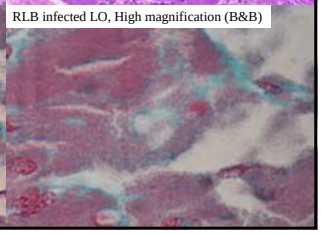
RLB infected Lymphoid Organ (H&E)

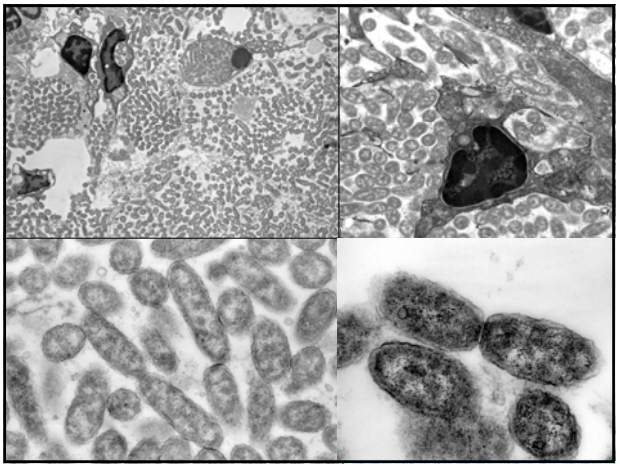


RLB infected LO (B&B)



RLB infected LO, High magnification (B&B)





PCR reaction for *Rickettsia Like Bacteria*

Madagascar Rickettsia

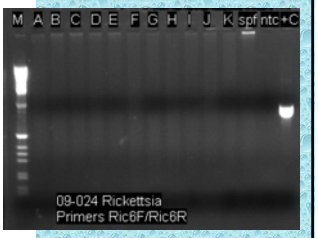
DNA Templates
1. Extracted frozen tissue
2. Extracted fixed tissue
3. Extracted hemolymph

PCR Reaction Mixture

Reagent	25 µl PCR Beads	Final Concentration
dH_2O	23.5 µl	dNTPs = 200 µM each Primers = 0.31 µM each $MgCl_2$ = 1.5 mM Enzyme = 2.5 U Buffer = 10 mM Tris, pH 9, 50 mM KCl
RIC6F	0.5 µl	
RIC6R	0.5 µl	
Template	0.5 µl	

PCR Cycling Parameters

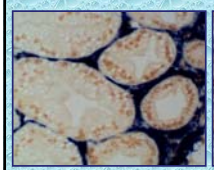
Time	Temperature (°C)	# of cycles
2 min	95	1
30 s, 2 min, 30 s	65, 72, 95	30
1 min, 2 min	45, 72	1
HOLD	4	



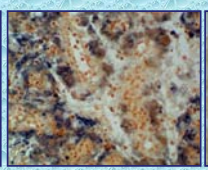
Madagascar Rickettsia Primers

Primers	Sequence	Amplicon size
RIC6F	TGG CAG TAC GGA ATA ACC TGA G	841 bp
RIC6R	ATC TGC GCG AGA GGC ATG TCA A	

In situ hybridization with Rickettsia Like Bacteria specific probe labeled with digoxigenin



Rickettsia : natural infection of *P. monodon by* RLB. Connective tissue of hepatopancreas 'intertubular)



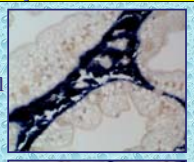
Rickettsia: experimental infection of *L. vannamei* by RLB, lymphoid organ

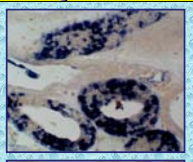
NHP: natural infection of *L. vannamei by NHP. No hybridization with RLB specific probe*

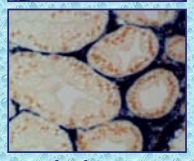
Specificity of RLB ISH and generic probes (differential diagnostic)

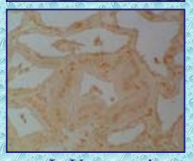
Generic bacterial probe

RLB probe





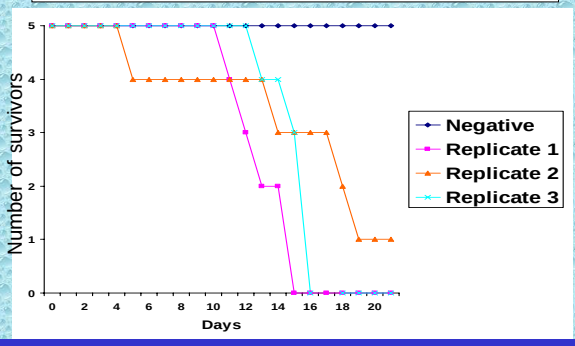




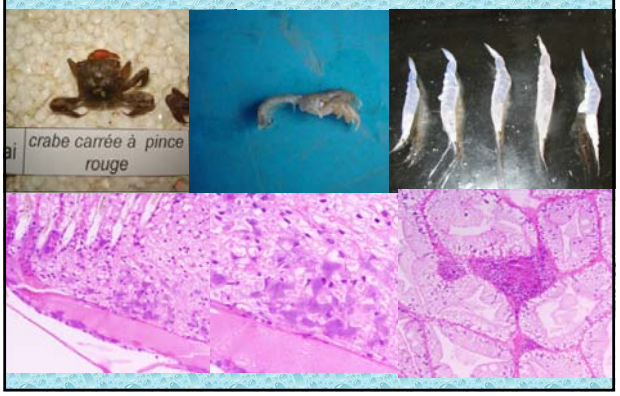
P. monodon hepatopancreas infected by RLB (Intertubular spaces)

L. Vannamei hepatopancreas infected by NHP

RLB experimental infection: injection challenge on *Litopenaeus vannamei*



Identified and suspected vectors for RLB



Microsporidian & Haplosporidian Parasites of Penaeid Shrimp

Microsporidian	Target Tissue(s)	Spores per Sporant	Spore Dimensions
Ameson =(Nosema) nelsoni	Muscle	1	2.0 x 1.2 µm
Ameson spp.			
Agmasoma (=Thelohania) penaei	CT, gonads, vessels, occasionally muscle	8	8.2 x 5 µm & 5.0 x 2.0 µm
Agmasoma duorara	Muscle, CT	8	5.4 x 3.6 µm
Agmasoma spp.			
Pleistophora spp.	Muscle, heart, CT, HP	16 to 40+	2.6 x 2.1 µm
HP microsporidian of *Penaeus monodon*	HP	multiple	~2 X 1 µm
HP haplosporidian of *Litopenaeus vannamei*	HP	multiple	~2 X 2 µm* (spores not known)

Microsporidia *Ameson michaelis*

Morphology	monomorphic, ovalum spore, refringent, Gram negative, non grouped
Dimensions	0.50 µm x 1 µm
Replication	Obligatory intracellular parasite. Cytoplasmic
Target Tissue	Muscle

COTTON SHRIMP CLINICAL SIGNS

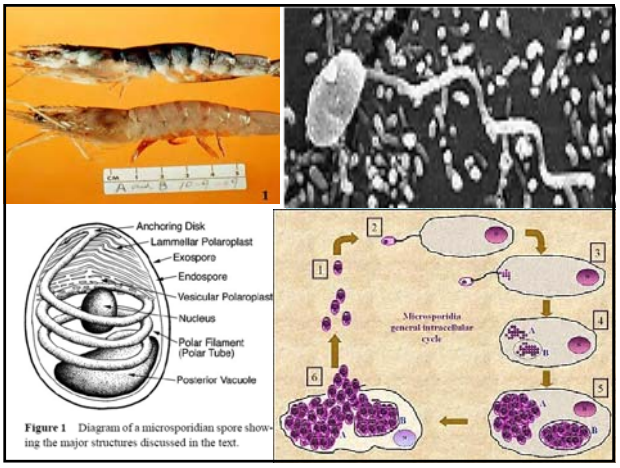
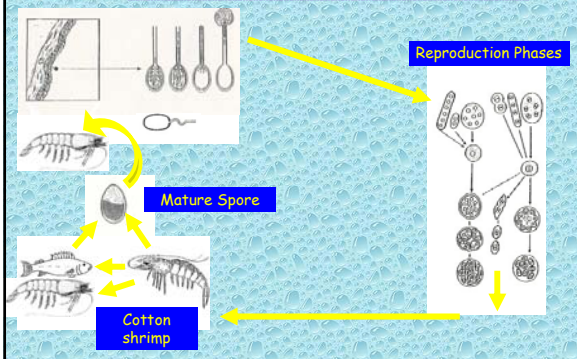
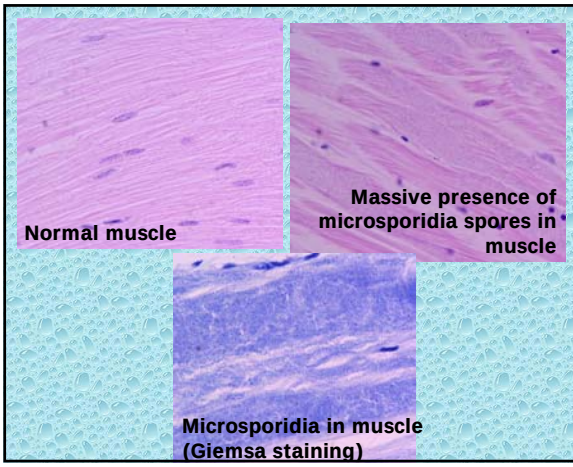


Figure 1 Diagram of a microsporidian spore showing the major structures discussed in the text.

MICROSPORIDIAN BIOLOGICAL CYCLE IN SHRIMP





MICROSPORIDIANS IN SOUTHERN AFRICA
Transmission Electronic Microscopy

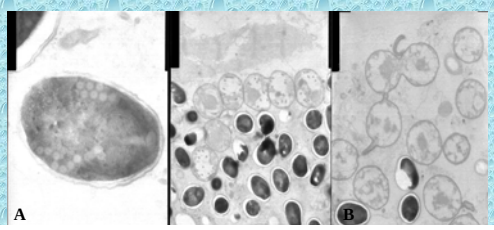


Figure 5 : *Penaeus monodon and Fenneropenaeus indicus.* A : spore with 9 spires, B: chains of sporoblasts

Conclusion: same species of parasites on *F. Indicus* and *P. Monodon*. Genus a priori *Ameson*, considering the grouping mode of sporoblasts.

Molecular tools for Ameson

The alignment of the sequence obtained is given below

Query: 69 caccaggttgattctgcctgacgtagacgctagtttcacagattgatccatgcaatttta 128

Sbjct: 1 caccaggttgattctgcctgacgtagacgctagtttcacagattgatccatgcaatttta 60

Conclusion: an amplification protocol of a specific portion of the genome has been designed. After testing its specificity and sensitivity, technology was finalized

Conclusions on National Focal points roles

Challenges for National Focal Points:

+ Participate in keeping Africa free from extremely deleterious pathogens such as WSD, YHD, TSD and IMND

+ Deal with emerging diseases of the Continent

+ Design and run successfully quarantines, zoning and compartmentalization

+ Find ways to involve actively private sector, using conviction and building confidence, notably using the help of private vets or aquatic sanitary agents, as the first front against diseases. Reactivity is key to success. Raising biosecurity levels is necessary.

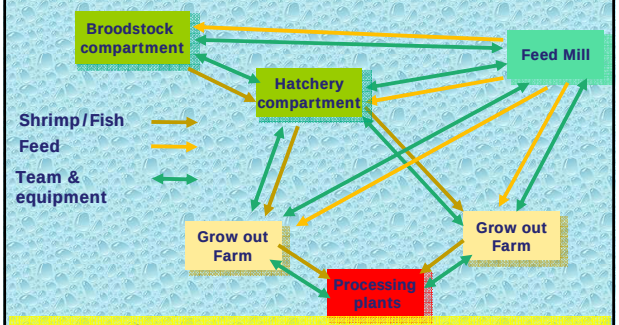
Huge responsibility for the future of crustacean aquaculture in Africa.

In parallel, domestication of local species should be actively promoted:

+ excellent exercise to quickly learn how to close the biological cycle, control all risks linked to aquaculture activities (pathologic, genetic, environmental, animal welfare).

+ It avoids the need of live animals importations.

A model of compartment for aquaculture industry



A possible future organization involving veterinarians for more efficient biosecurity practices in aquaculture: mimicking an integrated company through associations

The ultimate weapon for National Focal Points to fight against both exotic and local diseases

DOMESTICATON !

Thank you for your attention !

Some slides courtesy of Donald Lightner, UAZ, and Melba Bondad-Reantaso

Rickettsia Like Bacteria Disease

Clinical Signs and wet mount observations

1°) Moribond shrimp with no exterior signs (except a dirty gills aspect)

2°) Milky hemolymph and absence of clotting

3°) Hypertrophied hepatopancreas , pale

4°) Hypertrophied lymphoid organ

5°) Gram negative bacilli under microscope, observed even in hemocytes cytoplasm