FAO/ASTF Project: GCP/RAF/510/MUL:

Enhancing capacity/risk reduction of emerging Tilapia Lake Virus (TiLV) to African tilapia aquaculture: Intensive Training Course on TiLV

4-13 December 2018. Kisumu, Kenya

in cooperation with Kenya Marine Fisheries Research Institute (KMFRI) and Kenya Fisheries Service (KeFS)

Session 2:

Fish and shrimp viruses

ktangnelson@gmail.com

Food and Agriculture Organization of the United Nations

Fish viruses

- Infecting a closely related group of species
  Note: TiLV spcies name: *tilapia tilapinevirus*
- Mortality is temperature-dependent
- Mortality is age-dependent; young fish is more susceptible and has a higher mortality, the survivors became carriers
- Gross signs:
  - abdomenal swelling
  - hemorrhages on the body surface
  - exophthalmos (popped eyes)
  - necrosis in the infected organs
  - the presence of viral inclusion bodies by histology

How the viruses transmit

- Horizontal transmission through
  -shedding: viruses present semen or ovarian fluid, feces, urine, body fluid, and release into water
  -carriers: viruses present in the asymptomatic fish

- Vertical transmission
  -viruses present in the broodstock (ovary, testis), which pass the viruses to the offspring

Prevention and control strategies

- Avoidance
- Biosecurity

- Quarantine
- Destroy the infected stocks
- Disinfect water before discharge
- Not to stock the fish for 6-month
- Monitor the wild populations
- Filter the intake water
- Test the water with most susceptible species
- Stock virus-free fish

Management strategies

- Culture the fish in low (or high) temperature season
- Stock more fry and fingerings
- 2-stage culture: grow the young fish in the virus-free water for 1-6 months, then transfer them to the grow-out ponds
- stock fish species that are resistant to disease
- Reduce stress
  - Lower stocking density, lower handling stress
- Vaccination (vaccines need licenses)

Virus	Species	Host range	High mortality	Geographic distribution
IPNV	freshwater & marine	wide	High temperature	Widely spread
IHNV	salmon fish and trout	wide	Cold temperature (< 10^{o}C)	Widely spread
ISAV	marine	narrow	At <3^{o}C or >15^{o}C	Norway,UK,Canada,USA,Chile
VER (NNV)	primarily in marine species	wide	High temperature (e.g. 22^{o}C)	The Pacific region
EHNV	freshwater	narrow	Cold temperature	Australia
ISKNV	freshwater	wide	Warm water	China and the Pacific
KHV	freshwater	narrow	<13^{o}C or >23^{o}C	Asia Pacific

OIE-listed Fish Diseases (2018)

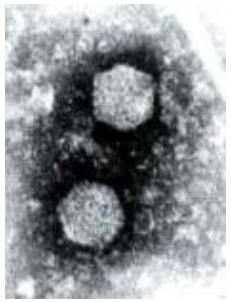
1. Infection with epizootic haematopoietic necrosis virus (**EHNS**)
2. Infection with *Aphanomyces invadans* (epizootic ulcerative syndrome)
3. Infection with *Gyrodactylus salaris*
4. Infection with infectious haematopoietic necrosis virus (**IHNV**)
5. infection with *HPR-deleted or HPR0* infectious salmon anaemia virus *(***ISAV***)*
6. infection with *salmonid alphavirus*
7. Koi herpes disease (**KHV**)
8. Red sea bream iridoviral disease
9. Spring viraemia of carp
10. Viral haemorrhagic septicaemia
11. *Oncorhynchus masou* virus disease
12. Viral encephalopathy and retinopathy (**VER)**

http://www.oie.int/standard-setting/aquatic-manual/access-online/

Major Fish Viruses

- **Infectious pancreatic necrosis virus (IPNV)**
- Infectious hematopoetic necrosis virus (IHNV)
- Infectous salmond anemia virus (ISAV)
- Nervous necrosis virus (NNV)
- Epizootic hematopoietic necrosis virus (EHNV)
- **Infectious spleen and kidney virus (ISKNV)**
- Koi herpes virus (KHV)

Infectious pancreatic necrosis virus (IPNV)



- IPNV is an aquatic birnaviurs, icosahedral shaped, with a size of 65 nm, no envelope
- The genome (4.5 kb) consists of 2 segments of dsRNA
  - Segment A: 3,099 bp
  - Segment B: 2,789 bp
  - A stable virus, remains infectious for 3 months in water
- Can be inactivated by chlorine, formalin, iodine, ozone, high pH (pH 12)

Susceptible species and mortality

- infects all salmonids; brook trout and rainbow trout are most susceptible
- acute infection, a major concern for salmonid aquaculture
- high mortality (upto 95%) in young salmonids (fry and fingerlings); reduced mortality in older fish.
- surviving fish can become carriers (90%), in some cases, viruses were not detected, but re-appear during the spawning
- Mortality is most severe at high temperatures

Clinical signs

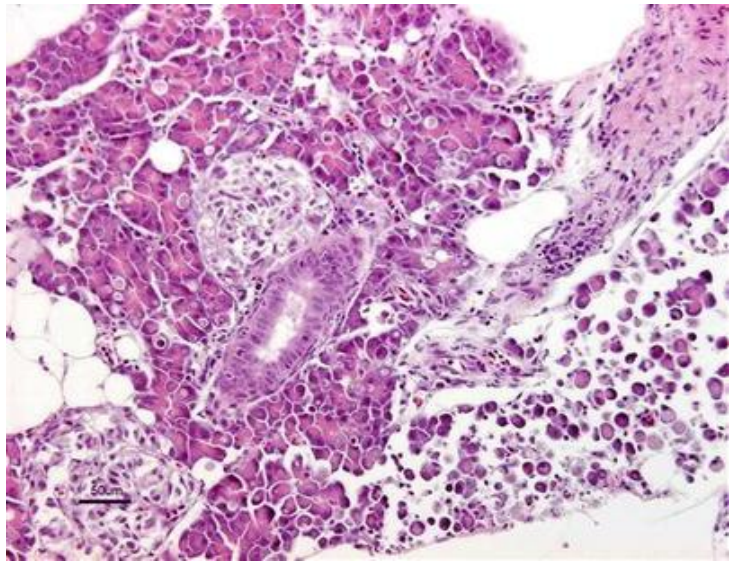
- Sudden mortalities in fry and fingerings of trout
- Neurological signs: whirling/spirally swimming
- Anorexia
- Dorsal darkening
- Swollen abdomen
- Exophthalmos
- Hemorrhage in the ventral part
- Internal pathology:

  The intestines are empty or filled with clear mucous, white fecal strings (pseudocast) from the vent.

Histopathology

IPNV

- Infect pancreatic acinar cells and cause acute necrosis
- Presence of McKnight cells (eosinophilic cells found in the lumen);
- Appearance of basophilic cytoplasmic inclusion bodies
- Virus replicates in pancreas, kidney, liver, gonad, spleen, intestine, and pyloric ceca



IPN infection in a farmed Atlantic salmon smolt. Necrosis of pancreatic cells (lower right) and normal tissue (upper left)

IPNV transmission

- Horizontal transmission

-IPNV present in the water and can infect naïve fish

-survivors became carriers (reservoir hosts), which can shed viruses in the feces and urine for over 2 years.

-viruses have been detected in the bird feces.

-viruses are detected in the mucoid white fecal strings.

- Vertical transmission

-from reproductive fluids and eggs, thus infected broodstock are risk populations

Infectious hematopoietic necrosis virus (IHNV)

- Infectious hematopoietic necrosis virus (IHNV) is a bullet-shaped, enveloped, virus.
- Its size is 110 nm long x 70 nm in dia.
- IHNV is a rhabdovirus, contains one negative-sense, ssRNA fragment. The genome size is 11-kb.
- Sensitive to heat and high pH

- Two other rhabdoviruses listed by OIE: VHSV (viral hemorrhage septicemia virus) and SVCV (spring viremia of carp virus)

Susceptible species and mortality

- Affects salmonids: rainbow trout, Altantic, sockeye and chinook salmon
- Coho salmond, brook trout, brown trout are resistant to IHNV
- 90-95% mortality in fry, reduced mortality in older fish, adult carriers are asymptomatic.
- Peak mortality (100%) at 10^{o}C, no disease when >15^{o}C

IHNV

Clinical signs

- Abnormal behavior: hyperactive, whirling
- Lethargic;
- Pale gills and viscera (from anemia);
- Dark discoloration in the dorsal
- Extensive haemorrhages on the abdomen and in the eyes around the pupil
- The presence of milky fluid in the stomach and intestines, white, thick, fecal strings trailing form the rectum

Histopathology

- extensive necrosis in hematopoietic tissue in kidney, spleen, and liver
- in hepatocytes (liver), there are cytoplasmic inclusion bodies and karyorrehetic nuclei

Transmission of IHNV

- Horizontal transmission

-ingesting infected tissue, or feces

-viruses were shed into water

-survivors became carriers, which can shed viruses in the feces and urine.

-adults shed viruses at spawning

- Vertical transmission

-from reproductive fluids and eggs,

-infected broodstock are the principle risk, eliminating infected stocks

Prevention and control measures

- use IHNV-free stock;
- disinfect eggs (25 ppm iodine for 5 min)

- quarantine
- disinfect water with UV, ozone,
- test and slaughter (broodstock culling)
- elevate culture temperature (>15^{o}C) for IHNV infected fish, with smaller operating facilities (hatchery producing eggs, fry)
- vaccination

Infectious salmon anemia virus (ISAV)

- ISA is a important pathogen for Atlantic salmon farming, listed by OIE
- ISA has an envelope with large surface glycoproteins spikes, polymorphic in shape
- Spherical: 90-140 nm in dia., filamentous: >300 nm long,
- ISA is an isavirus, contains 8 segments of negative-sense ssRNA (sizes ranging from 1.0 to 2.4 kb), the total genome size is 14.3 kb.
- Virus is stable for 20 hr in the seawater
- Sensitive to heat, very low/high pH, drying, lipid soluble solvents (e.g. chloroform, SDS)

Mortality

- infect Altantic salmon (*Salmo salar*), discovered in Norway in 1984
- Sea trout and rainbow trout are asymptomatic carriers
- Mainly chronic infection (e.g. 3% per day), but sometime acute with an 100% mortality
- Outbreak only occurs when the salmond in the seawater
- The more virulent strain (*ISAV-HPRΔ*) has been replaced by low pathogenic strains *ISAV-HPR0*

Clinical signs

ISAV

- high mortalities in young fish
- lethargy
- hanging head on water surface
- pale gills (anemia)
- hemorrhage on skin or fins
- swollen abdomen (acites);
- exophthalmia

Internal pathology

- darken and larger liver
- petechiae in the internal fat
- splenomegaly

Viral shedding

- ISAV infect blood cells and endothelial cells
- Cause a gradual drop in hematocrit as the hemoptoic tissues fail
- Multifocal hemorrhagic hepatic necrotic foci
- Necrosis in kidney tubules and sinusoidal congestion
- Infection often leads to release of virions into the bloodstream and urine. Shedding via urine is probably responsible for initial spreading of the virus from fish to fish in the early stages of an ISA outbreak.

Transmission

- Horizontal transmission

  -viruses are present in skin mucus, feces, urine, blood

  -from subclinical salmonids which are carriers for ISAV

- Vertical transmission-not demonstrated

- Mechanical vector, e.g. sea lice, can transfer the ISAV to susceptible species

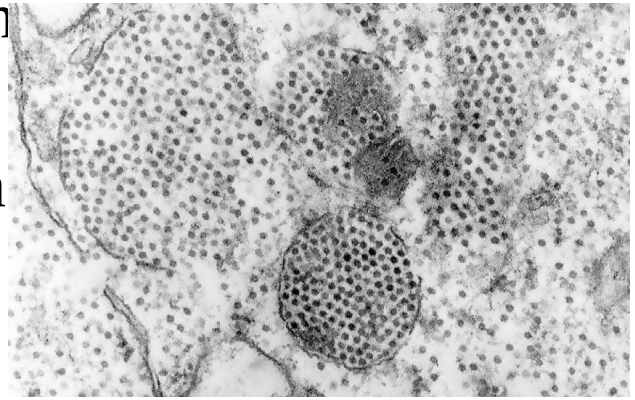
Prevention and control measures

- fish parts (viscera, muscle, trimmings) are risk factors for ISA
- ISAV is highly contagious in seawater, keep the culture pen at >5-6 km apart
- set up the ISA-free zone
- control production and processing facilities
- Restriction on the movement of infected salmon fish and equipment to the ISA-free zone
- slaughter all the infected salmon, disinfect, and fallow the production site for 6-month
- vaccination

Encephalopathy and retinopathy (VER)

VER

- Viral nervous necrosis disease (VNND) is also known as viral encephalopathy and retinopathy disease (VER)
- The etiological agent is nervous necrosis virus (NNV), a betanodavirus. The virion is spherical shaped, no envelope, with a size of ~30 nm in diam.
- Betanodavirus, contains 2 positive-sense ssRNA, RNA1 (3,103 nt) encodes the RNA-dependent RNA polymerase (RdRp), RNA2 (1,433 nt) encodes the capsid proteins, the total gen size: 4.5 kb
- Virus replicates in central nervous systems, including spin cord, brain, retina



Pathology and mortality

- NNV replicate in nerve cells and cause necrosis
- 80-100% mortality in larvae and young juveniles; affect older fish and result in significant production losses
- Has a wide host ranges, including ~ 40 marine species (e.g. striped jack, flounder, tiger puffer, grouper, Atlantic halibut) and freshwater fish (e.g. guppies, catfish).
- No clinical sign at 16^{o}C, increase to 22^{o}C, viral loads elevated and results in mortality

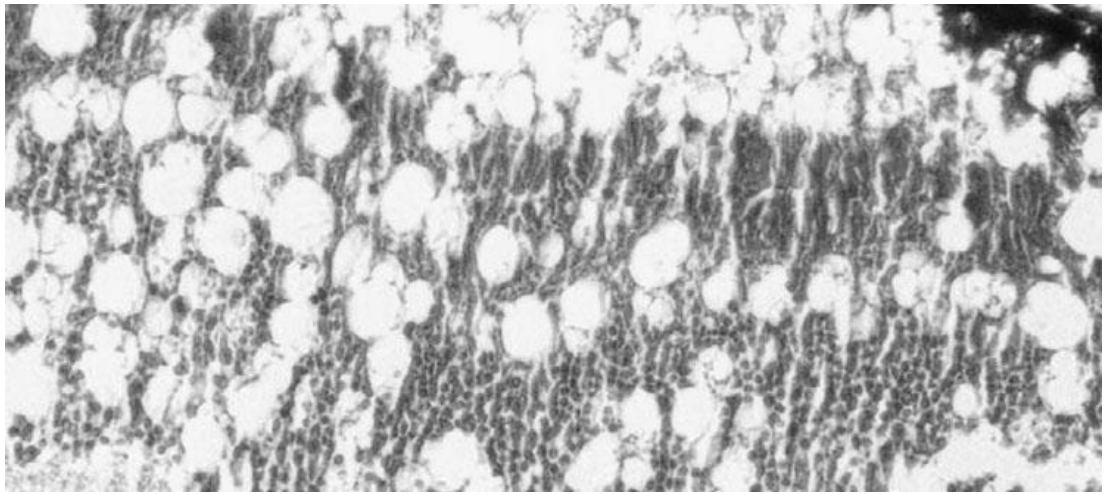
Fish mortality caused by VER

VER in seven-banded grouper (*Epinephelus septemfasciatus*). Dark fish are affected; light fish are normal.

Clinical signs

Abnormal swimming behaviors (eg, corkscrew swimming and abrupt darting) from the nervous systems damage

dark coloration in body
loss of appetite
lethargic
float near the edge of the pond



The histopathological characteristic of VNN is the vacuolation of the central nervous system, especially the brain and the retina.

VER transmission

- **Horizontal transmission**

-co-inhabitation
-contamination *Artemia* biomass
-viruses present water
-carriers

- **Vertical transmission**

-viruses found in the ovary, eggs, hatched larvae of the infected broodstock

Prevention and control measures

- Good husbandry and biosecurity

- the use of virus-free spawners and reducing the spawning frequency

-egg washing

-disinfecting seawater with ozone

- Vaccination

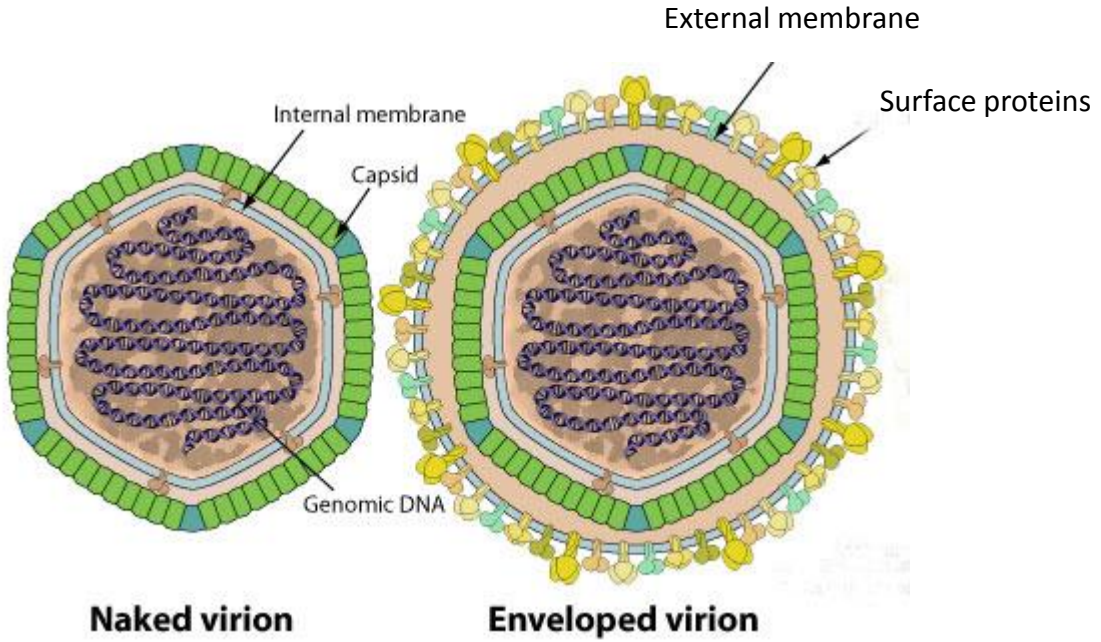
-immersion immunization using inactivated NNV, or oral immunization NNV encapsulated in the *Artemia* biomass

-vaccination of broodstock by intramuscular injection 1 month before the breeding season to lower the risk of vertical transmission

Epizootic hematopoietic necrosis virus (EHNV)

- EHNV infects freshwater fish, redfin perch (*Perca fluviatilis*) and rainbow trout (*Oncorhynchus mykiss*) in Australia.
- Negatively correlated with water temperature, higher mortality in low temperature (12^{o}C)
- EHNV is resistant to drying, can survive for 113 days in dry fish tissue

- EHNV is an enveloped, spherical, virus, under the family *Iridoviridae*.
- iridovirus are large, from 150 nm in diameter.
- EHNV genome: dsDNA, 127 kb
- Virus replicate in both cytoplasmic area and nucleus



Systemic, most severe in hematopoietic tissue
Viremia to disperse the viruses to other tissues

Internal pathology:
-petechial hemorrhages at the base of fins,
-excessive amounts of peritoneal fluid,
-swelling in the kidney and spleen,
-pale white spots appear in the liver

Redfin perch showing liver lesions caused by EHNV (Photo: Richard Whittington, University of Sydney).

Histopathology

- vascular damage
- multifocal necrosis in the liver, spleen and hematopoietic tissue of the kidney
- basophilic **intracytoplasmic** inclusion bodies are found within hepatocytes (Virus replicate in both cytoplasmic area and nucleus)

Prevention and control

- Biosecurity

  -disinfect the equipment

  -birds can be vectors, keep them away from culture facility
- The use of virus-free stocks

- Quarantine
- Difficult to control the infected wild populations
- No vaccine

Infectious spleen and kidney necrosis virus (ISKNV)

- Iridoviral disease, caused by ISKNV, the genus M*egalocytivirus*
- Virions: 160-200 nm, smaller genome size: 110 kb
- Infectious with ISKNV was recognized in mandarin fish (*Siniperca chuatsi*), an economically important freshwater species in China since 1994 (He *et al.*)
- Host: wide range of freshwater fish, including tilapia

Mortality and clinical signs

- Mortality is typically between 20% and 60% and sometimes reaches 100% of fish in intensive culture coditions.
- Disease is more severe in juvenile fish, but mortality also occurs in market-size fish

Clinical signs:

- Lethargic
- Loss of appetite
- pale gills
- petechial haemorrhages in fins and operculum

Internal pathology

- enlarged spleen and kidney

Tilapia iridovirus

- A case in USA (Subramaniam et al. 2016)
- Affect tilapia fry/fingerlings, had a mortality 50-75%
- ISKNV was also found in Thailand
- Multiple infections of ISKNV/Iridovirus was reported in cage culture
- A semi-nested PCR was developed (Dong et al. 2016)
- This viruses can be vertically transmitted (Suebsing et al., 2016)

Koi herpes virus

- KHV is a enveloped, spherical, ds DNA virus
- Viral particles are 157 nm in size,
- KHV is a member of *Alloherpesviridae*
- Renamed as *Cyprinid HV 3* (CyHV3) under the genus *Cyprinivirus*
- cause epidermal hyperplasia
- The disease is restricted to common carp (*Cyprinus carpio)* and Koi × crucian carp (*Carasius carasius*) hybrids

Mortality and clinical signs

- high mortality in all strains of common carp (*Cyprinus carpio*), at all ages. In the field, adults are more susceptible.
- Lethargic and enophthalmia (sunken eyes)
- white patches on the skin and gills due to epithelial hyperplasia and necrosis
- Secondary bacterial infection, such as *Flavobacterium columnare*, is common in affected fish.

Clinical signs of KHV infection:
(A) gill damage such as whitish gills; (B) severe gill necrosis; (C) some fish also showed blister-like lesions on the skin and white patches on the gills.

Histopathology

- epithelial hyperplasia,
- hypertrophy and necrosis in the affected gill and epidermal tissue
- epithelial cells within the affected areas will have enlarged nuclei with marginated chromatin
- necrosis in the hematopoietic tissues of kidney, spleen, liver and lamina propria of the intestine.

Prevention and control

Prevention:

- avoidance, vigilant screening for KHV-free stock
- biosecurity
- eliminate vectors

Control:

- vaccine, KoVax, attenuated live vaccine
- recombinant vaccine

Virus	Species	Host range	High mortality	Geographic distribution
IPNV	freshwater & marine	wide	High temperature	Widely spread
IHNV	salmon fish and trout	wide	Cold temperature (< 10^{o}C)	Widely spread
ISAV	marine	narrow	At <3^{o}C or >15^{o}C	Norway,UK,Canada,USA,Chile
VER	primarily in marine species	wide	High temperature (e.g. 22^{o}C)	The Pacific region
EHNV	freshwater	narrow	Cold temperature	Australia
ISKNV	freshwater	wide	Warm water	China and the Pacific
KHV	freshwater	narrow	<13^{o}C or >23^{o}C	Asia Pacific

Shrimp viral diseases

OIE Listed Crustacean Diseases
(9 listed as of 2018)

1. Acute hepatopancreatic necrosis disease (AHPND)
2. Infection with *Aphanomyces astaci* (Crayfish plague)
3. Infection with *Hepatobacter penaei* (Necrotising hepatopancreatitis-NHP)
4. Infection with **infectious hypodermal and haematopoietic necrosis virus** (IHHNV)
5. Infection with **infectious myonecrosis virus** (IMNV)
6. Infection with ***Macrobrachium rosenbergii nodavirus*** (White tail disease) (MrNV)
7. Infection with **Taura syndrome virus** (TSV)
8. Infection with **white spot syndrome virus** (WSSV)
9. Infection with **yellow head virus** genotype 1 (YHV-1)

4.8 MMT

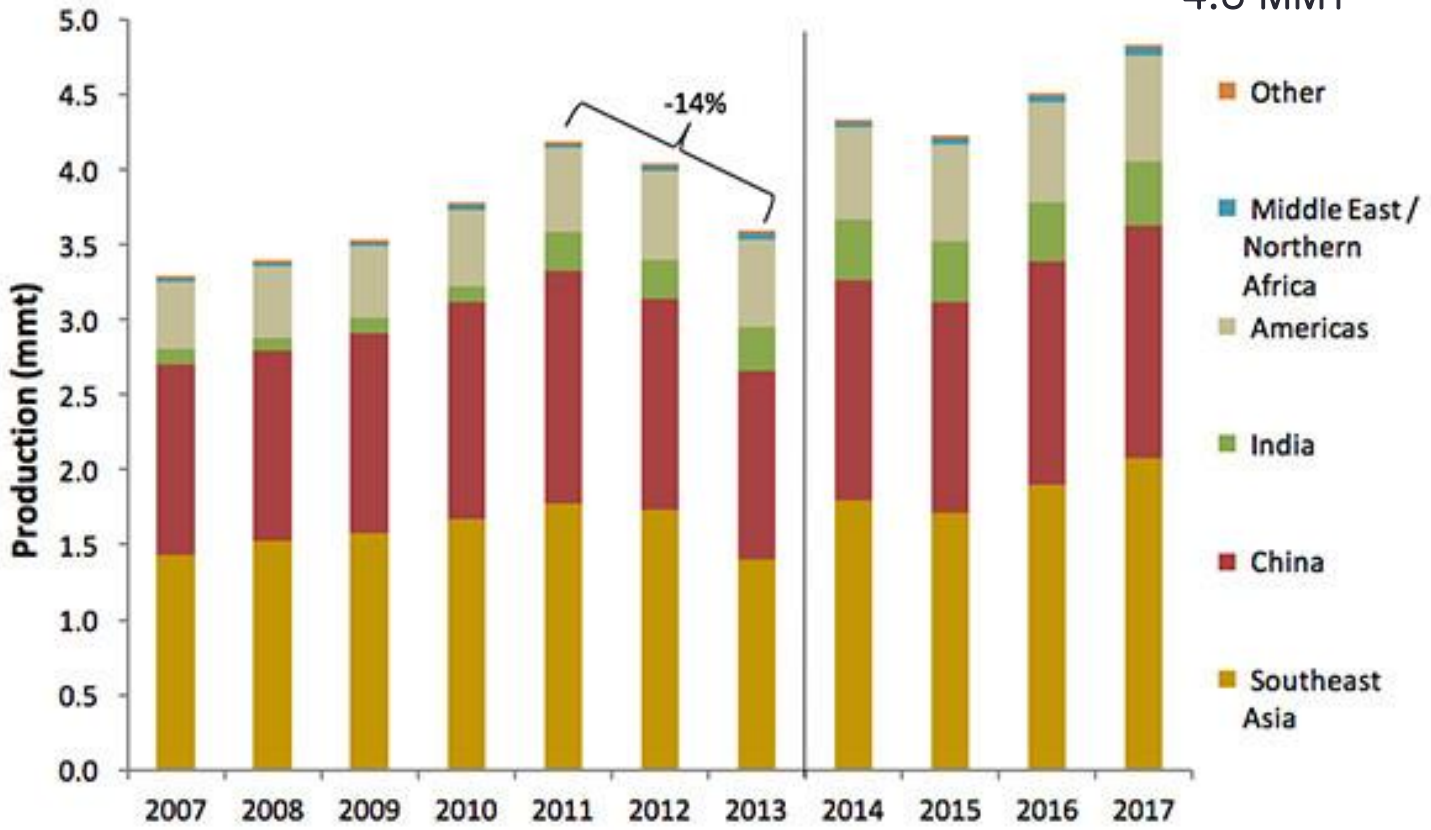
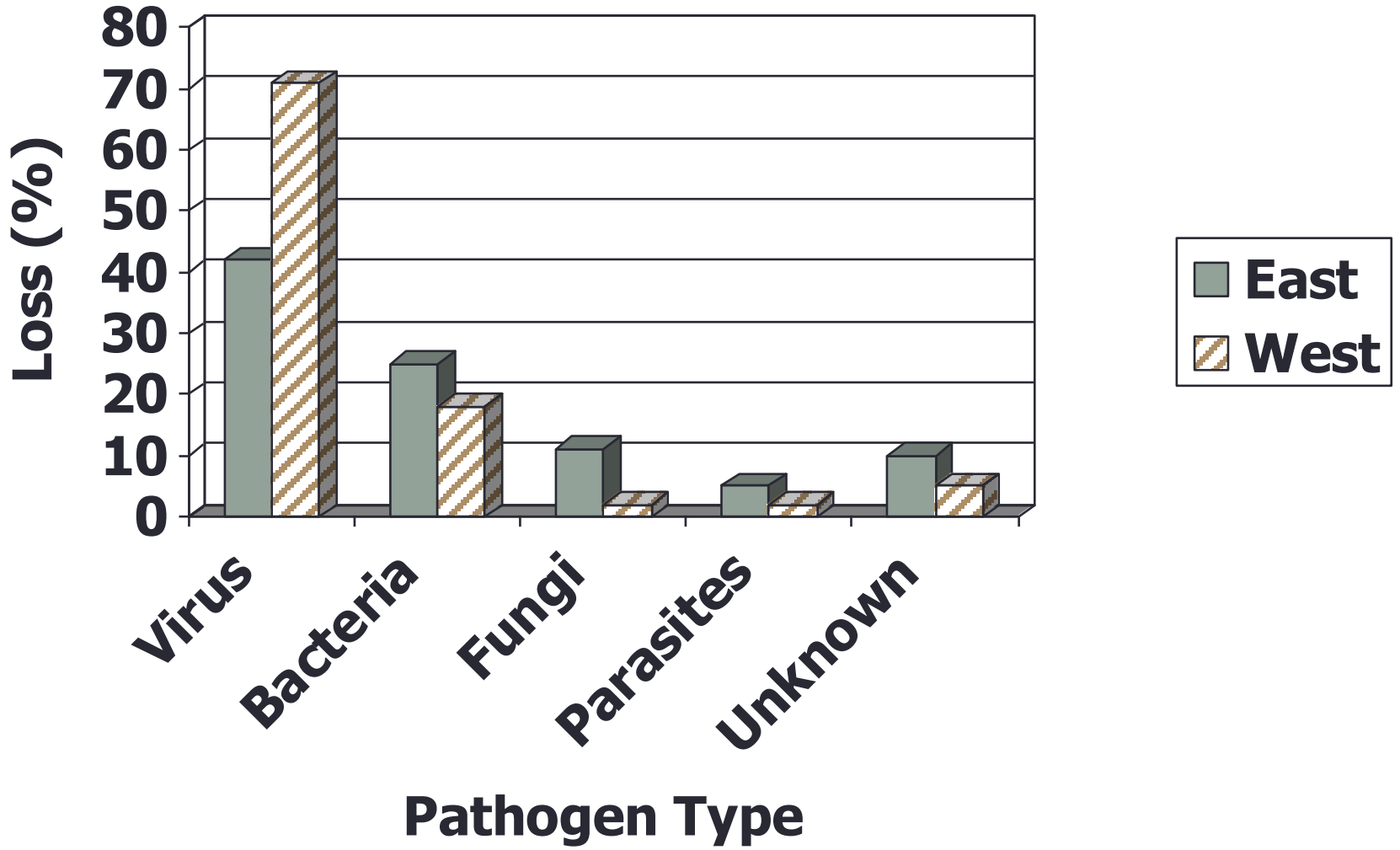


Figure 1. Shrimp farming production by region. Sources: FAO (2007-2011); FAO & GOAL 2014 (2012-2013); GOAL 2015 (2014-2017).

Proportional losses attributed to different pathogen types

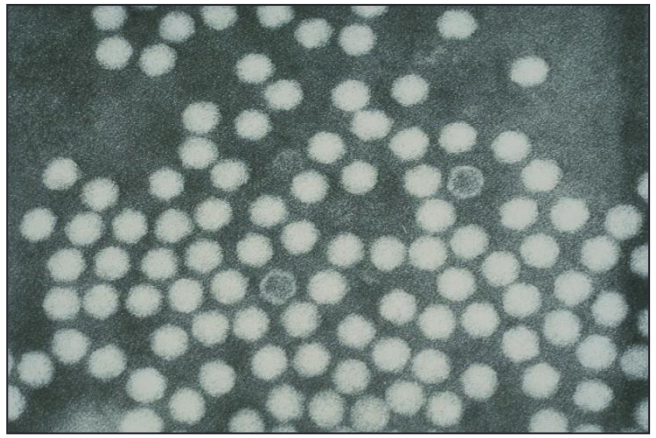


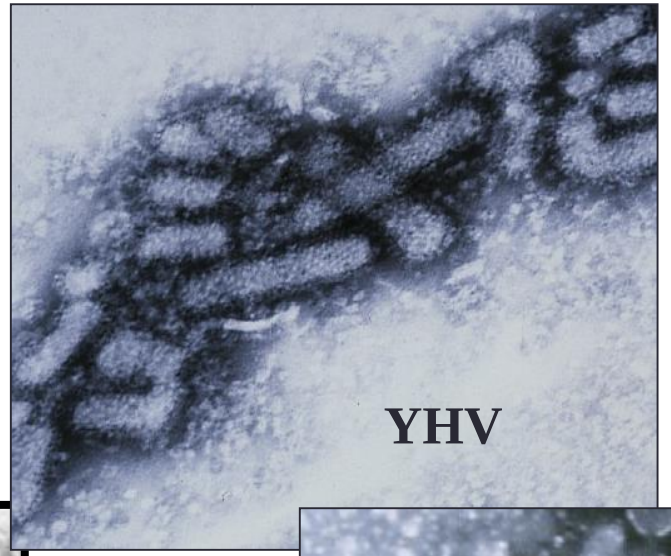
Flegel, 2006, GAA Report

GAA Report: Importance of disease by agent type

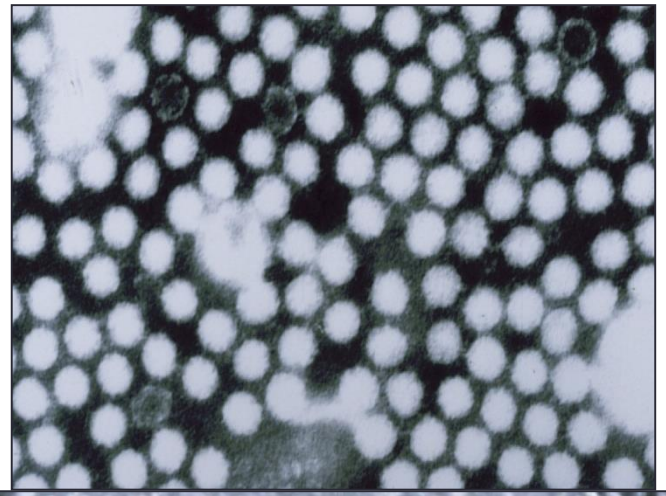
- Global production loss estimates:
  - Viruses ~65%
  - Bacteria ~20%
- Viruses are the most important disease agents by almost 3:1 over bacterial diseases.
- It follows that most of the currently OIE listed shrimp diseases have viral etiologies.

IHHNV

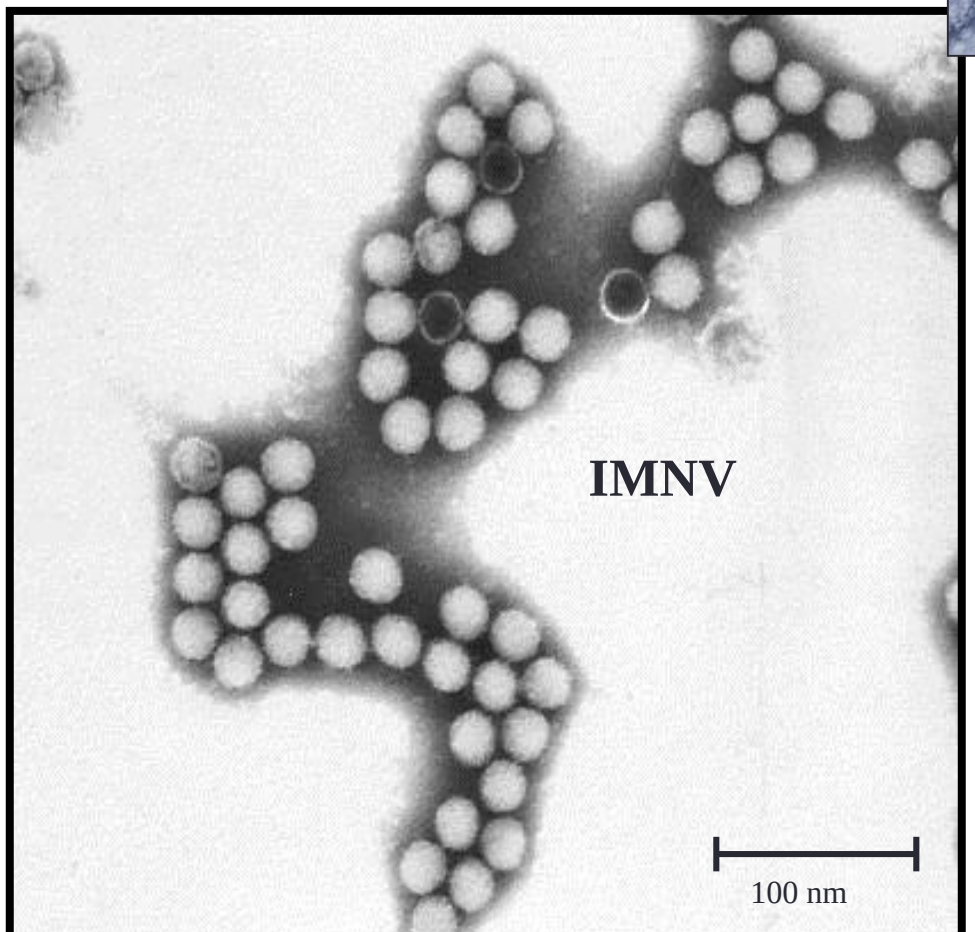




YHV

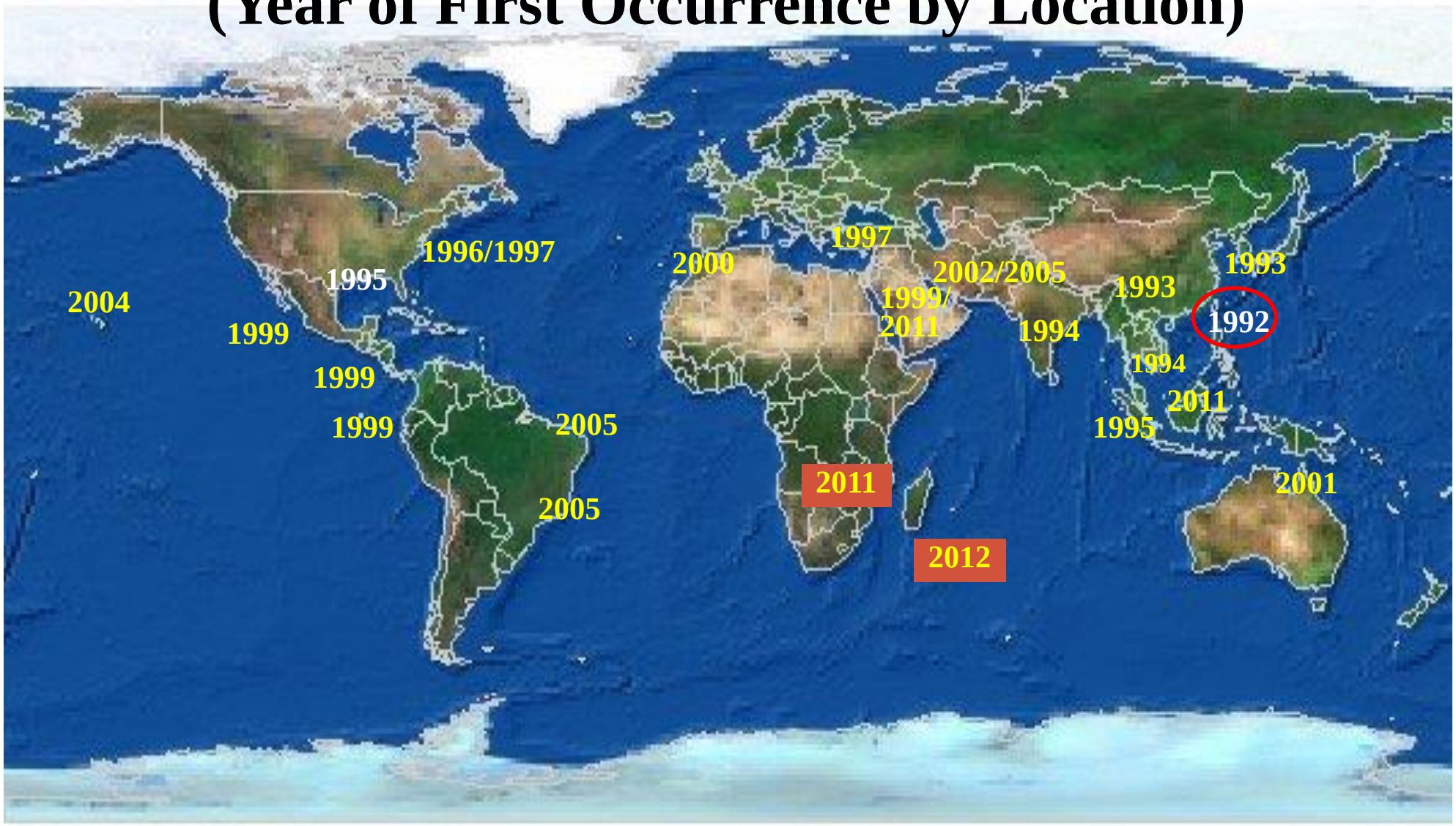


TSV



WSSV

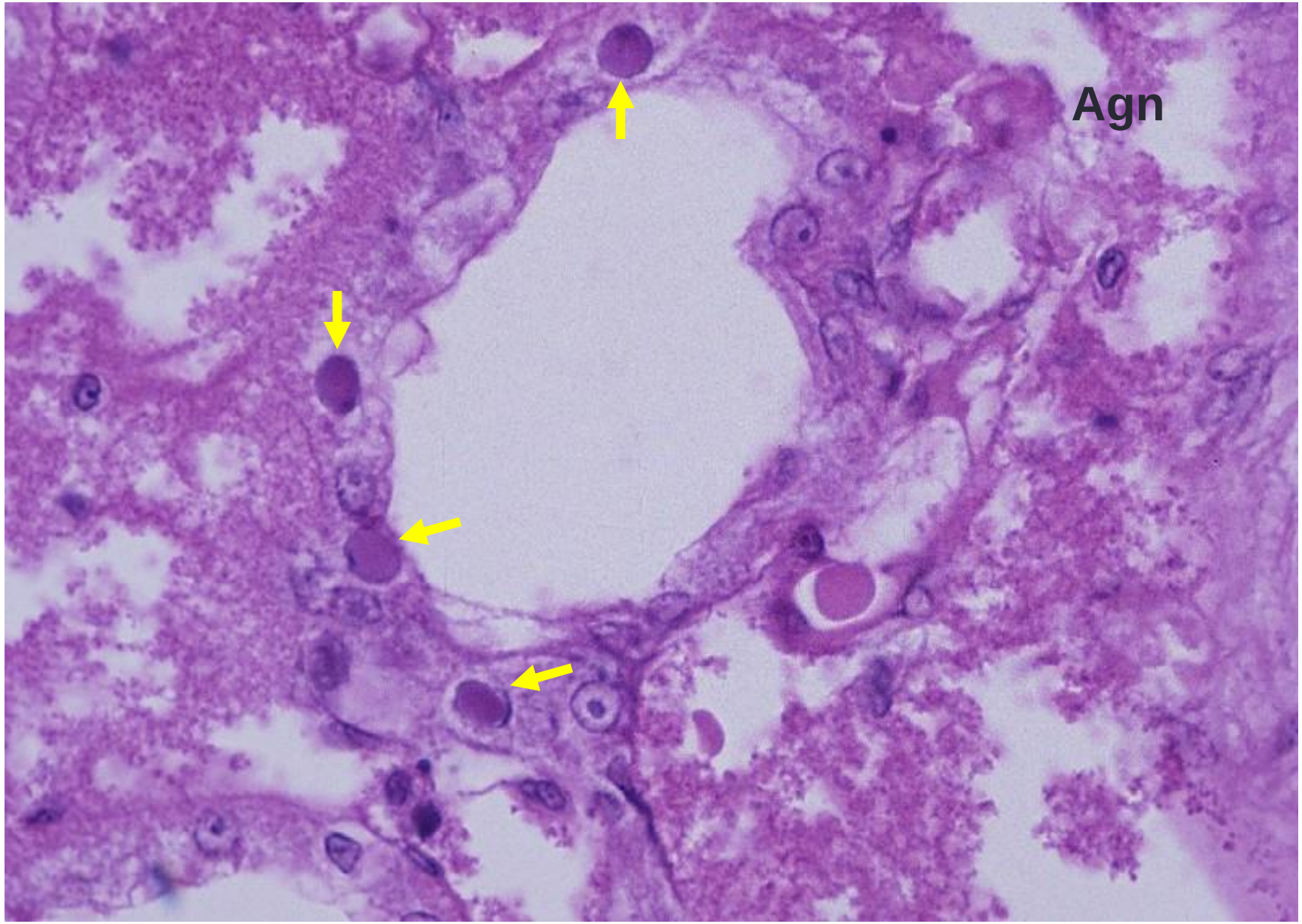
THE WHITE SPOT VIRUS PANDEMIC
(Year of First Occurrence by Location)



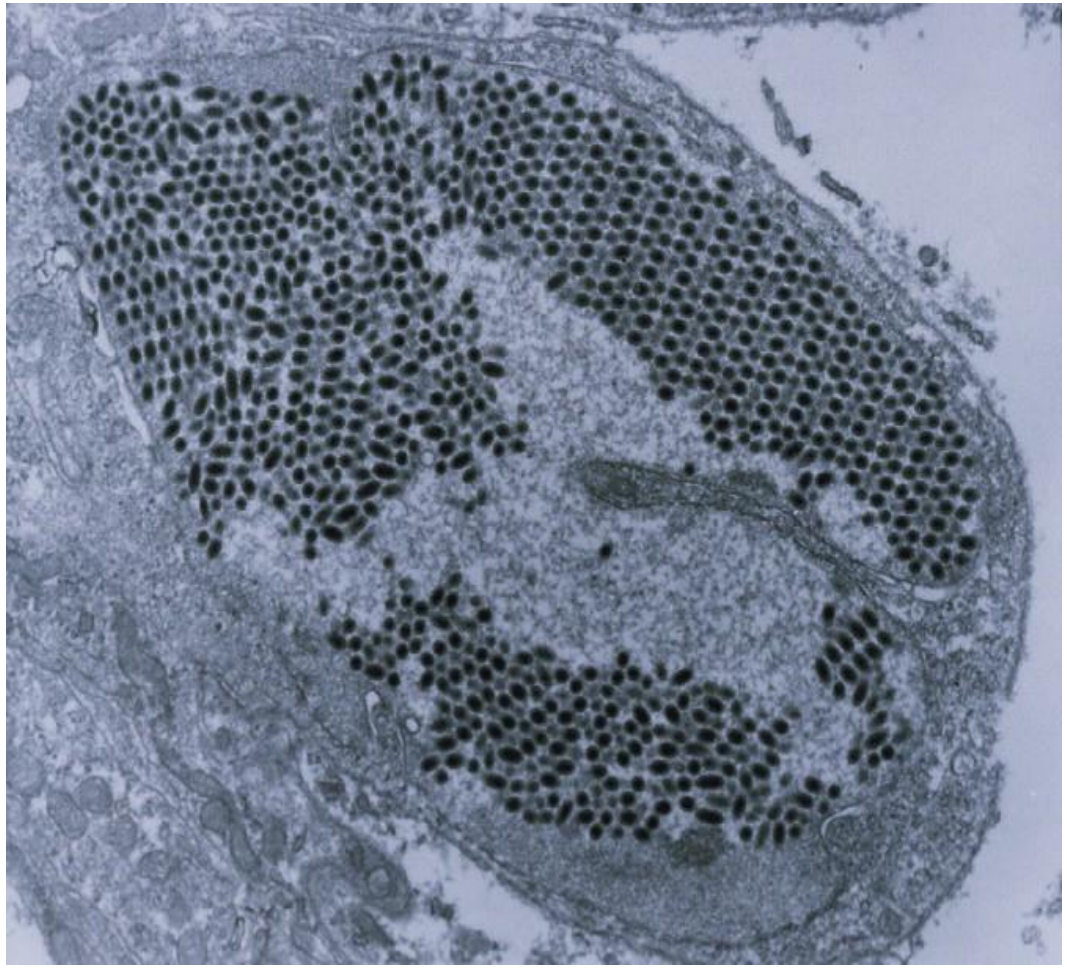
White Spot Disease Clinical Signs

- Sudden reduction in feeding, lethargic.
- Red discoloration in *P. monodon*, *P. vannamei*, *P. 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.

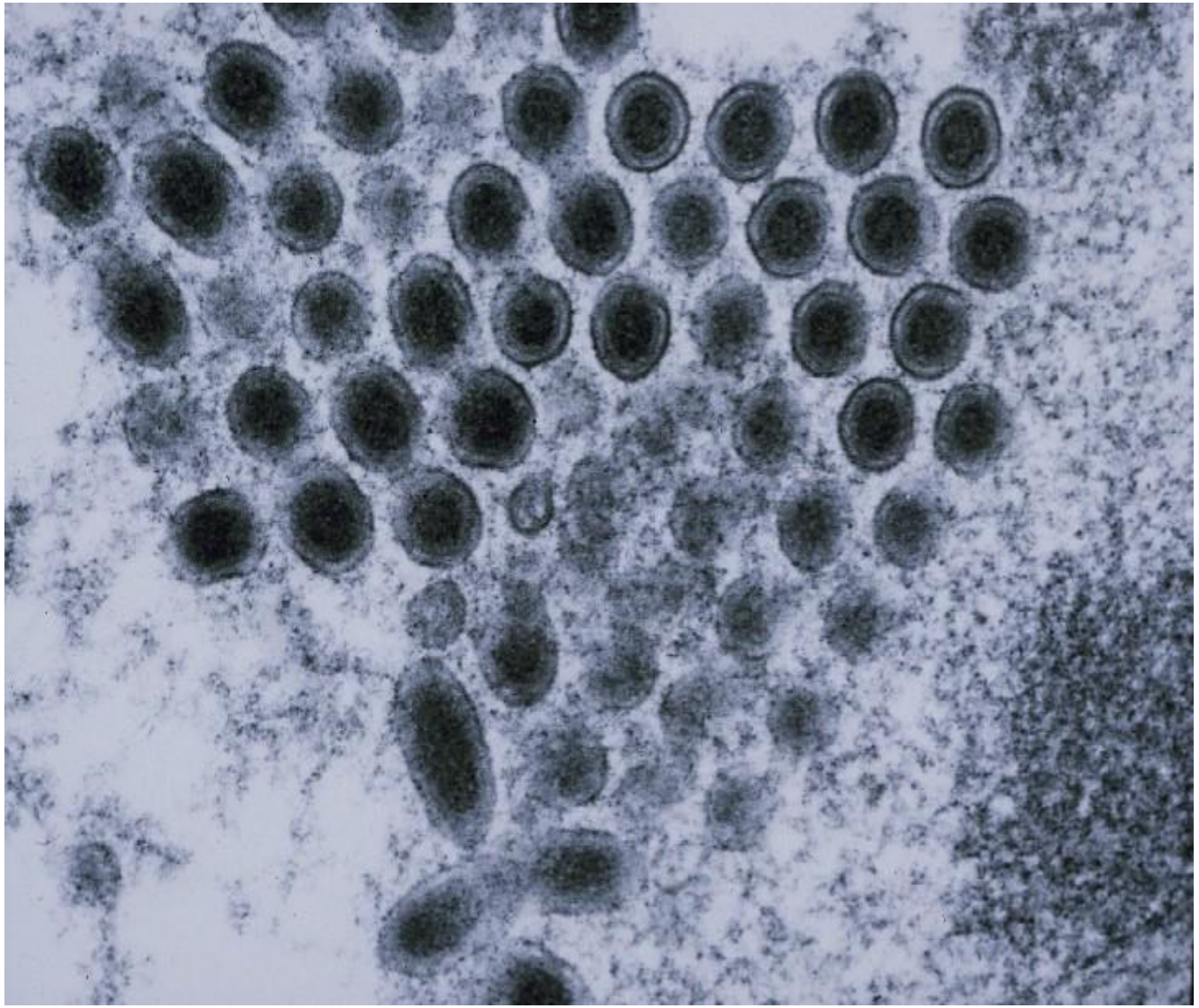
WSSV in *P. monodon*, Madagascar, April 2012
Photo courtesy of Marc LeGroumellec, UNIMA



H&E Histology of antenna gland (Agn) of a WSSV-infected *Penaeus vannamei*. Arrows indicate examples of dark basophilic (mature) intranuclear inclusions



Transmission electron micrograph of a WSSV-infected cell in the cuticular epithelium of a *Penaeus vannamei*. Most of the virions are sectioned across their long axis and only a very few show the elliptical morphology of the intact virion.



Transmission electron micrograph of a WSSV-infected cell, the dense central nucleocapsid and outer envelope of the virions is readily apparent.

Aquamen Farm, South Madagascar May 2012

WSSV in *Penaeus vannamei* – Ecuador, 1999

WSSV Natural and Experimental Hosts

List of known natural & experimental hosts for WSSV is 104 species (all are decapods, except artemia):

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

Fish Market

Black Tiger Shrimp

Frozen, Shell On
41-50 ct.

lb. 5.99

THIS AD GOOD JANUARY	WED. 17	THUR. 18	FRI. 19	SAT. 20
SUN. 21	MON. 22	TUES. 23	(1996)	

SEAFOOD

Fresh Dungeness Crab
USDC Lot Inspected
2.99 LB.

Black Tiger Shrimp
51/60 Count, Raw, Shell-On, Frozen/Thawed
USDC Lot Inspected
5.99 LB.

Seafood Specials

Shell-On Shrimp
71/90 count

3.99 lb.

Prices Effective April 15th Through April 21st, 1998

Current Management Strategies for White Spot Disease in the Americas

- Increased biosecurity
  - ✓Source water treatment to exclude wild vectors
  - ✓Reduced water use with higher culture densities
- SPF (WSSV-free) shrimp stocks
- Use SPR (resistant) lines of shrimp
- Hyperthermia & WSSV
  - ✓Reduction of farming in cold season
  - ✓Use of greenhouses


Covered ponds in Guangzhou Province, China

Covered ponds in Guangzhou Province, China

FAO/ASTF Project: GCP/RAF/510/MUL:

Enhancing capacity/risk reduction of emerging Tilapia Lake Virus (TiLV) to African tilapia aquaculture: Intensive Training Course on TiLV

4-13 December 2018. Kisumu, Kenya

in cooperation with Kenya Marine Fisheries Research Institute (KMFRI) and Kenya Fisheries Service (KeFS)

Thank you for your attention

Hakuna Matata!!!

Food and Agriculture Organization of the United Nations