

Documentation of an Emerging Disease (Early Mortality Syndrome or Acute Hepatopancreatic Necrosis Disease) in SE Asia & Mexico

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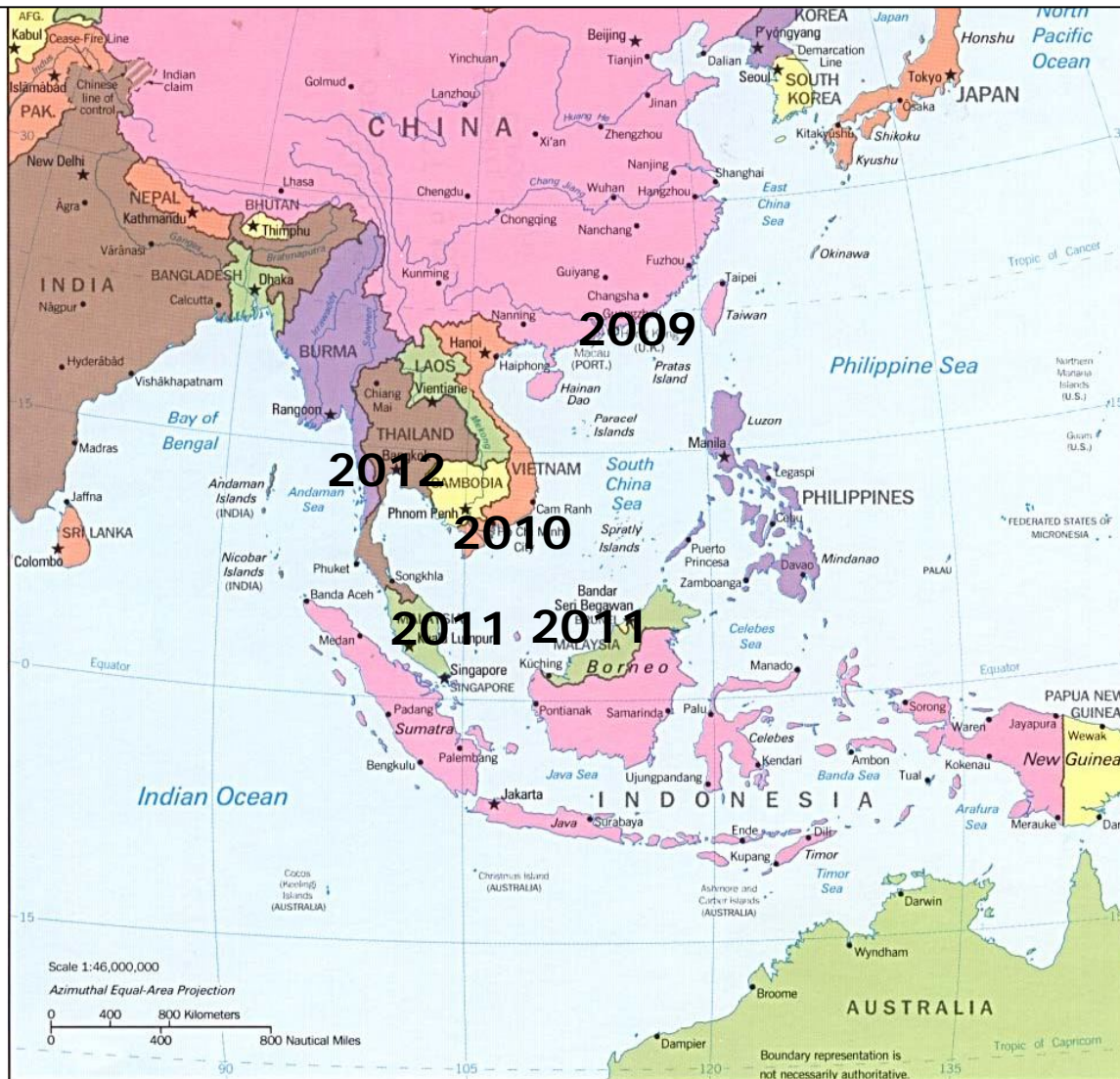
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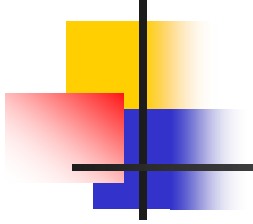


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Spread of EMS/AHPND in East and SE Asia





OCCURRENCE OF EMS/AHPND IN MEXICO

Spread of "New Disease" in western Mexico





EMS in Sonora, Mexico – Photo by Ms. Silvia Gomez



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

Juvenile *Penaeus vannamei*
Left with normal HP; right with EMS/AHPND



Comparison of EMS in Asia vs. EMS in Mexico

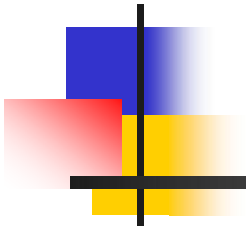
EMS/AHPND shrimp in Vietnam



EMS/AHPND shrimp from a shrimp pond near Mazatlan



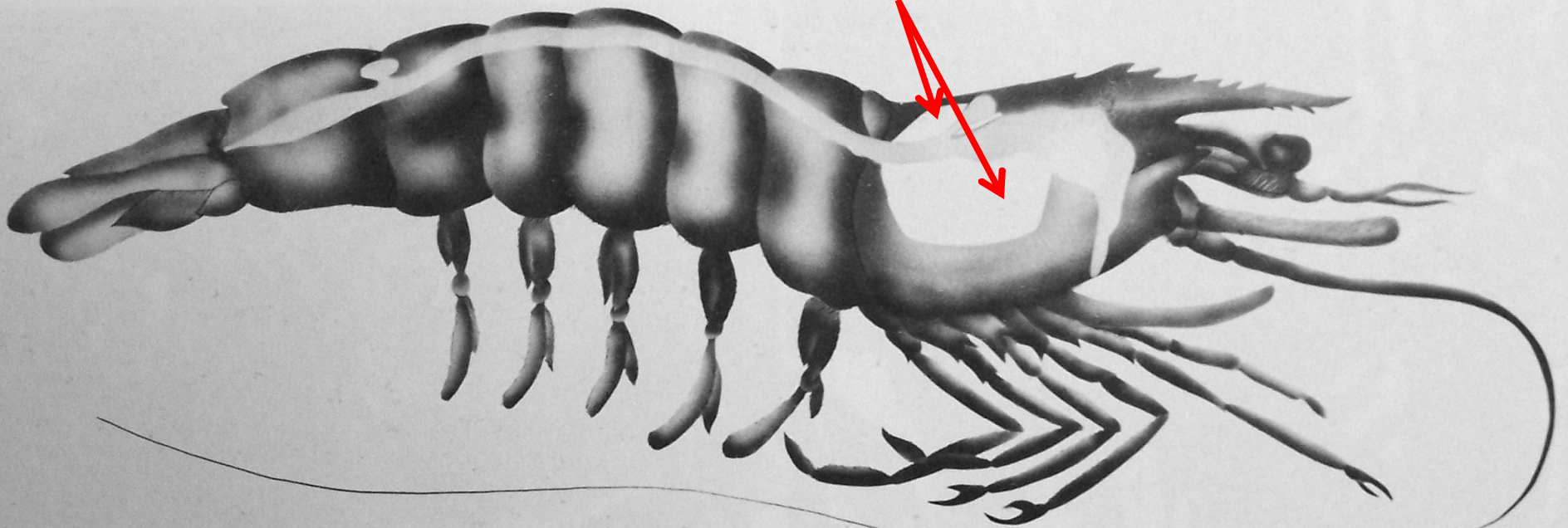
The hepatopancreas is the target organ for EMS (= AHPND)



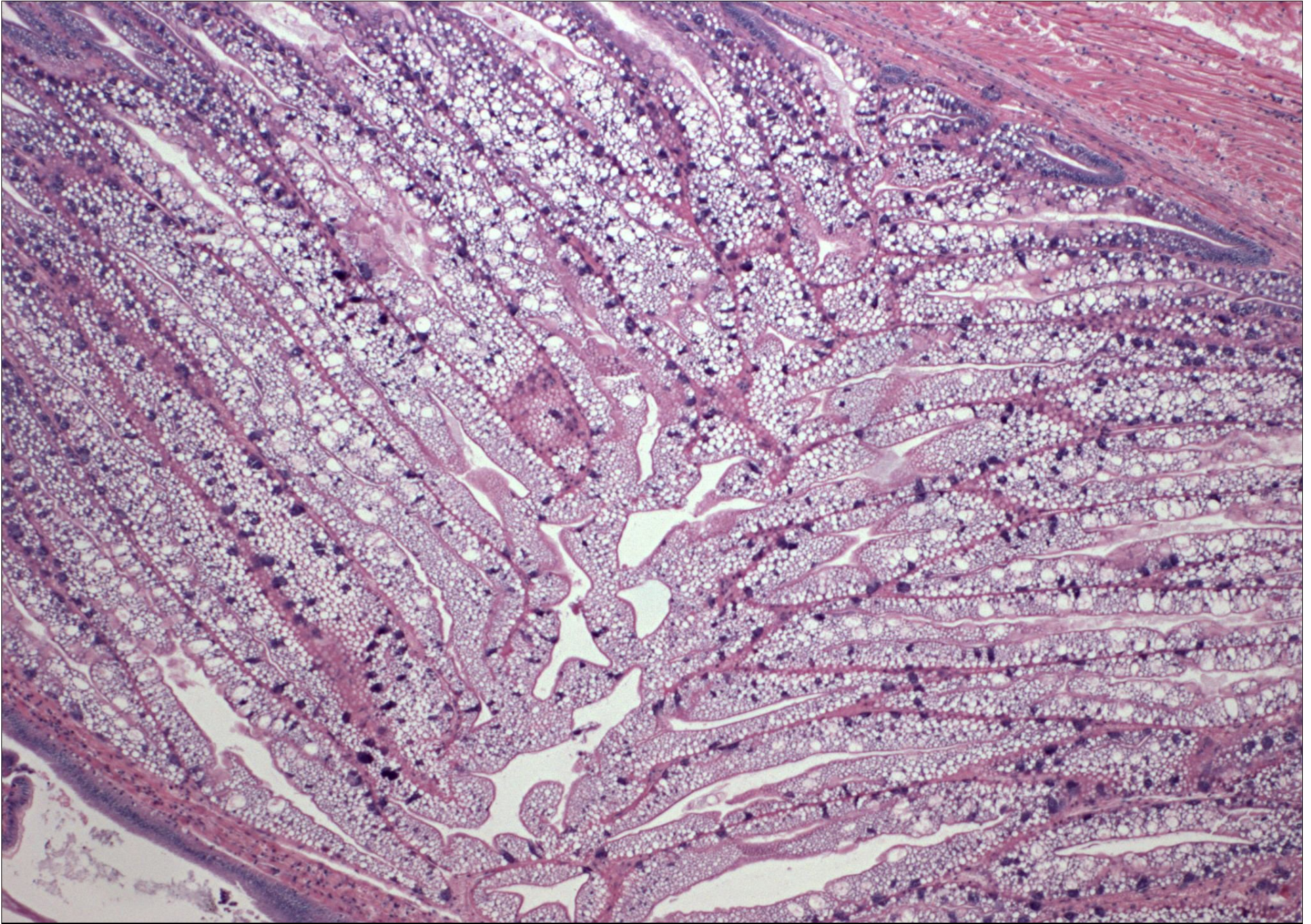
Normal histology of the hepatopancreas

HP is the target organ for EMS

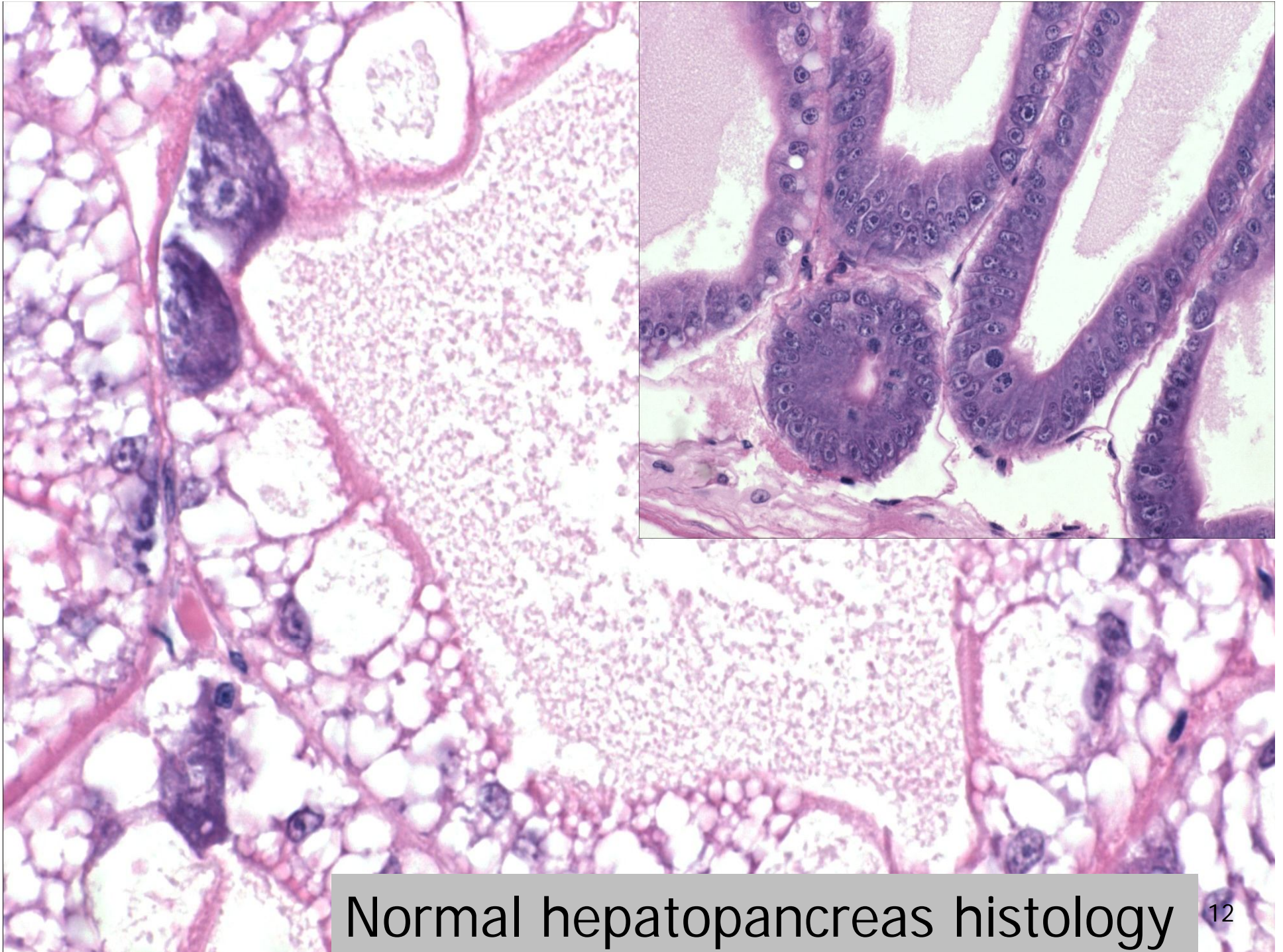
Hepatopancreas



PENAEID SHRIMP DIGESTIVE SYSTEM



Case 11-041. Normal hepatopancreas (HP)



Normal hepatopancreas histology



Gross Signs of EMS/AHPNS

- Significant atrophy of the hepatopancreas (HP).
- Often pale, yellowish or white within the HP connective tissue capsule.
- Black spots or streaks sometimes visible.
- HP does not squash easily between thumb & finger.



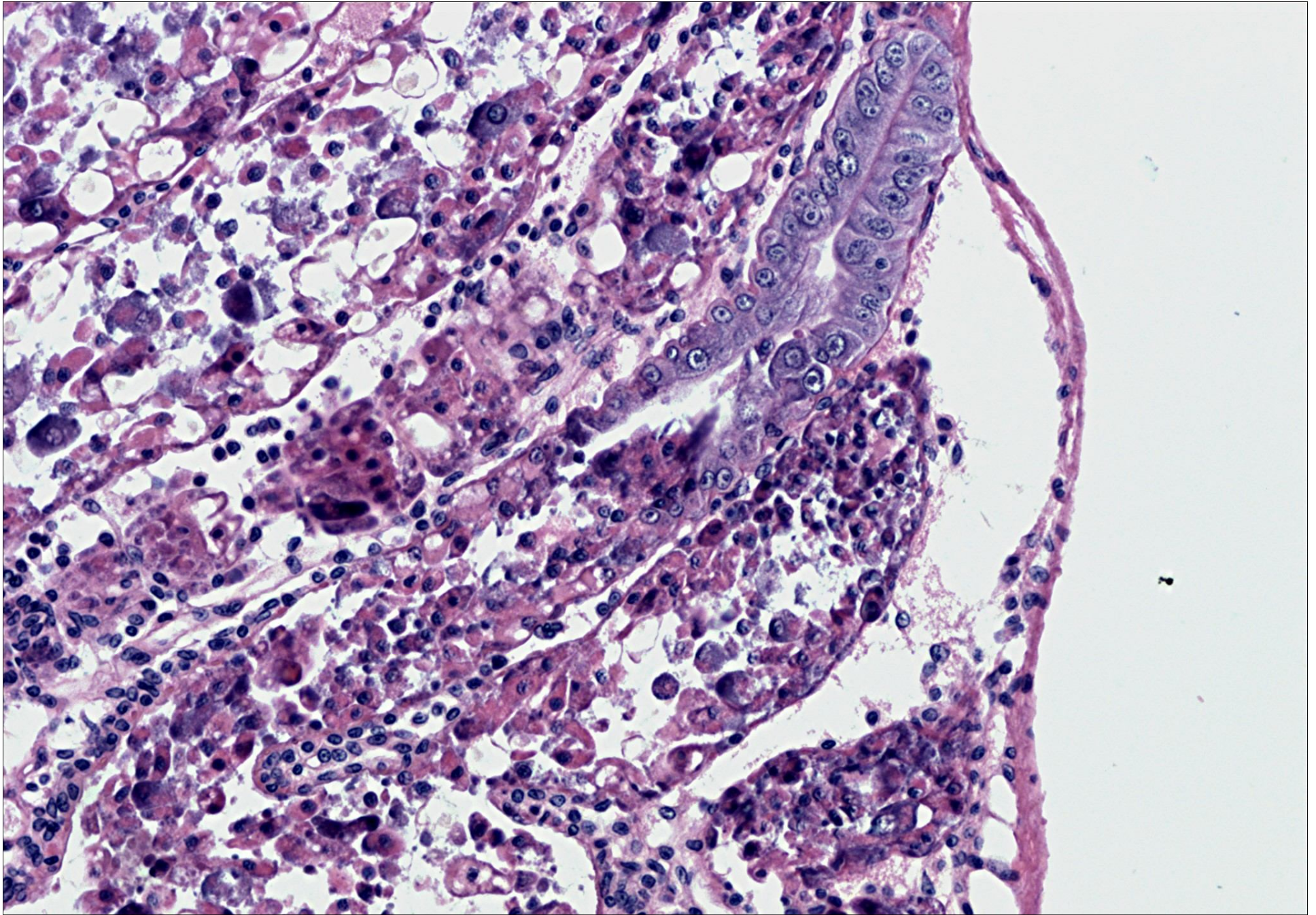
Acute Hepatopancreatic Necrosis Disease Has Two Distinct Phases:

- An acute phase
 - Acute Hepatopancreatic Necrosis Disease or AHPND.
 - HP tubule cells (R, B, F & later E-cells) show acute loss of function.
 - **Significant acute sloughing of HP tubule epithelial cells.**
 - Abundant bacteria in the hepatopancreas at this stage are not easily demonstrated.
- Terminal phase ends with destruction of the HP by opportunistic *Vibrio* spp.



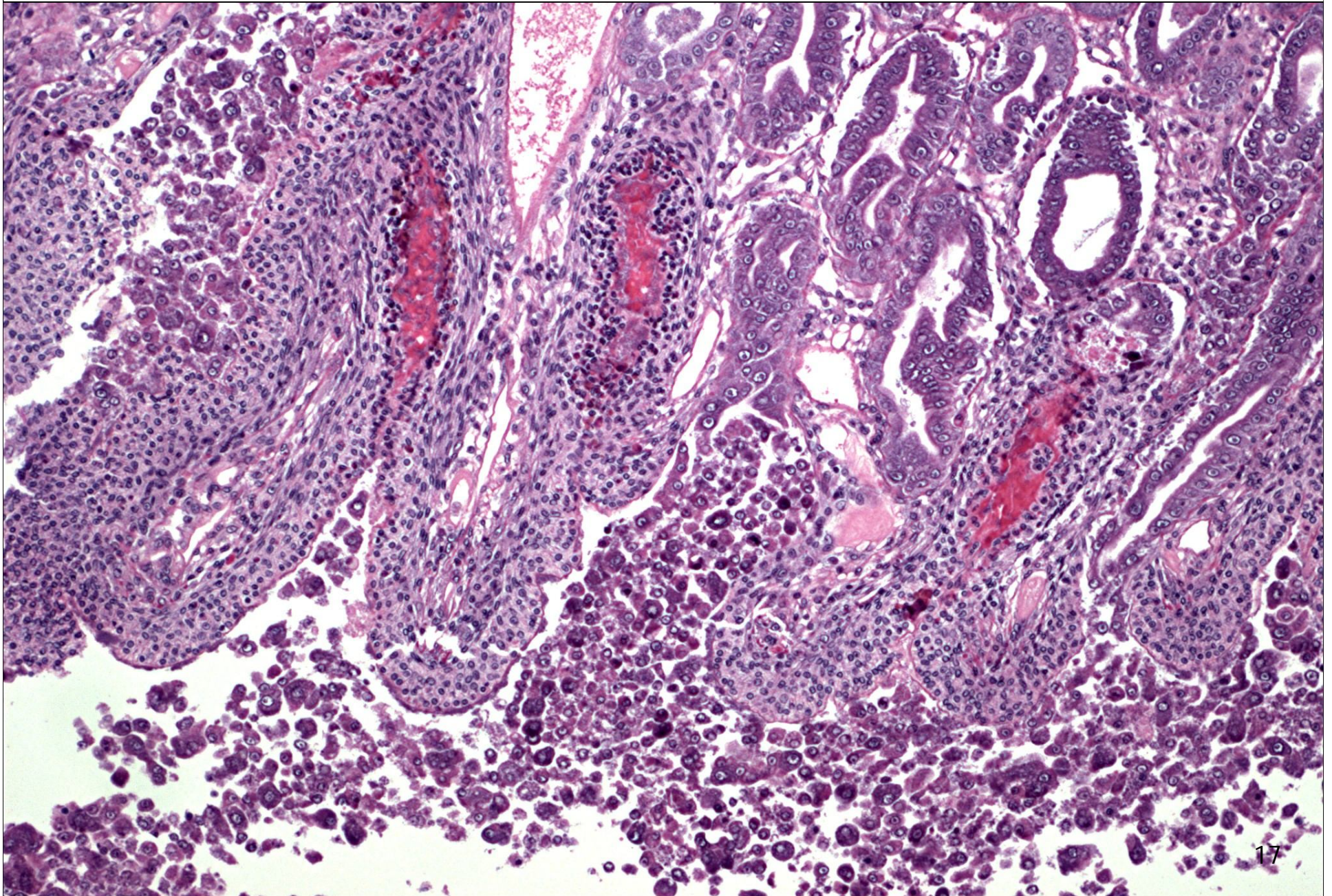
Histopathology showing acute phase HP dysfunction

- Samples from South China
August/September 2010.
- Vietnam July 2011 & 2012 &
December 2011 & 2012.



Case 11-214. *P. monodon*. Vietnam; Note proximal to distal progression of lesions. 20x

Case 11-254. *P. vannamei*. Vietnam; HP tubule epithelium sloughing, significant proximal hemocytic inflammation & some tubules with putative vibriosis; 10x



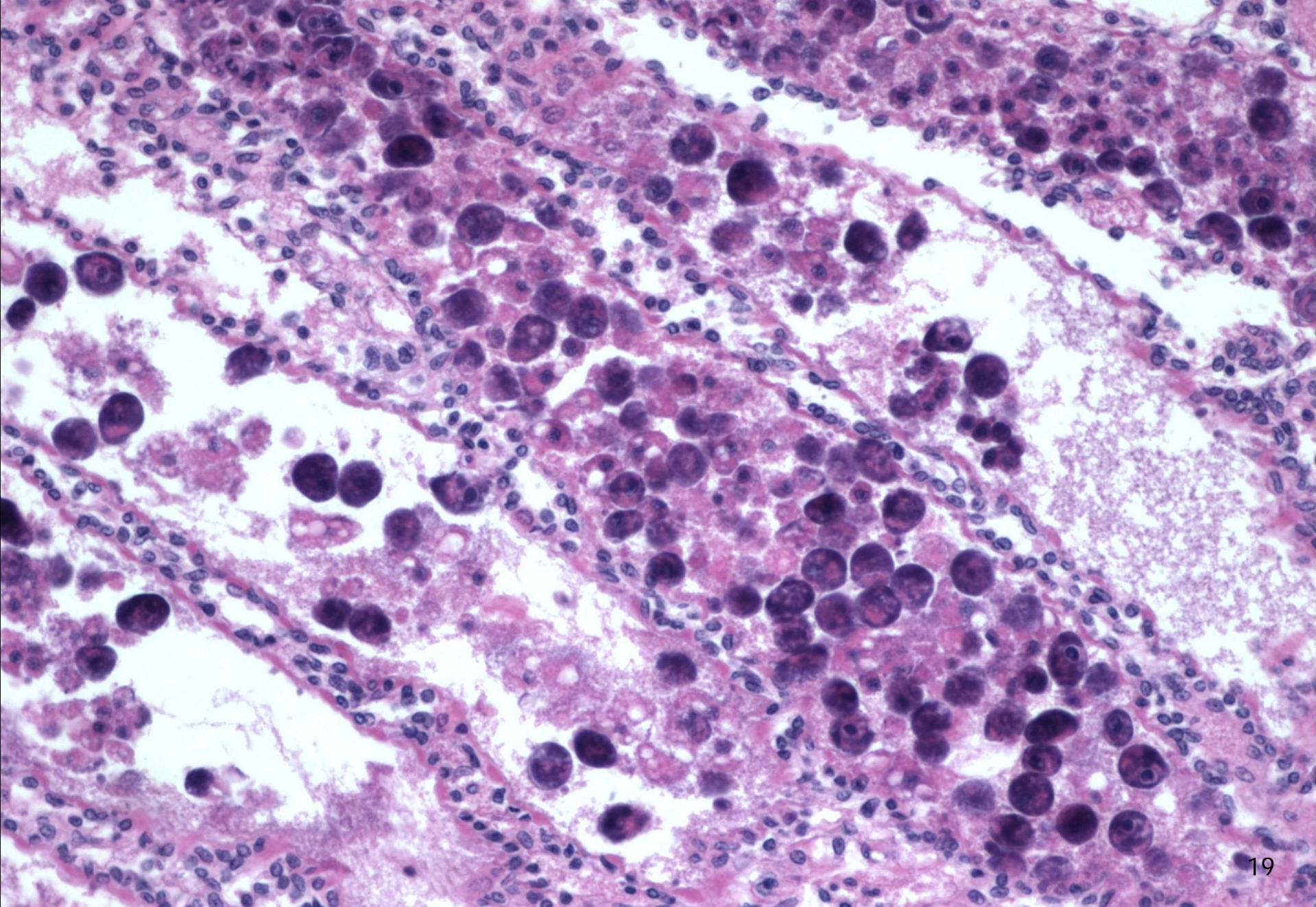


Samples from Mexico

May 2013

- Acute phase samples from Mexico showing EMS/AHPND.
- Chronic phase of EMS/AHPND.

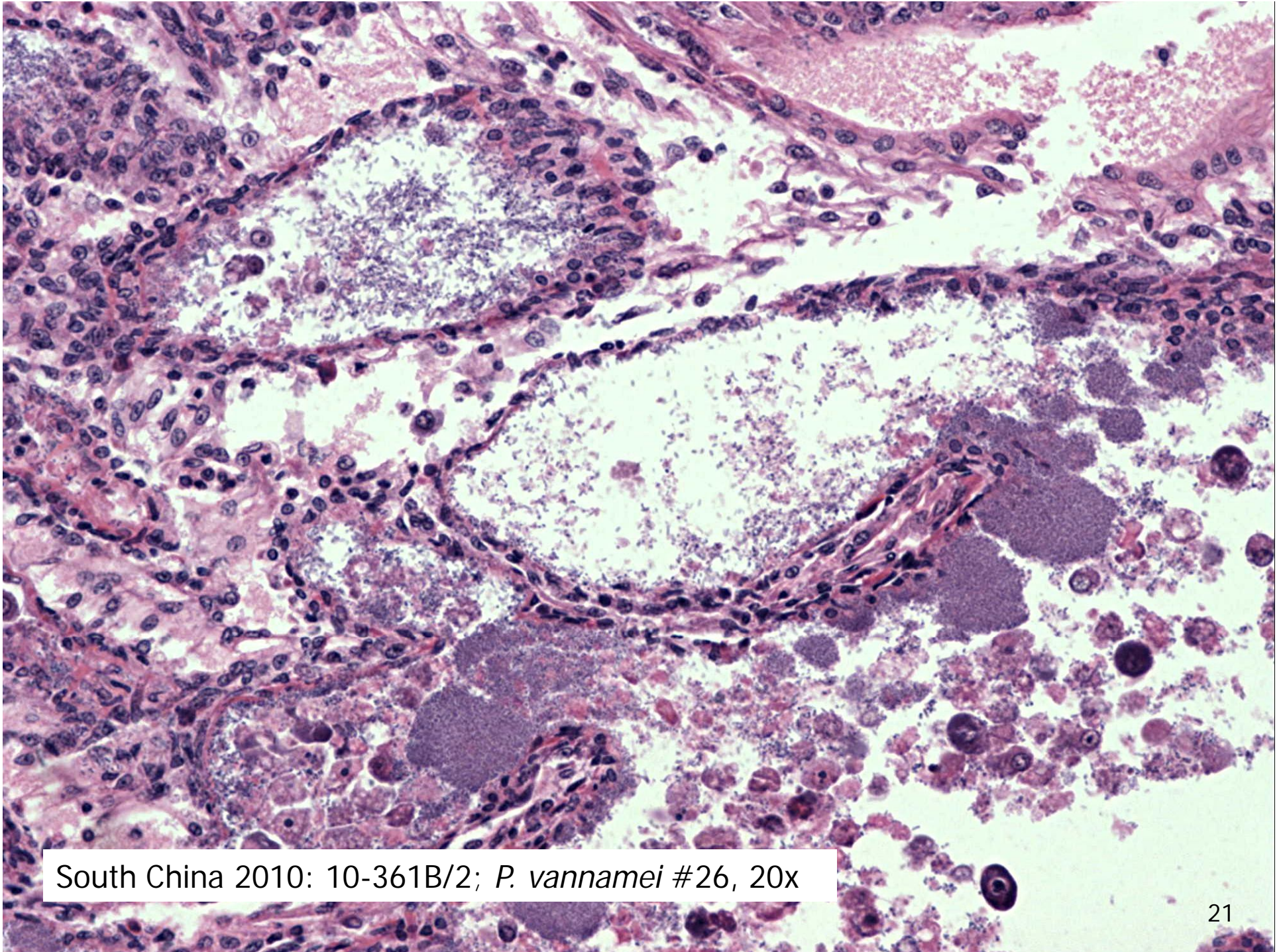
Mexico - Acute Phase of AHPND; UAZ-APL 13-220; 20x





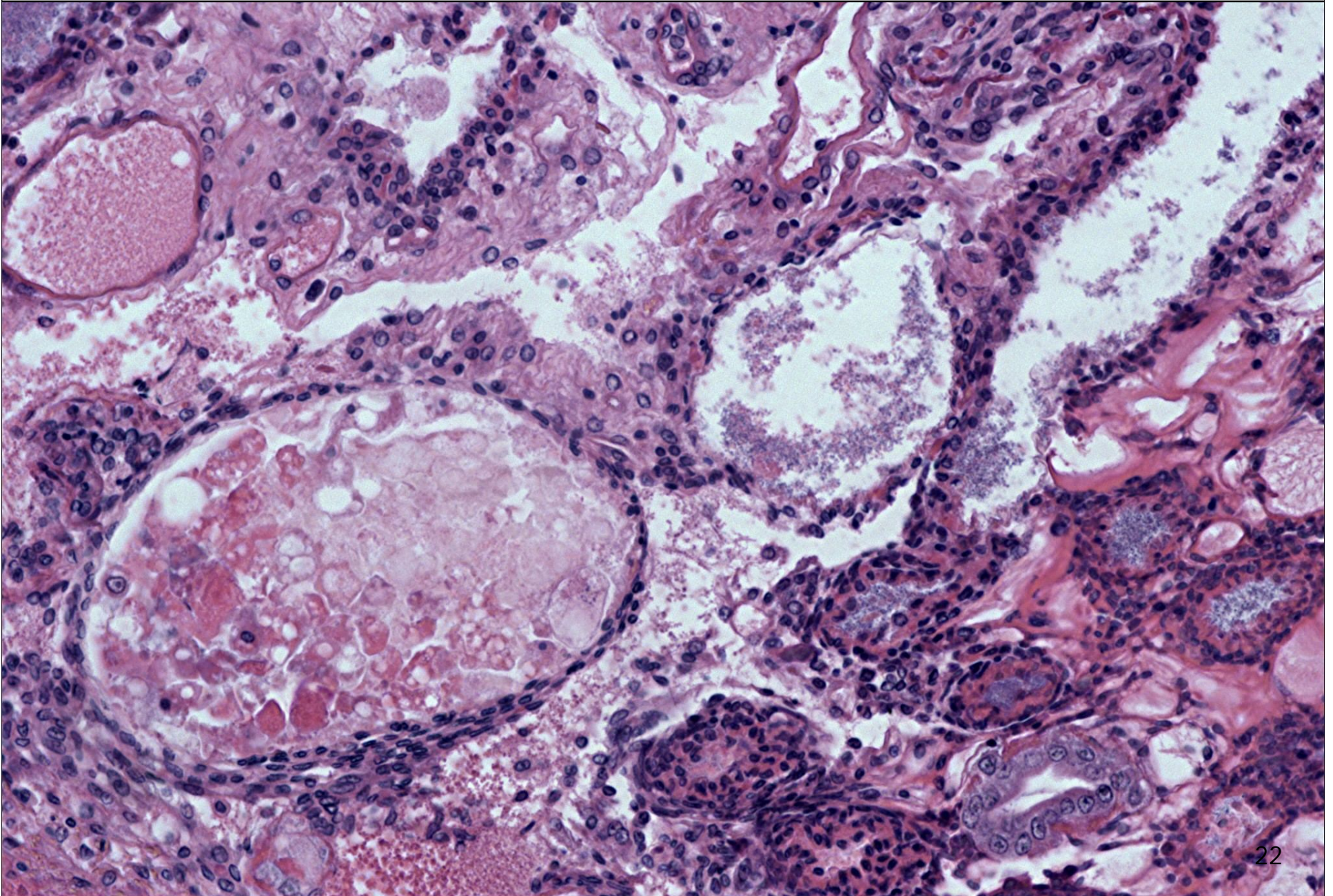
Histopathology showing terminal phase of HP destruction due to Vibriosis

- Samples from South China
August/September 2010.
- Vietnam July 2011 & 2012 &
December 2011 & 2012.



South China 2010: 10-361B/2; *P. vannamei* #26, 20x

Case 11-214. *P. monodon*. Vietnam; Terminal phase of EMS. Most HP tubules are destroyed. Massive bacterial infection by a probable *Vibrio* spp.



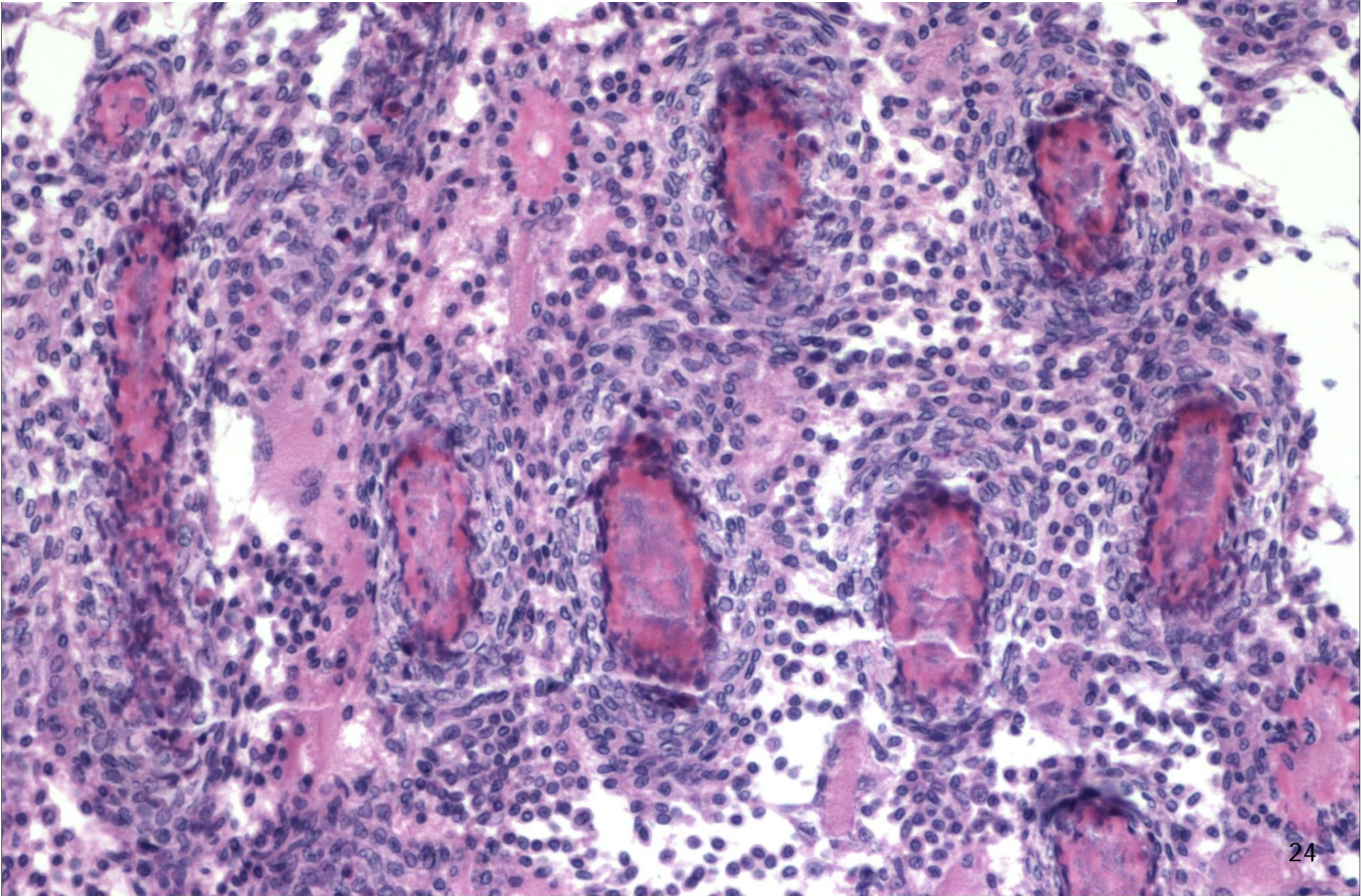


Samples from Mexico

May 2013

- Acute phase samples from Mexico showing EMS/AHPND.
- Chronic phase of EMS/AHPND.

Mexico – *Penaeus vannamei* - Terminal Phase of AHPND;
UAZ-APL 13-220A-3; 20x

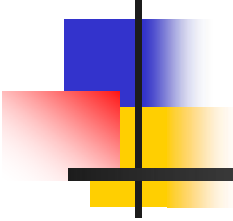


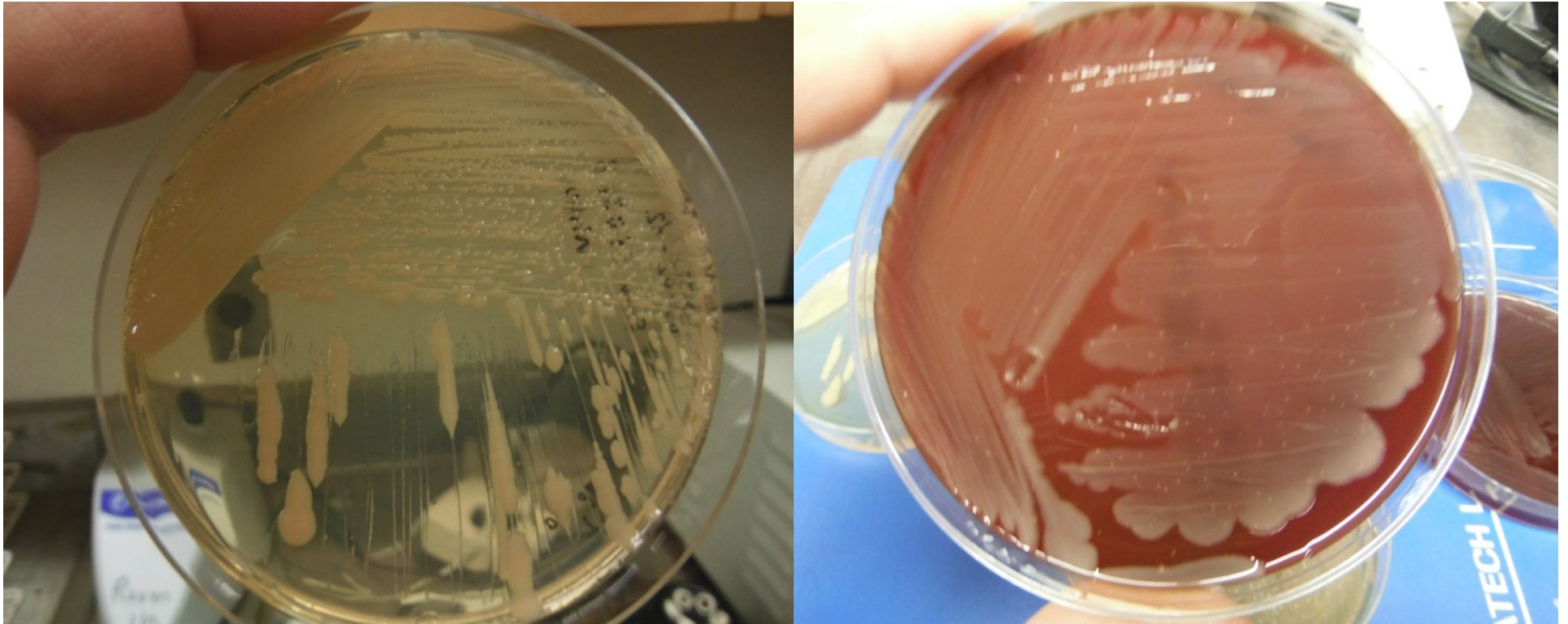
Proposed Case Definition for EMS/ AHPND



- Idiopathic – no specific disease causing agent (infectious or toxic) was identified until March 2013.
- Pathology:
 - ✓ acute progressive degeneration of hepatopancreas (HP) from medial to distal with dysfunction of all HP cells, prominent necrosis & sloughing of these tubule epithelial cells.
 - ✓ terminal stage shows marked inter- & intra-tubular hemocytic inflammation & development of massive secondary bacterial infections that occur in association with necrotic & sloughed HP tubule cells.

Recent work on the Agent of AHPND/EMS

- 
-
- Biochemical characterization.
 - Molecular characterization of extra chromosomal elements of the VP that causes AHPND/EMS.



The agent found to induce EMS/AHPND pathology was identified as a strain of ***Vibrio parahaemolyticus***.

Biochemical comparison of SE Asian & Mexican VP isolates that cause AHPND

API 20 NE Test Result	VP A/3 SE Asia	VP from Mexico
NO ₃ => NO ₂	+	+
NO ₂ => N ₂	-	-
Indole	+	+
Glucose fermentation	- (usually +)	- (usually +)
Arginine dihydrolase	-	-
Urease	-	-
Esculin hydrolysis	-	-
Gelatin liquefaction	+	+
B-Galactodiase	+	+
Assimilation of: D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, maltose, L-malate	+	+
D-gluconate, caprate, citrate, phenyl acetate	-	-
Oxidase	+	+



Five *Vibrio parahaemolyticus*
isolates from S.E. Asia & Mexico

Designation	Causes AHPND
13-028A/2	NO
13-028A/3	YES
12-297B	YES
1335	YES
13-306D/4	YES



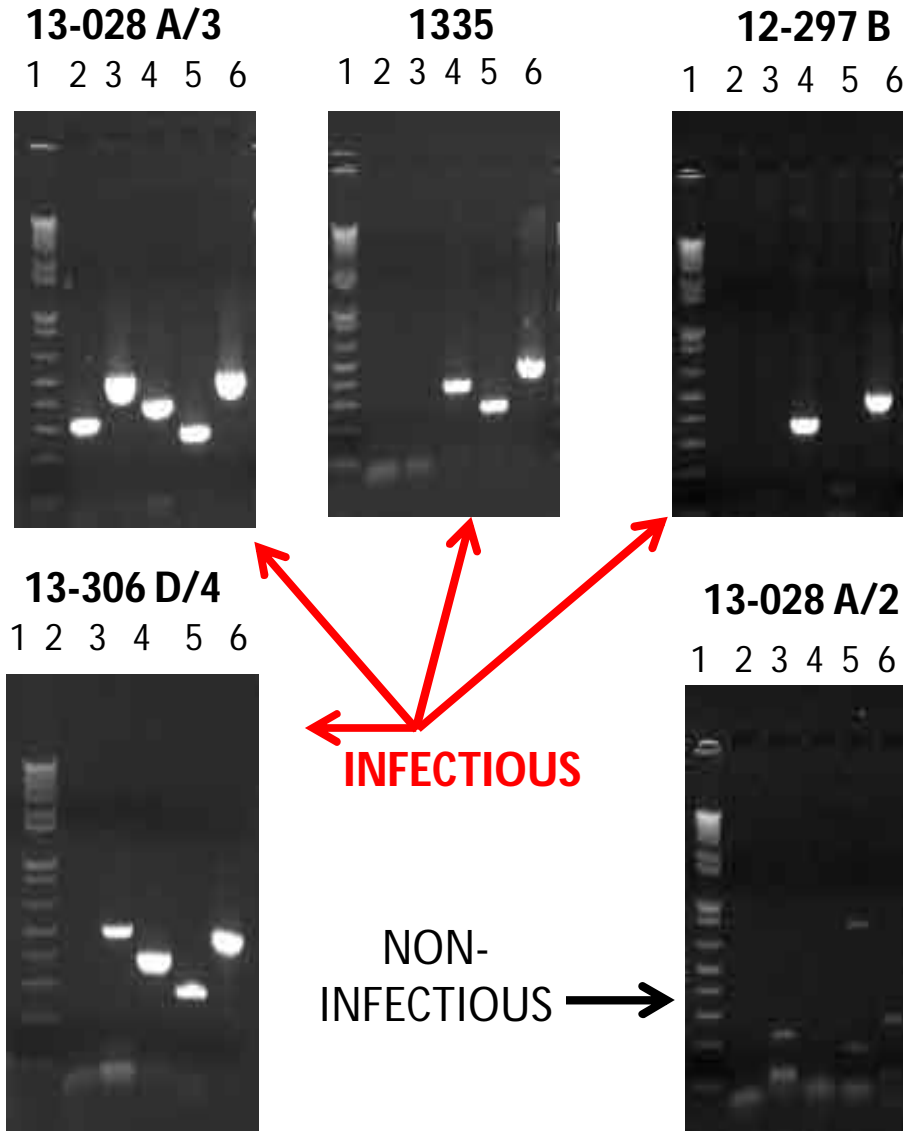
Two VP isolates underwent metagenomic sequencing

- Sequenced were VP A/2 & VP A/3.
- VP A/2 does not cause AHPND/EMS.
- VP A/3 does cause AHPND/EMS.
- Primers were designed from the metagenomic sequencing data for the extra-chromosomal genetic material that was found.
- These primers gave the following results:

PCR profile of each VP isolate

Samples:

1. 1 Kb marker
2. Phage (contig 9)
3. Contig 32
4. Contig 52
5. Contig 73
6. Contig 89

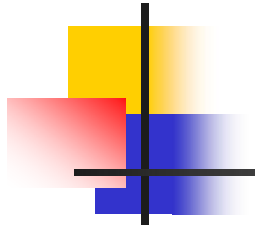




Contigs

- Contigs 52 & 89 are the only consistent amplicons present among the three AHPND-causing isolates.
- A Gene Reach PCR kit is being developed for the VP agent of AHPND based on Contig 89.

Detection of Geographic Isolates Using Contig 89



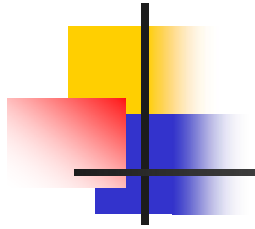
Samples:

1. 1 kb marker
2. Vietnam
3. China
4. Malaysia
5. Thailand
6. Mexico

1 2 3 4 5 6



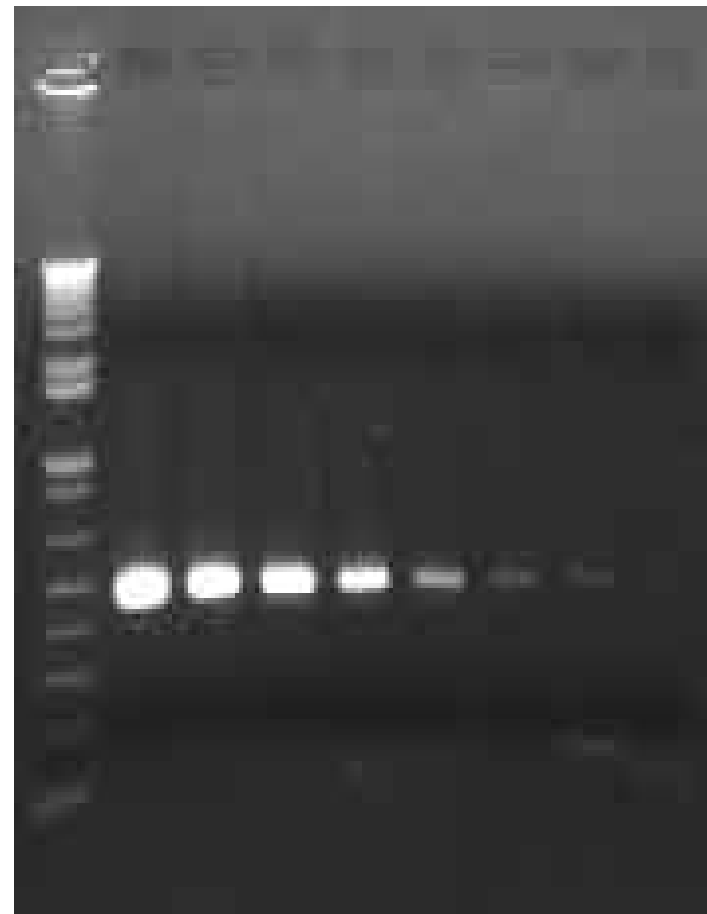
Sensitivity of PCR Using Contig 89

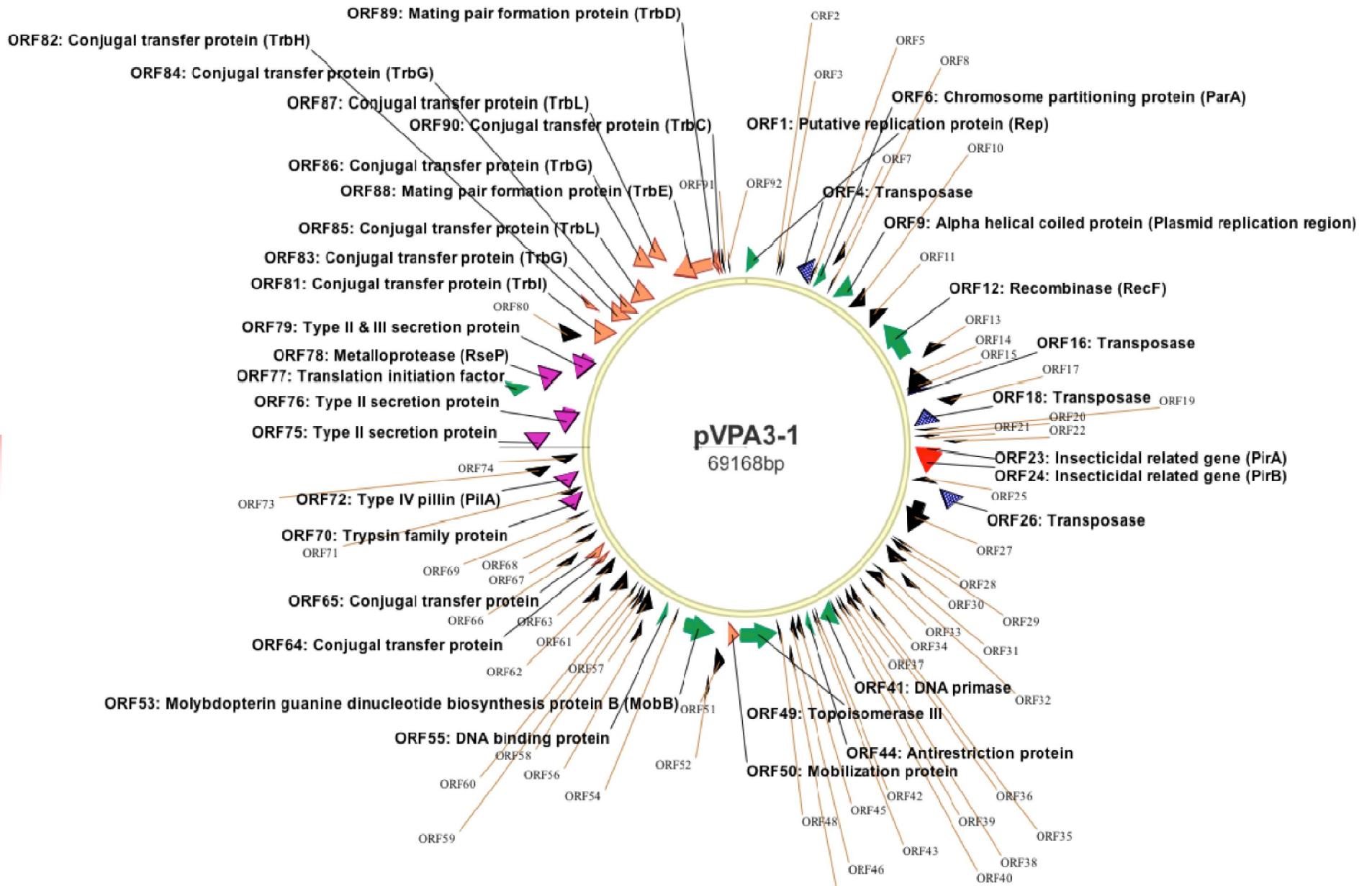


Samples/Dilution of VP Culture:

1. 1 Kb marker
2. A/3 (10^{-2})
3. A/3 (10^{-3})
4. A/3 (10^{-4})
5. A/3 (10^{-5})
6. A/3 (10^{-6})
7. A/3 (10^{-7})
8. A/3 (10^{-8})
9. A/3 (10^{-9})

1 2 3 4 5 6 7 8 9





Transposase



Hypothetic protein



Plasmid structural protein



Virulence associated protein

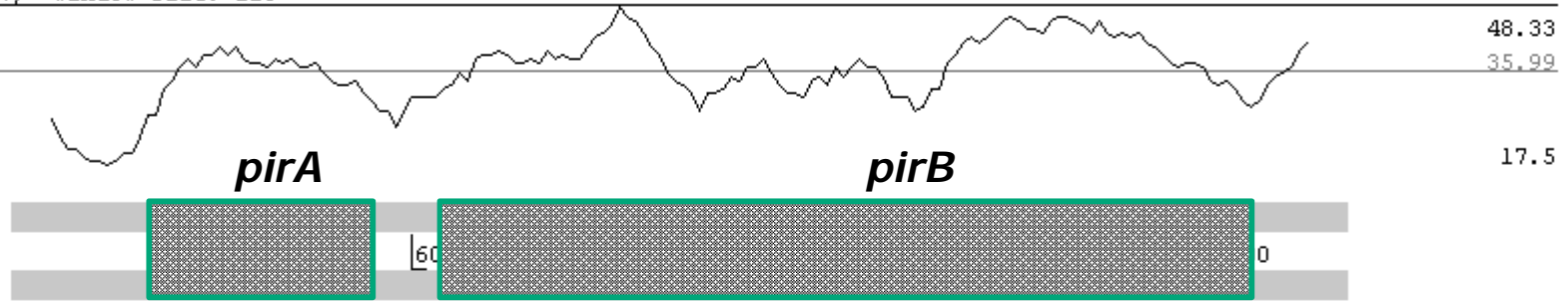


Plasmid mobilization protein



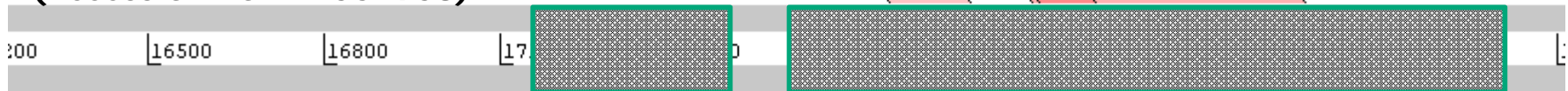
Insecticidal related protein

GC Content (%) Window size: 120

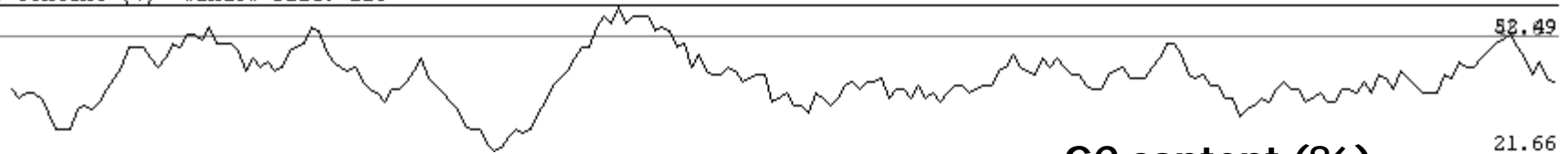


Photorhabdus luminescens *PirA* and *PirB* genes
(Accession no. DQ459368)

Vibrio parahemolyticus *PirA* and *PirB* genes
In the plasmid pVPA3-1
(Accession no. KM067908)

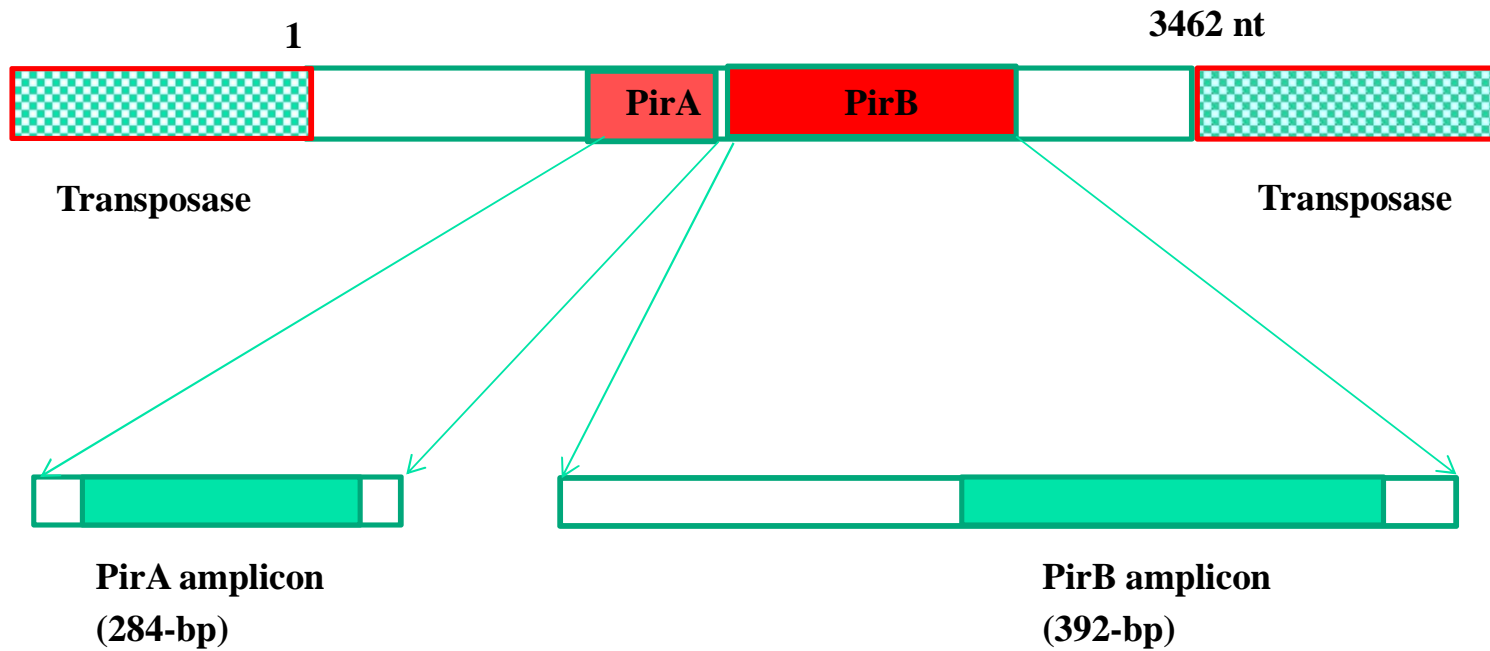


GC Content (%) Window size: 120



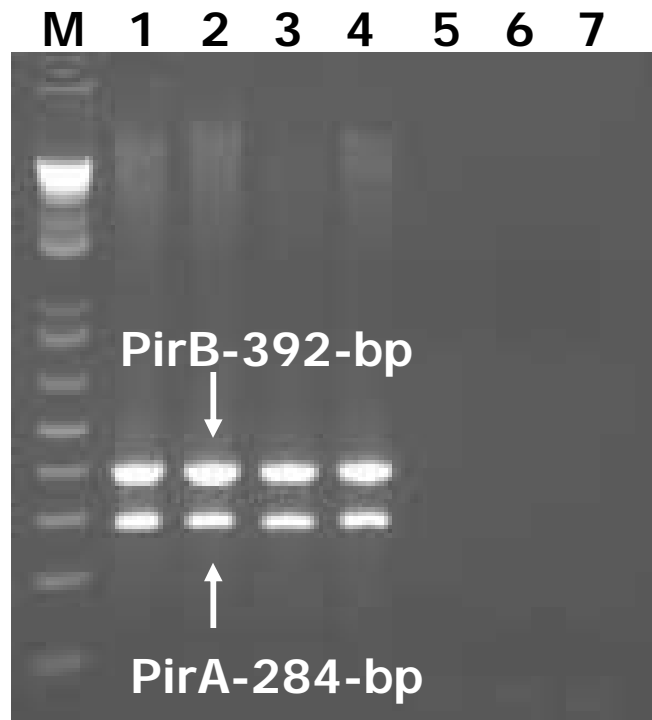
GC content (%)

Pir (Photorhabdus insect related) A- and PirB-like genes



Plasmid (69.1-kb)	G+C%
3.4-kb PirA & B region	37
Remaining plasmid region	45

PCR detection of PirA- and PirB-like genes in *V. parahaemolyticus*, the causative agent of AHPND

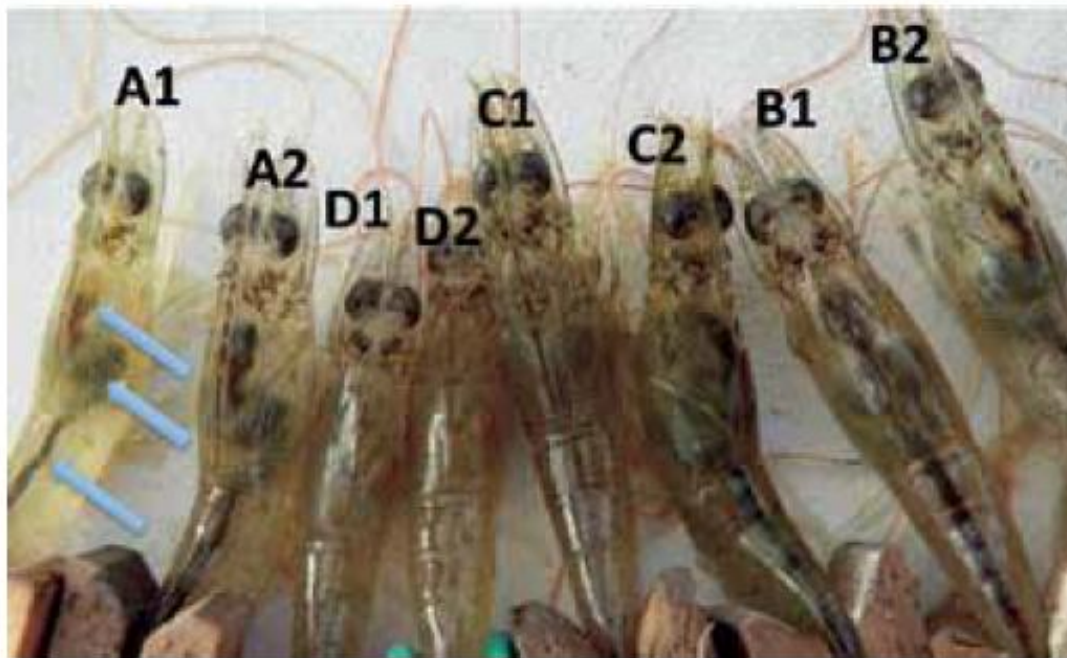


PIR B is 52 kd
PIR A is 14 kd

Lane #	Strain	AHPND	Origin
1	13-511A/1	Positive	MX
2	A3	Positive	VN
3	13-306D/4	Positive	MX
4	12-194G	Positive	VN
5	A2	Negative	VN
6	13-488L	Negative (SHPN+)	India
7	13-431/1	Negative (SHPN+)	US-TX

production

Tilapia Could Enhance Water Conditions, Help Control EMS In Shrimp Ponds



Ten days after exposure to pathogenic *Vibrio parahaemolyticus*, shrimp A1, A2, C2, B1 and B2 show normal stomachs, hepatopancreases and midguts (arrows from top to bottom). The remaining shrimp show signs of AHPN infection: empty stomachs, pale hepatopancreases and empty midguts.

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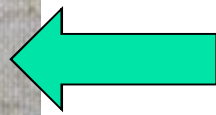
Donald V. Lightner, Ph.D

School of Animal and Comparative
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AHPN Challenge Study

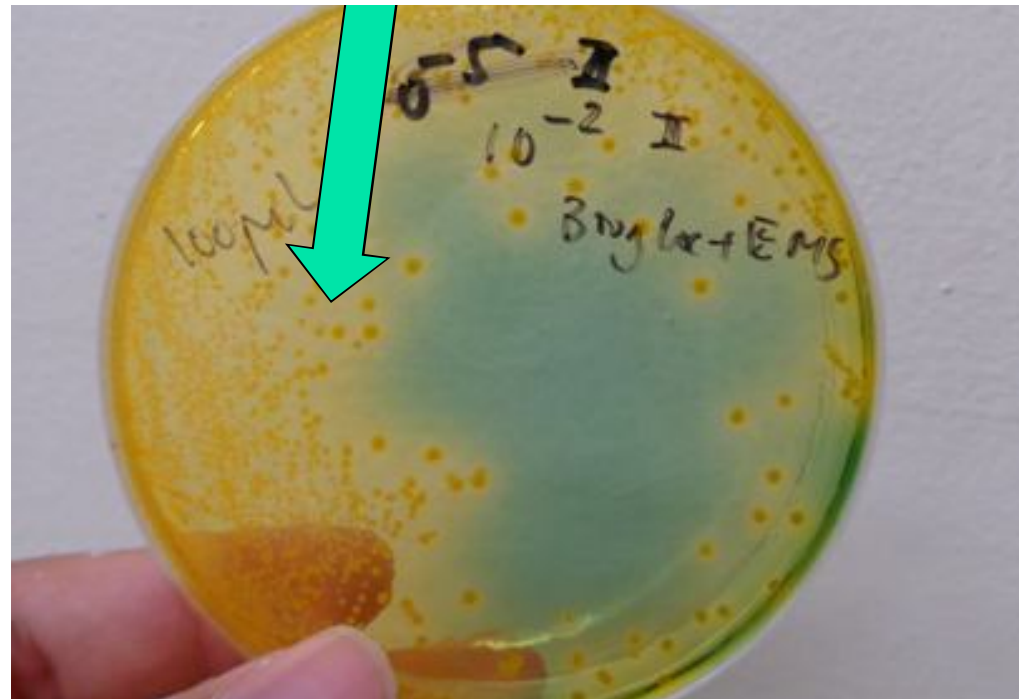
A laboratory study was conducted at

How to Explain the Effects of Tilapia?



Dense blooms of *Chlorella* spp. are typical with tilapia.

TCBS yellow (as opposed to green) colonies dominate cultures from tilapia tanks.





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Thank you
for your attention!



Reference Lab for Crustacean (Shrimp) Diseases