Food and Agriculture Organization of the United Nations

FAO/China Intensive Training Course on Tilapia Lake Virus (TiLV)
Sun Yat Sen University, Guangzhou, China
18-24 June 2018

Session 2

Ha Thanh Dong[1,2]

Emerging, re-emerging and new diseases of tilapia

[1]King Mongkut's University of Technology Thonburi, [2]Fish Health Platform, Centex Shrimp (Mahidol University/BIOTEC), Thailand.

Objective

- To update on emerging, re-emerging and new diseases of tilapia
  - ❖Emerging viral infections
  - ❖Emerging bacterial infections
  - ❖Emerging parasitic infection
  - ❖Emerging unknown pathogen

Emerging Viral Infections

Viral Infections in Tilapia

Agent	DNA/ RNA	Geographical Distribution	Ref.
Lymphocystis disease virus (LCDV)	DNA	North Tanzania	Paperna, 1973
Infectious pancreatic necrosis virus (IPNV)	RNA	Taiwan	Hedrick et al. 1983
Bohle virus	DNA	Australia	Ariel and Owens, 1997
Iridovirus-like		Canada	McGrogan et al.1998
Viral nervous necrosis (VNN)	RNA	France, Indonesia and Thailand	Bigarre´ et al. 2009; Prihartini et al. 2015; Keawcharoen et al. 2015
Infectious spleen and kidney necrosis virus (ISKNV)	DNA	US Midwest, Thailand	Subramaniam et al. 2015; Suebsing et al. 2016
Tilapia larvae encephalitis virus (TLEV)	DNA	Israel	Shlapobersky et al. 2010
Tilapia lake virus (TiLV)	RNA	Asia, Africa, and South America	e.g. Eyngor et al. 2014; Jansen et al. 2018

Emerging, re-emerging, new viral infections of tilapia

	Lymphocystis disease virus (LCDV)	*Lymphocystivirus*	Case reports with little concern
Re-emerging	Infectious pancreatic necrosis virus (IPNV)	*Aquabirnavirus*	Case reports with little concern
	Bohle virus	*Ranavirus*	Case reports with little concern
	Iridovirus-like	*Iridoviridae*	Case reports with little concern
Emerging	Viral nervous necrosis (VNN)	*Betanodavirus*	Case reports with little concern
Emerging	Infectious spleen and kidney necrosis virus (ISKNV)	*Megalocytivirus*	Case reports with little concern
New/ newly emerging	Tilapia larvae encephalitis virus (TLEV)	*Herpesvirus*	Case reports with little concern
New/ newly emerging	Tilapia lake virus (TiLV)	*Tilapinevirus*	→ Global concern

IPNV re-emerged in tilapia

1983: Subclinical infection of IPNV in tilapia in Taiwan was reported
1987: Experimental challenge indicated that IPNV is pathogenic to tilapia (killed 25% fish)

……………

……………

2018: IPNV re-emerged in tilapia

- Subclinical infection cases
- Its impact remains unknown
- Investigation should be initiated in tilapia farming countries

Received: 1 January 2018 | Revised: 27 February 2018 | Accepted: 28 February 2018
DOI: 10.1111/jfd.12807

ORIGINAL ARTICLE

WILEY Journal of Fish Diseases

Infectious pancreatic necrosis virus isolated from farmed rainbow trout and tilapia in Kenya is identical to European isolates

I R Mulei[1,2] | P N Nyaga[2] | P G Mbuthia[2] | R M Waruiru[2] | L W Njagi[2] | E W Mwihia[2] | A.A.A. Gamil[1] | Ø Evensen[1] | S Mutoloki[1]

TABLE 1 Results of samples screened for infectious pancreatic necrosis virus by PCR and immunohistochemistry

Species	Farm	Samples collected	Ratio positive by PCR and sequencing	Ratio positive by Immunohistochemistry
Rainbow trout	RTH	15	7/9	4/7
	RTM	14	9/13	1/9
	RTT	6	2/5	2/7
	RTK	12	5/10	6/8
	RTJ	15	3/5	1/5
Tilapia	TGA	15	5/8	Not examined
	TCD	12	4/8	Not examined
	TOF	16	3/10	Not examined

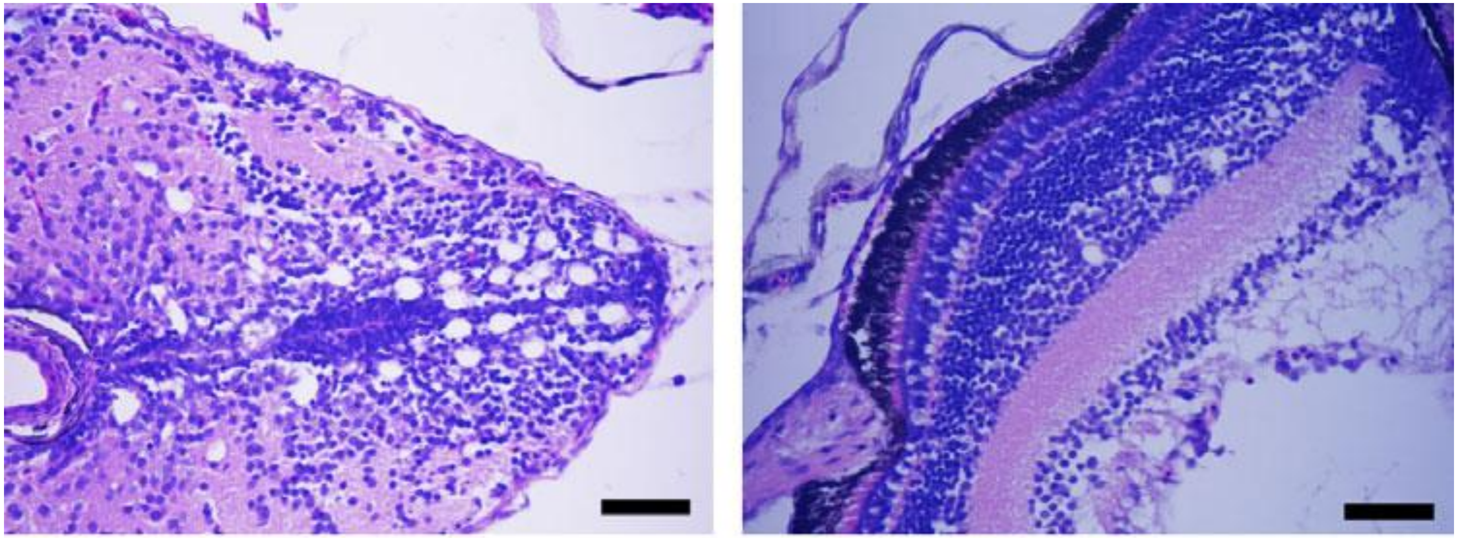
Viral Nervous Necrosis (VNN) disease

- ❖ **Causative agent**: *Betanodavirus*
- ❖ **Clinical signs:** signs of neurological disorders: loss of balance, erratic swimming
- ❖ **Host:** >30 species, mainly in marine fish
- ❖ **Geographical distribution**: worldwide
- ❖ **Cases in tilapia** (France, Thailand & Indonesia)

  e.g. a case in tilapia hatchery
  - 10 days-old larvae of tilapia
  - Mortality 90-100%
  - Histopathological manifestation of VNN disease
  - 93.07–93.88% similarity to red-spotted grouper nervous necrosis virus (RGNNV)

Keawcharoen et al. JFD 2015, 38, 49-54

Viral Nervous Necrosis (VNN) disease

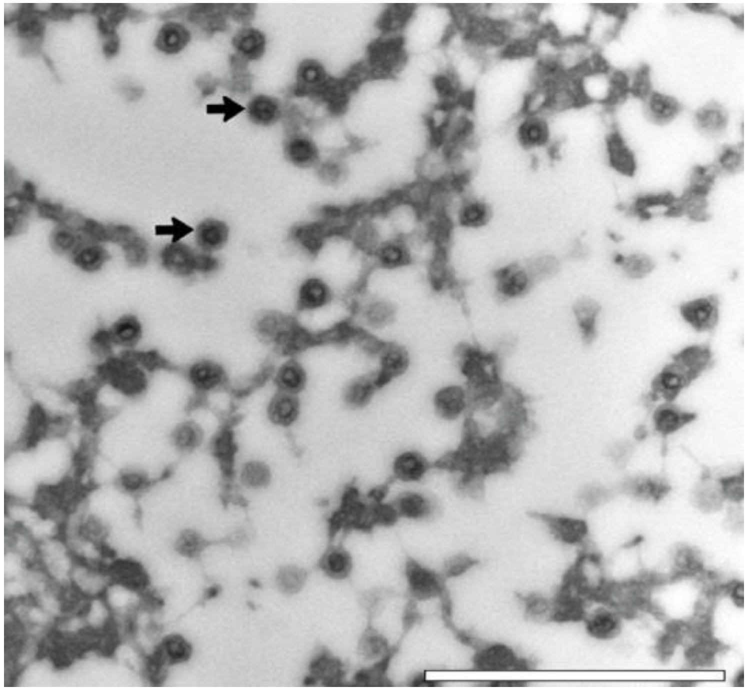


Keawcharoen et al. JFD 2015, 38, 49-54

- **Histopathological feature:** Vacuolation was observed in brain, eye and spinal cord of diseased fish
- **Detection methods:** PCR methods (OIE disease card)

Infectious spleen and kidney necrosis disease (ISKND)

- **Synonym**: Iridoviral disease (common name), red sea bream iridoviral disease (OIE)
- **Causative agent**: *Megalocytivirus* ISKNV
- **Clinical signs**: darkening, pale gills
- **Host**: wide range of both marine and freshwater fish, including tilapia



Subramaniam et al. (2016)

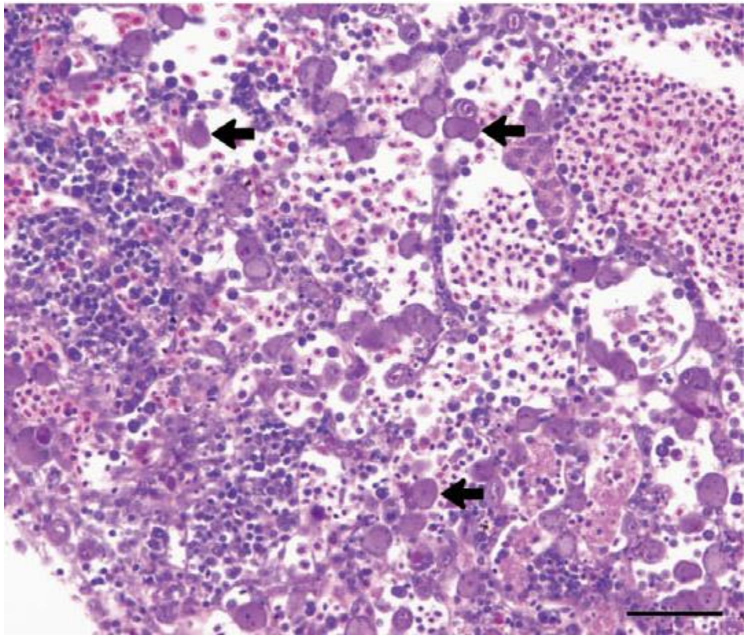
Infectious spleen and kidney necrosis disease (ISKND)

A case in USA (Subramaniam et al. 2016)

- Tilapia fry/fingerlings
- Mortality 50-75%

In Thailand

- Multiple infections of ISKNV/Iridovirus was reported in cage culture & a semi-nested PCR was developed (Dong et al. 2016)
- Recent reports: vertical transmission & LAMP detection method (Suebsing et al. 2016)



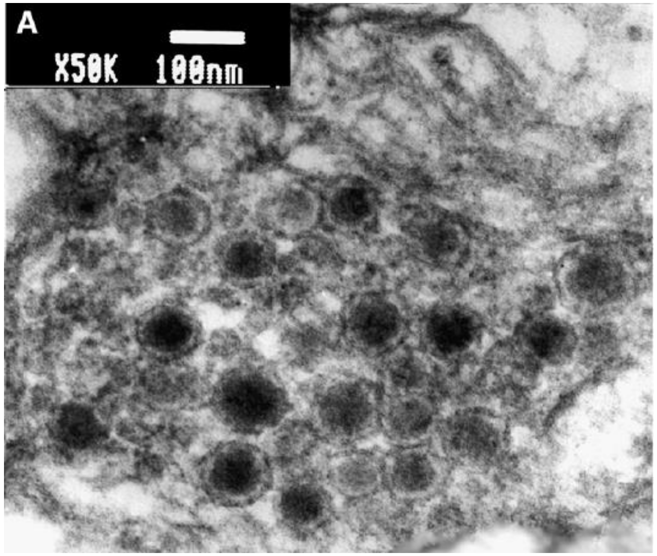
Presence of basophilic hypertrophied cells
(Subramaniam et al. 2016)

Tilapia larvae encephalitis virus (TLEV) disease

- **Causative agent**: TLEV/Herpes-like virus
- **Clinical signs**: spiral swimming
- **Host:** blue tilapia (*O. aureus*), red tilapia (*Oreochromis* sp.), Nile tilapia (*O. niloticus*)
- **Mortality**: reach up to 98%
- **Susceptible stages:** 32-34 days post fertilization
- **Geographical distribution**: Israel
- **Histopathological feature:** Not available
- **PCR detection:** available

TLEV-1(5′ TCGTGGGCCTTATCCCGCGT 3’)

TLEV-2 (5′ GAGACCAGAAAGTGCTTCTC 3′)



Lack of investigation in other countries

Shlapobersky et al. Virology (2010) 399: 239-247

Tilapia lake virus disease (TiLVD)

REVIEWS IN Aquaculture

Reviews in Aquaculture, 1–15 doi: 10.1111/raq.12254

Tilapia lake virus: a threat to the global tilapia industry?

Mona Dverdal Jansen[1], Ha Thanh Dong[2] and Chadag Vishnumurthy Mohan[3]

1 Norwegian Veterinary Institute, Oslo, Norway
2 Department of Microbiology, Faculty of Science, King Mongkut's University of Technology Thonburi (KMUTT), Bangkok, Thailand
3 WorldFish, Penang, Malaysia

Will be presented by other speakers

Emerging Bacterial Infections

Bacterial Diseases in Tilapia

- Streptococcosis – Emergence of S. agalactiae serotype IX
- Columnaris – Complexity of *F. columnare*
- Francisellosis – Emerging/re-emerging in some countries
- Hemorrhagic septicemia
  - *A. hydrophila*
  - Non-*A. hydrophila* (*A. veronii* & *A. jandaei*) (Dong et al. JFD 2017)
- Edwardsiellosis caused by *E. ictaluri*
- *Aerococcus viridans* infection (Ke et al. Aquaculture 2012)
- Hahellosis/red egg disease (Senapin et al. Aquaculture 2016)
- Unknown diseases

} New to tilapia

S. agalactiae serotype IX emerged in tilapia

- GBS have been classified to 10 serotypes (Ia, Ib, II–IX)
- In aquatic animals: 4 serotypes Ia, Ib, II and III
- Serotype IX is new to tilapia
- Killed 10-90% fish in challenged experiments
- Investigation of serotype IX should be investigated in other countries

Table 2. The geographical distribution of the *S. agalactiae* isolates

Areas	Serotype III	Serotype IX	Total
Hui Zhou	15	0	15
Zhan Jiang	5	1	6
Zhao Qing	53	5	58
Zhu Hai	0	1	1
Total	73	7	80

Zhang *et al*. 2018
Microbial Pathogenesis,
doi: 10.1016/j.micpath.2018.05.053.

Complexity of *F. columnare* in tilapia

- *F. columnare* is causative agent of columnaris disease
- *F. columnare* in tilapia is a complex of several unclassified taxa

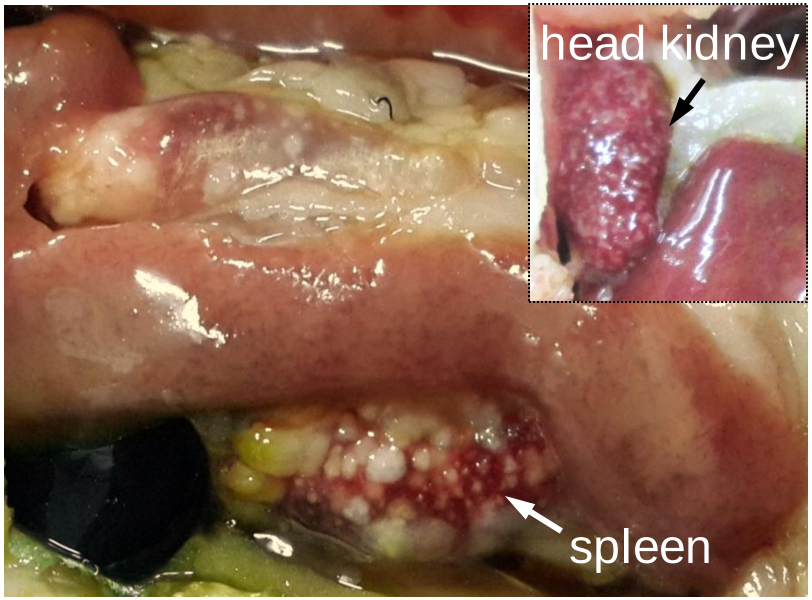
Genomovar	II				I		I/II	II			
Strains	1362	CF1	94-081	C#2	ATCC49512	Pf1	1215	1214	NK01	*F. johnsoniae* UW101	*F. psychrophilum* JIP02/86
1362											
CF1	92.9 0.15										
94-081	97.6 0.19	91.3 0.04									
C#2	93.2 0.36	92.1 0.21	93.1 0.17								
ATCC49512	42.6 0.85	43.5 0.71	42.6 0.66	43 0.5							
Pf1	42.8 0.96	43.4 0.82	42.7 0.77	43.3 0.61	95.9 0.11						
1215	30.1 0.02	30.4 0.12	30 0.17	30.2 0.33	29.8 0.83	29.8 0.94					
1214	27.8 0.62	28.1 0.76	28.3 0.81	27.9 0.98	28 1.47	28.2 1.58	28 0.64				
NK01	27.7 0.74	28.1 0.89	28.3 0.93	27.9 1.1	28 1.6	28.1 1.71	28.1 0.77	94.5 0.12			
F. johnsoniae UW101	22.6 3.5	21.9 3.36	22.7 3.31	24 3.14	22.6 2.65	22.9 2.54	20.7 3.48	21.5 4.12	20.9 4.24		
F. psychrophilum JIP02/86	20.4 1.92	20.7 1.78	21.1 1.73	22.2 1.56	21.5 1.07	21.7 0.96	20.1 1.9	20 2.54	20 2.66	22 1.58	

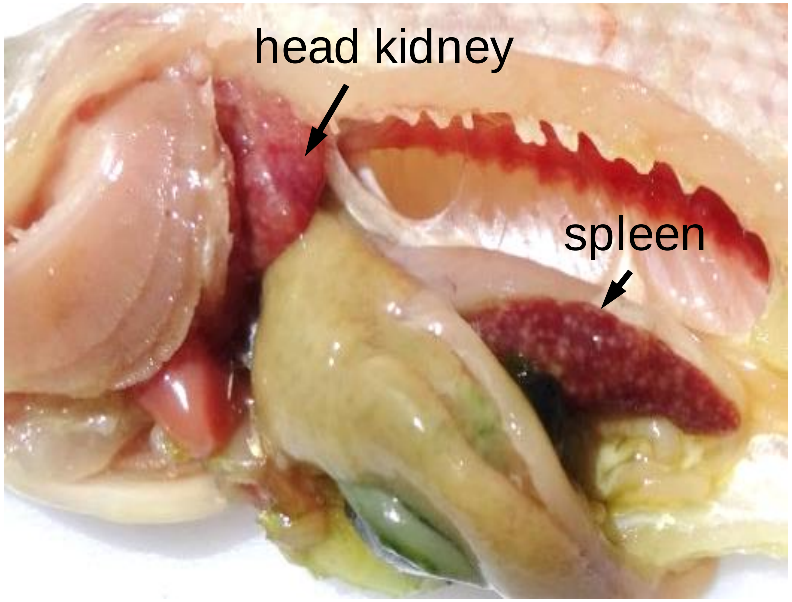
%DDH
% G+C content difference

dDDH supports taxonomic reclassification of Fc originated from tilapia

Dong et al. J Fish Dis (2015) 38:901-913
Kayansamruaj et al. Infection, Genetics and Evolution 54 (2017) 7–17

Same same…but different…





What disease you think about?

Francisellosis of tilapia

Causative agent:

- *Francisella noatunensis* subsp. *orientalis*
- Previously known as Rickettsia-like organism, RLO
- Fastidious intracellular bacterium

Host range:

- Susceptible tilapia, ornamental cichlids
- Infection but does not kill the hosts: striped catfish, common carp

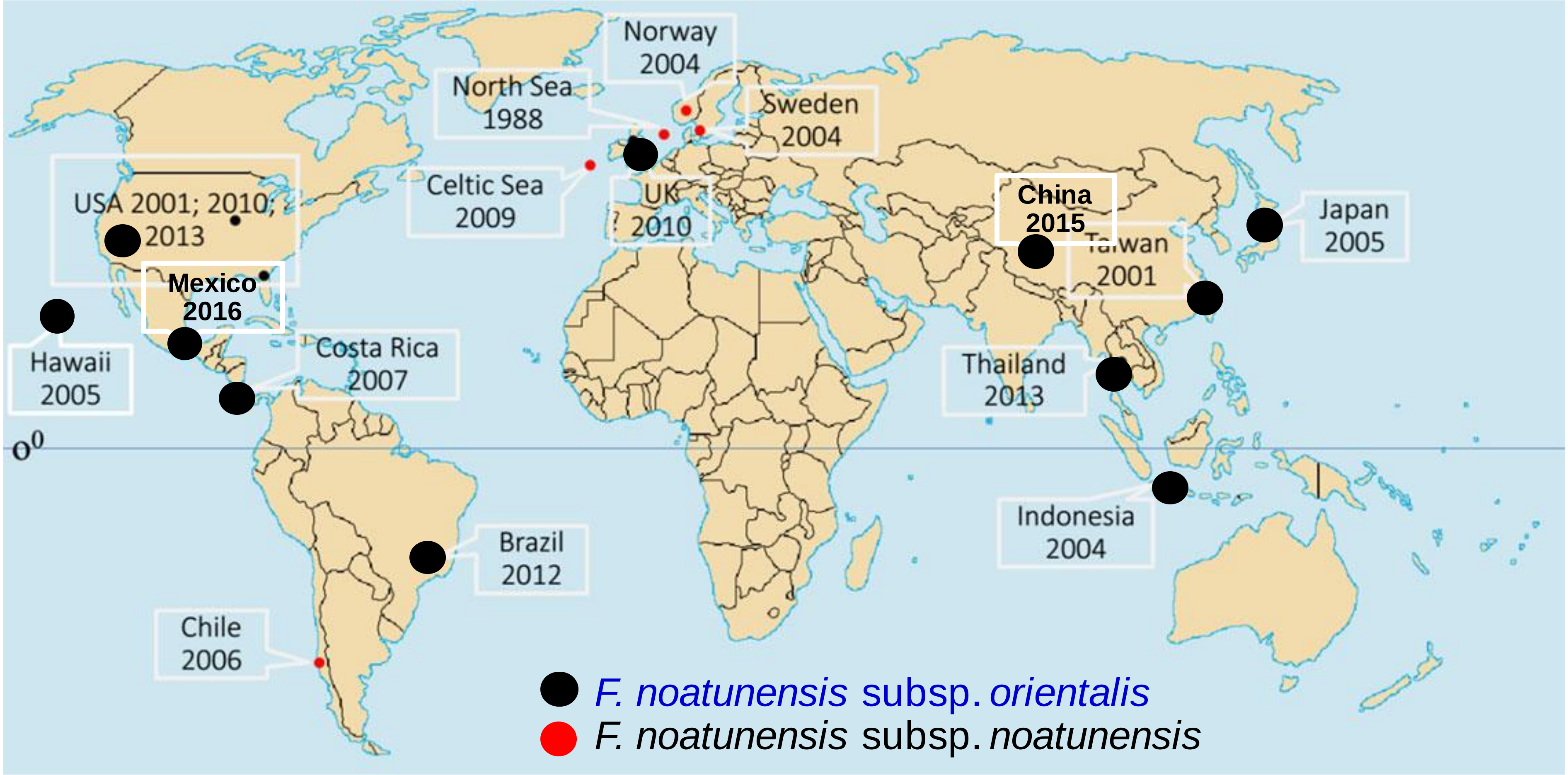
Cumulative mortality: 40-50%

Clinical signs: visceral white spots (eg spleen & head kidney)

Season: Cool weather (25-28 ^{o}C)

Francisellosis of tilapia

Geographical distribution

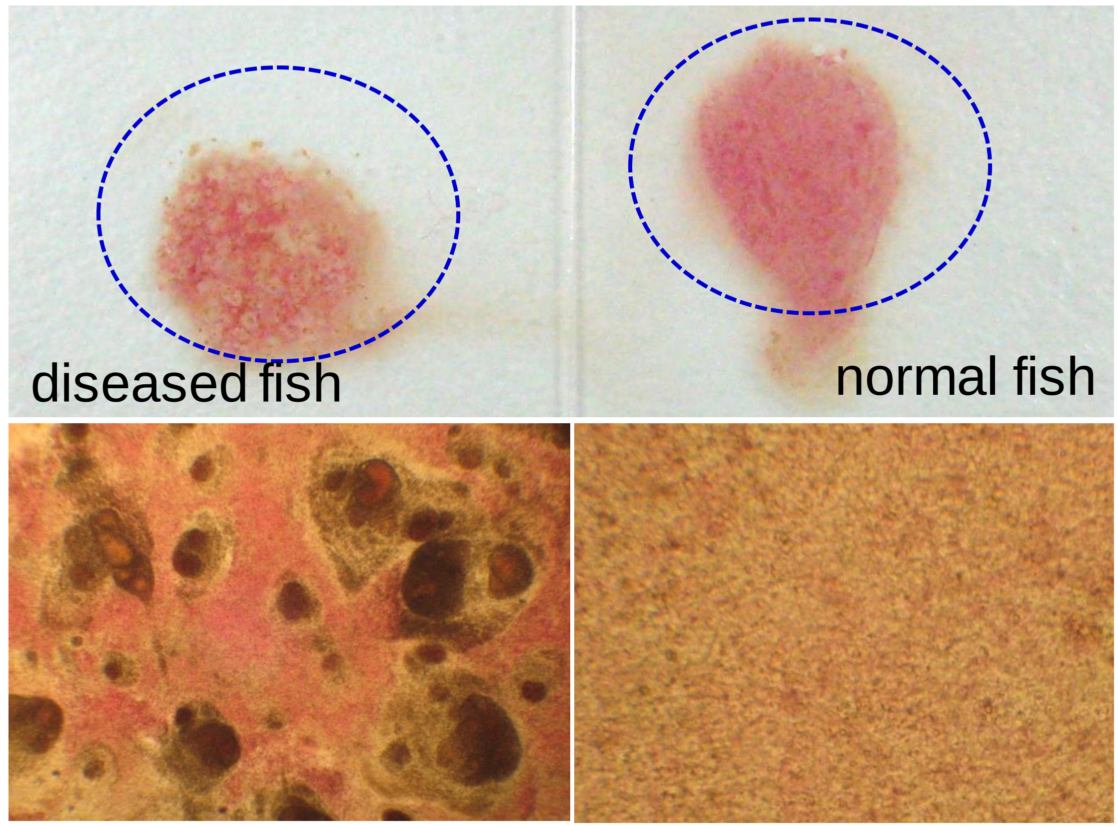


Adapted from Nguyen et al. 2015 Aquac Res. doi:10.1111/are.12802

Francisellosis of tilapia

Presumptive Diagnosis

Clinical sign

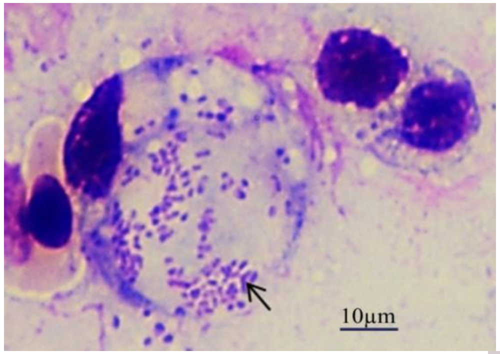


Wet mount examination

Photographs were taken in conjunction with the outbreaks described in Nguyen et al. 2015. Aquac Res & Dong et al. 2016. Dis Aquat Org.

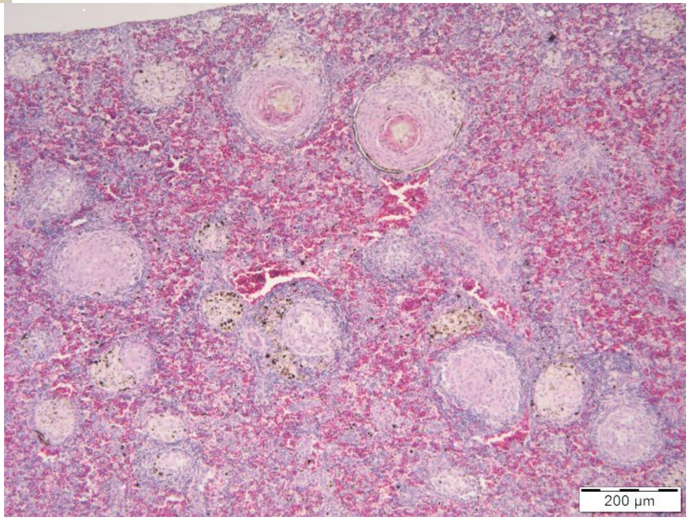
Francisellosis of tilapia

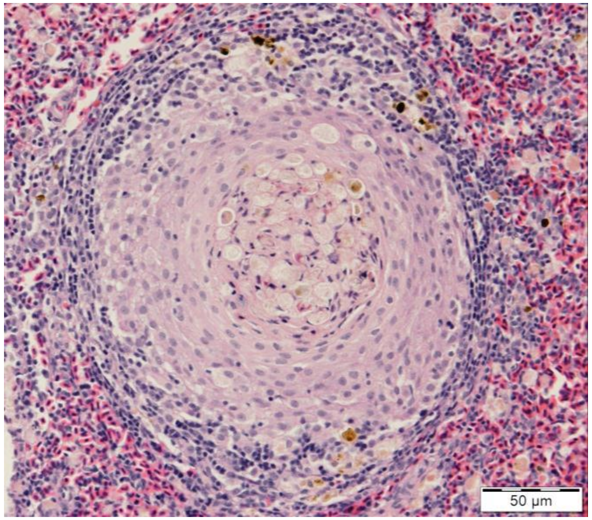
Diagnosis



Rapid staining of smeared-head kidney with Giemsa revealed presence of both intra- and extra-cellular bacteria

Micrographs of H&E stained sections of the spleen showed typical granulomas



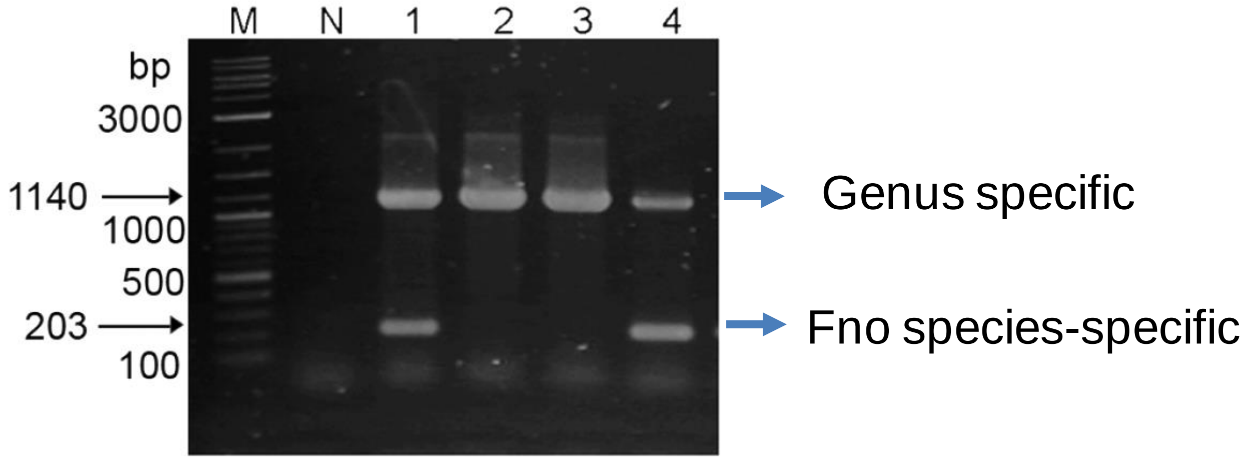


Photographs were taken in conjunction with the outbreaks described in Nguyen et al. 2015. Aquac Res & Dong et al. 2016. Dis Aquat Org.

Francisellosis of tilapia

Molecular Diagnosis

- Genus specific PCR (Forsman et al. 1994)
- Real-time PCR (Duodu et al. 2012);
- ISH, genus-specific (Hsieh et al. 2007)
- Immunohistochemistry (Soto et al. 2012)
- Duplex PCR and ISH (Dong et al. 2016)
- Colorimetric LAMP (Pradeep et al. 2016)
- Recombinase polymerase amplification (RPA) (Shahin et al. 2018)



Which one infected with *F. noatunensis* subsp. *orientalis* ?

Photograph was taken in conjunction with the outbreaks described in Nguyen et al. 2015. Aquac Res

Edwardsiellosis of tilapia

Causative agent:

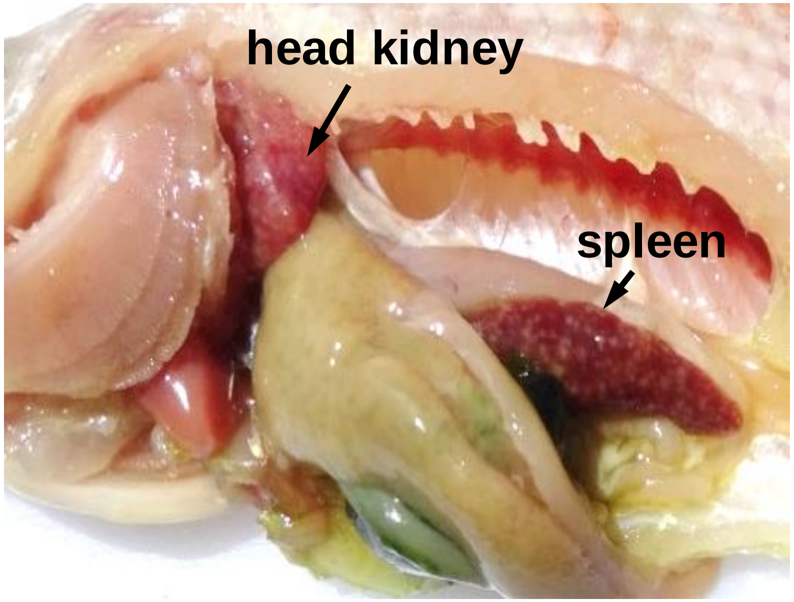
- *Edwardsiella ictaluri*
- Common in catfish but not common in non-catfish
- Does not kill tilapia in striped catfish ponds (personal observation)
- 2012: first report of *E. ictaluri* in Nile tilapia in Western Hemisphere (Soto et al. 2012)
- No reported in other countries

Edwardsiellosis of tilapia

Recent cases in Southeast Asia

- Red tilapia juveniles
- Killed 40-50% fish in the first month after stocking
- Presence of white spots in multiple internal organs

- Presumptive diagnosis based on clinical sign: Francisellosis
- PCR negative for *Fno*

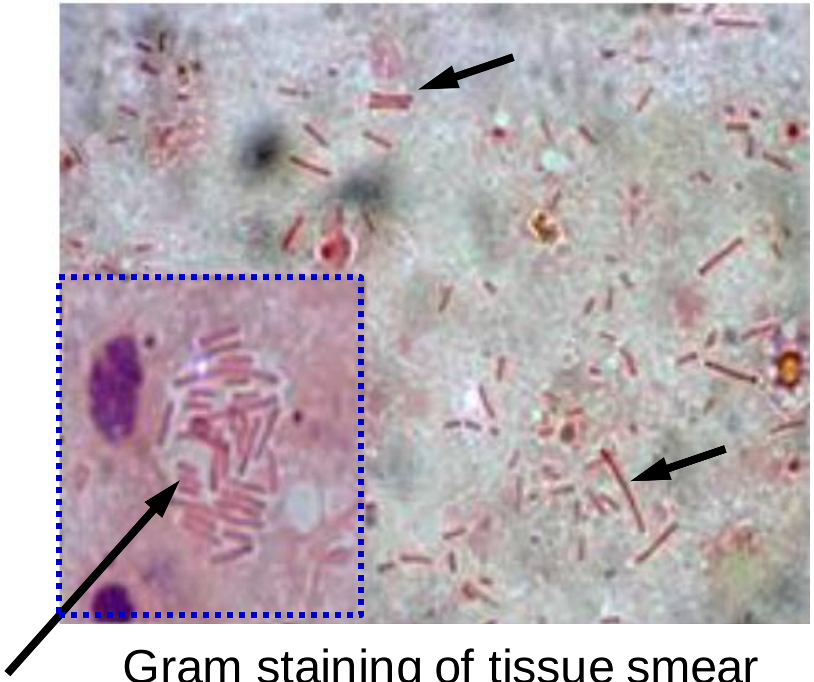


MS submitted

Edwardsiellosis of tilapia

Presumptive diagnosis

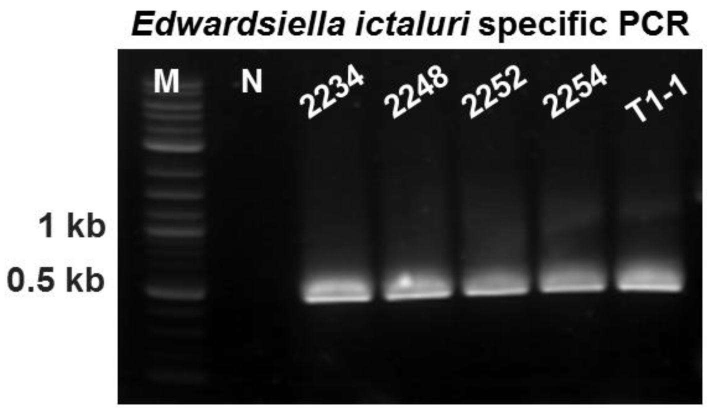
- Tissue smear, Gram staining (take 5 min)
- Numerous Gram negative, rod-shaped bacteria
- Suspected bacterial infection

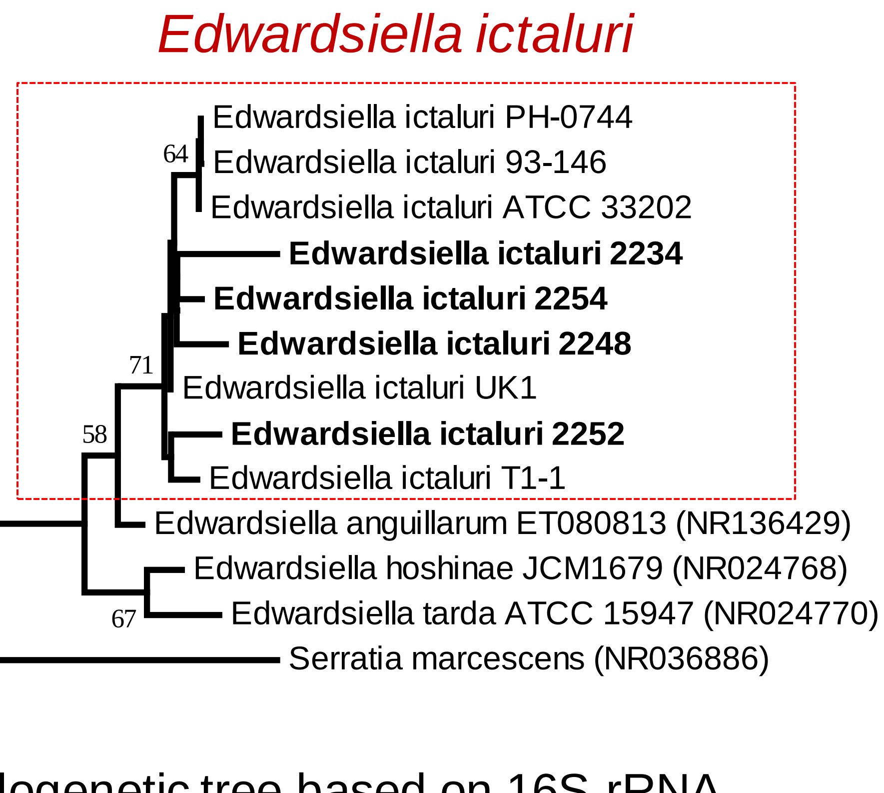


Gram staining of tissue smear

Edwardsiellosis of tilapia

- Bacterial isolation: pure pinpoint colonies on TSA
- Gram negative, rod-shaped bacteria





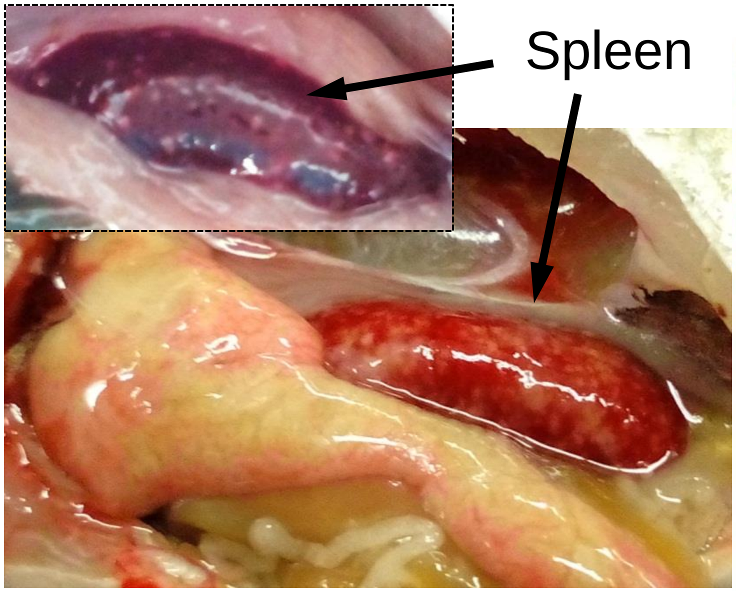
Phylogenetic tree based on 16S rRNA

MS submitted

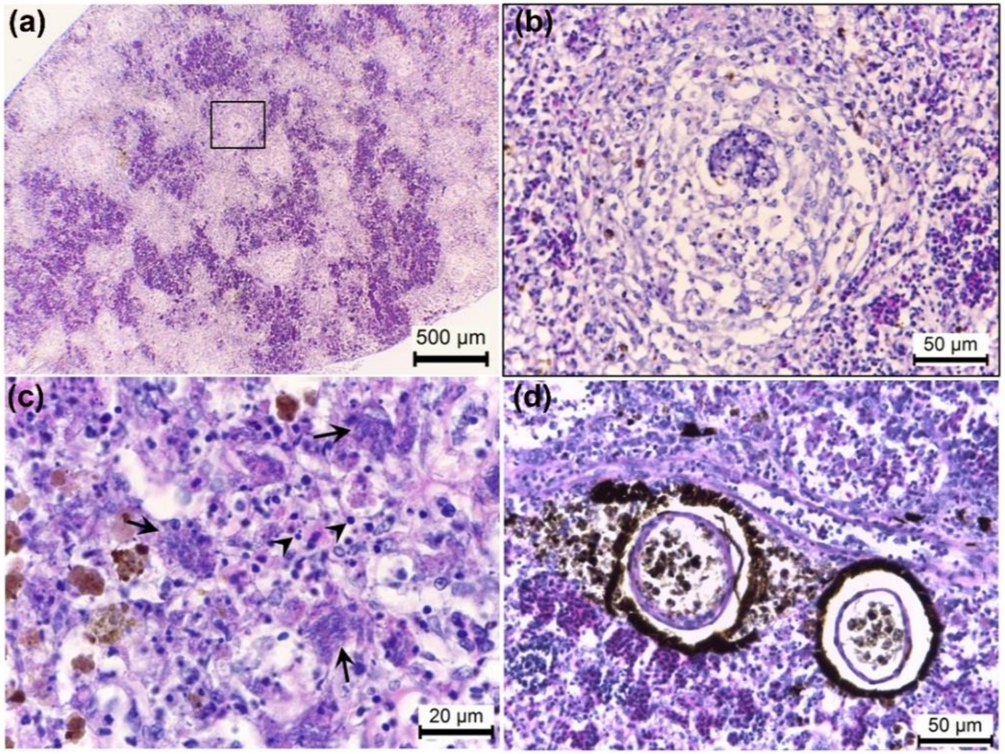
Edwardsiellosis of tilapia

Challenged experiments fulfilled Koch's postulates

- Fish reproduce the same clinical signs
- 95-100% mortality in 3-9 days (dose-dependent)



Experimental fish



Histopathological features of edwardsiellosis in the experimental fish

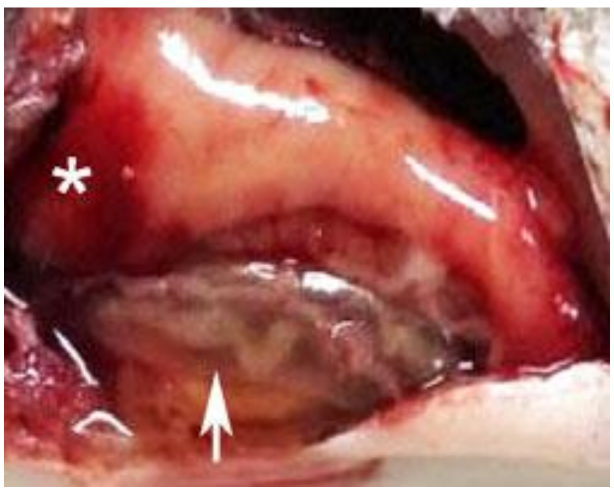
MS submitted

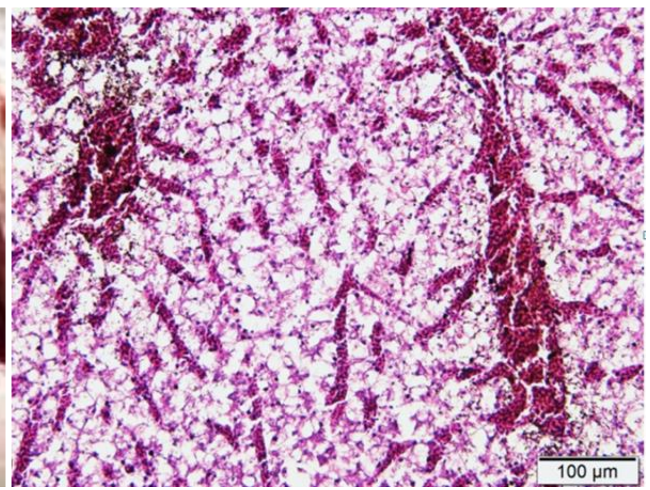
Edwardsiellosis of tilapia

- *E. ictaluri* is an emerging pathogen of tilapia aquaculture in Southeast Asia
- *E. ictaluri* infections in tilapia may have been overlooked due to similar clinical signs between Francisellosis & Edwardsiellosis
- Should be put on disease watchlist

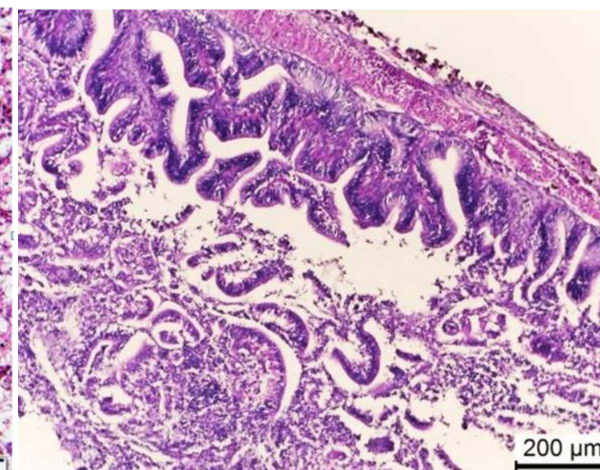
A. veronii & A. jandaei infection

- are newly reported pathogens of tilapia
- may have been misidentified as *A. hydrophila* or previously overlooked
- both cause “hemorrhagic septicemia”
- Coinfections with other pathogens are very common





Blood congestion



Intestinal necrosis

Dong et al. 2015 Aquaculture 448:427-435
Peepim et al. 2016 Aquaculture 464:399-409
Dong et al. 2017 J Fish Dis 40:1395–1403

Aerococcus viridans infection

Aquaculture 342–343 (2012) 18–23

Contents lists available at SciVerse ScienceDirect

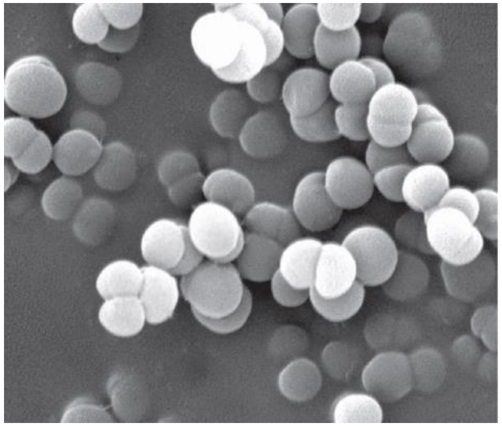
Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Recovery and pathogenicity analysis of *Aerococcus viridans* isolated from tilapia (*Orecohromis niloticus*) cultured in southwest of China

Xiaoli Ke, Maixin Lu *, Xing Ye, Fengying Gao, Huaping Zhu, Zhanghan Huang

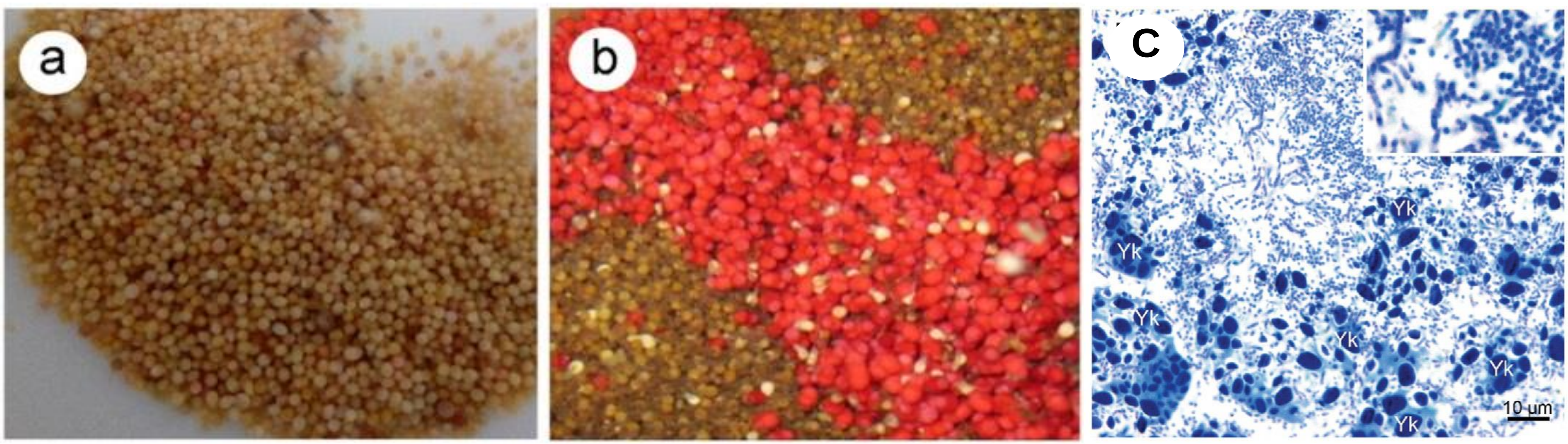
Key Laboratory of Tropical & Subtropical Fishery Resource Application & Cultivation, Ministry of Agriculture, Pearl River Fisheries Research Institute, Chinese Academy of Fishery Science, Guangzhou, 510380, China



- This work firstly reports the infection and histopathological changes of *A. viridans* in tilapia
- Associated with 30-40% loss in Guangdong Province, China, 2010
- **The major symptoms**: serious congestion of the gill and the abdomen, swelling gallbladder and a severe diffusion in liver. Some fish show exophthalmia and spiral swimming.
- Experimental infection caused 45-85% mortality, fulfilled Koch's postulates

Hahellosis/Red egg disease

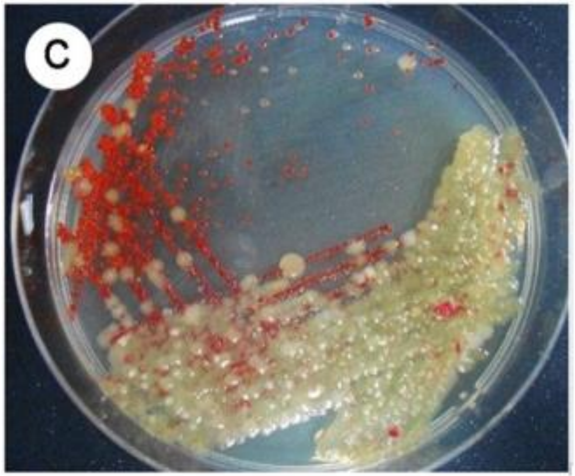
- ❖ Occurred in a tilapia hatchery in Thailand since 2010
- ❖ Mortality 10-50%
- ❖ Occur during cold season (<24 ^{0}C)
- ❖ Causative agent: unknown



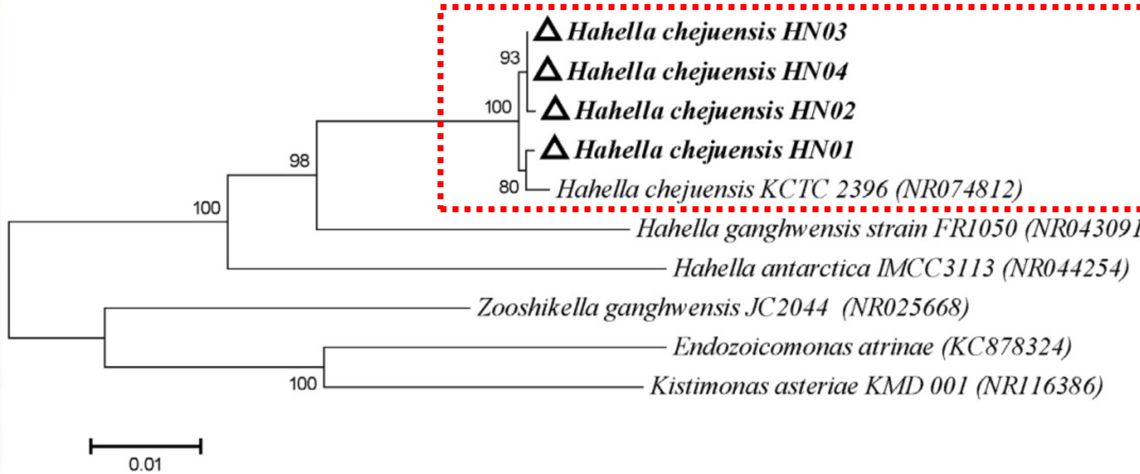
Normal eggs | Red eggs | Bacteria in the egg

Senapin et al. Aquaculture (2016) 454:1-7

Hahellosis/Red egg disease



Bacterial isolation using TSA



Red pigmented bacteria was identified using 16S rRNA

Hahella chejuensis **is a marine bacteria**

…occurred in tilapia hatcheries?

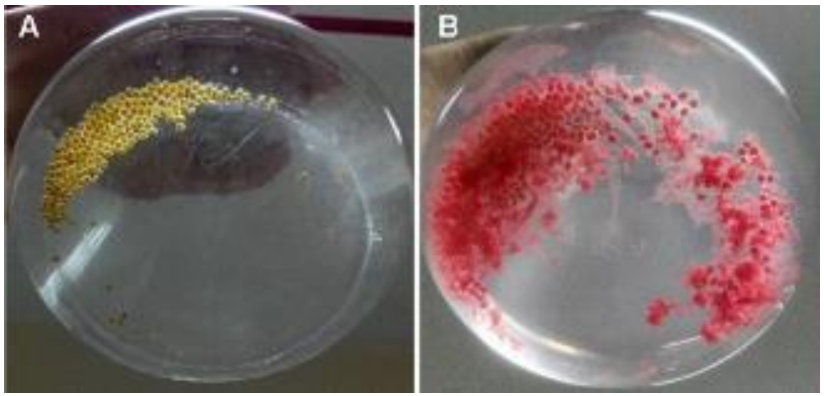
Int J Syst Evol Microbiol. 2001 Mar;51(Pt 2):661-6.

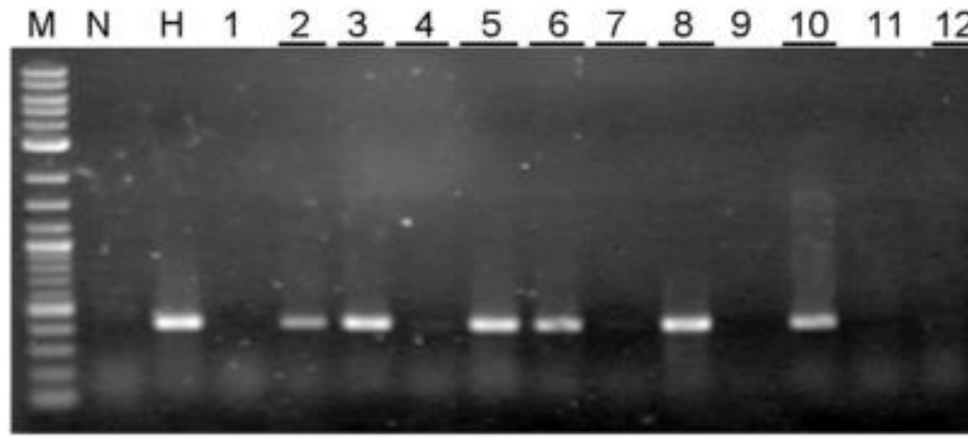
Hahella chejuensis gen. nov., sp. nov., an extracellular-polysaccharide-producing marine bacterium.

Lee HK[1], Chun J, Moon EY, Ko SH, Lee DS, Lee HS, Bae KS.

Senapin et al. Aquaculture (2016) 454:1-7

Hahellosis/Red egg disease



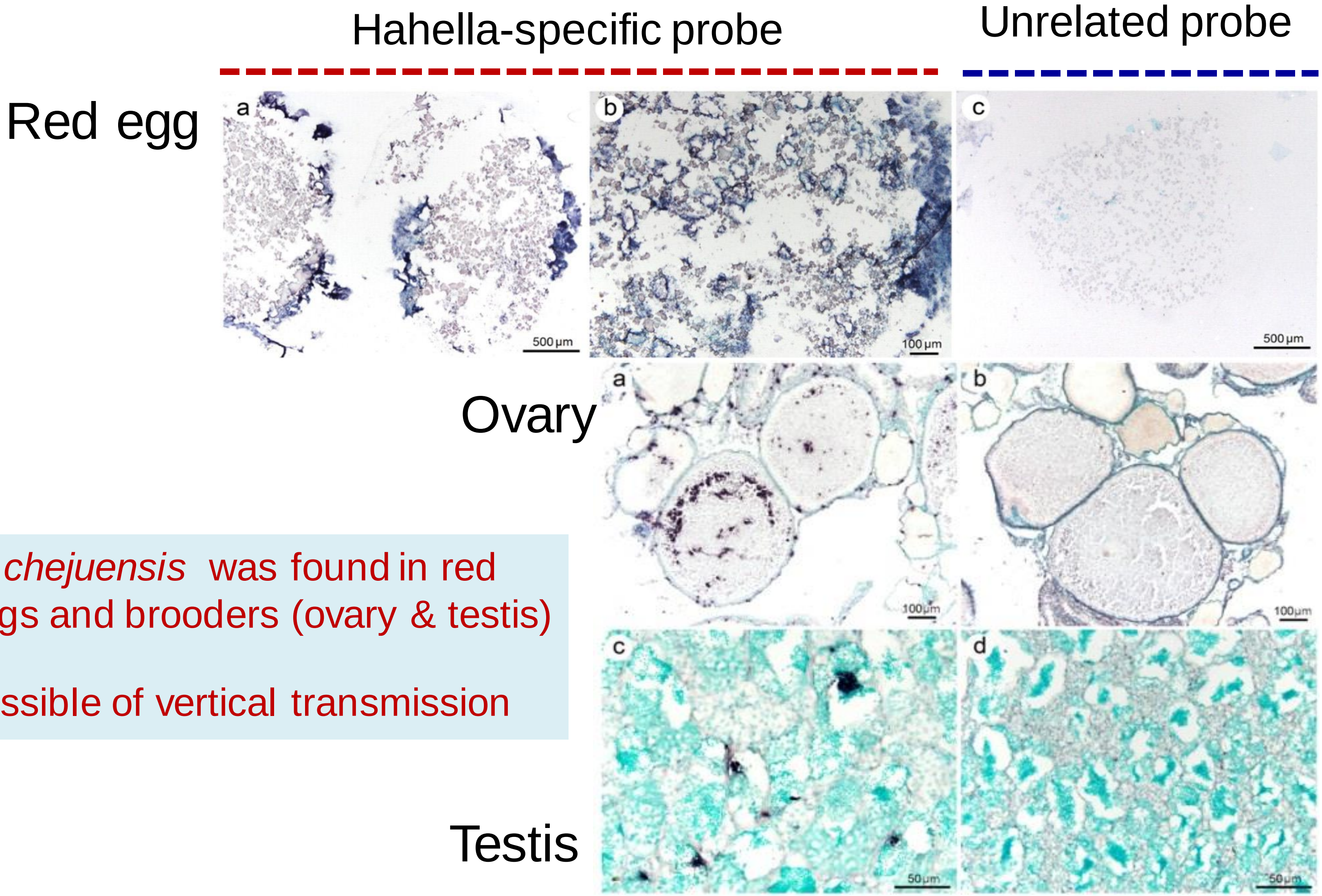


H. chejuensis caused red egg disease & reduced hatching rate in experimental challenge

Specific PCR detection methods were developed targeting 16S rRNA

Senapin et al. Aquaculture (2016) 454:1-7

Hahellosis/Red egg disease



- *H. chejuensis* was found in red eggs and brooders (ovary & testis)
- Possible of vertical transmission

Senapin et al. Aquaculture (2016) 454:1-7

How the farmer solve this problem?

- ✓ Reduce salinity from 7 ppt to 4 ppt
- ✓ Expose sand from the filter system to sunlight
- ✓ Wrap the hatcheries with plastic to increase temperature (30 °C)

- ➢ Reduction of loss: ~ $ 600,000 /year
- ➢ Calculation based on 30% mortality (range from 10-50%)

Emerging parasitic infection

Aquaculture 491 (2018) 169–176

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

ELSEVIER

Aquaculture

Trypanosomiasis causing mortality outbreak in Nile tilapia intensive farming: Identification and pathological evaluation

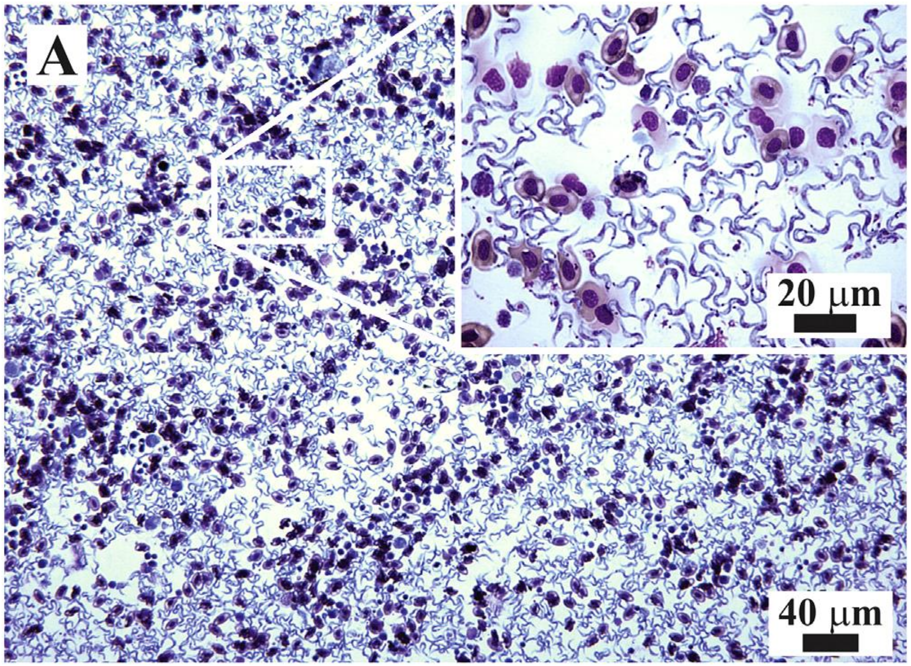
Check for updates

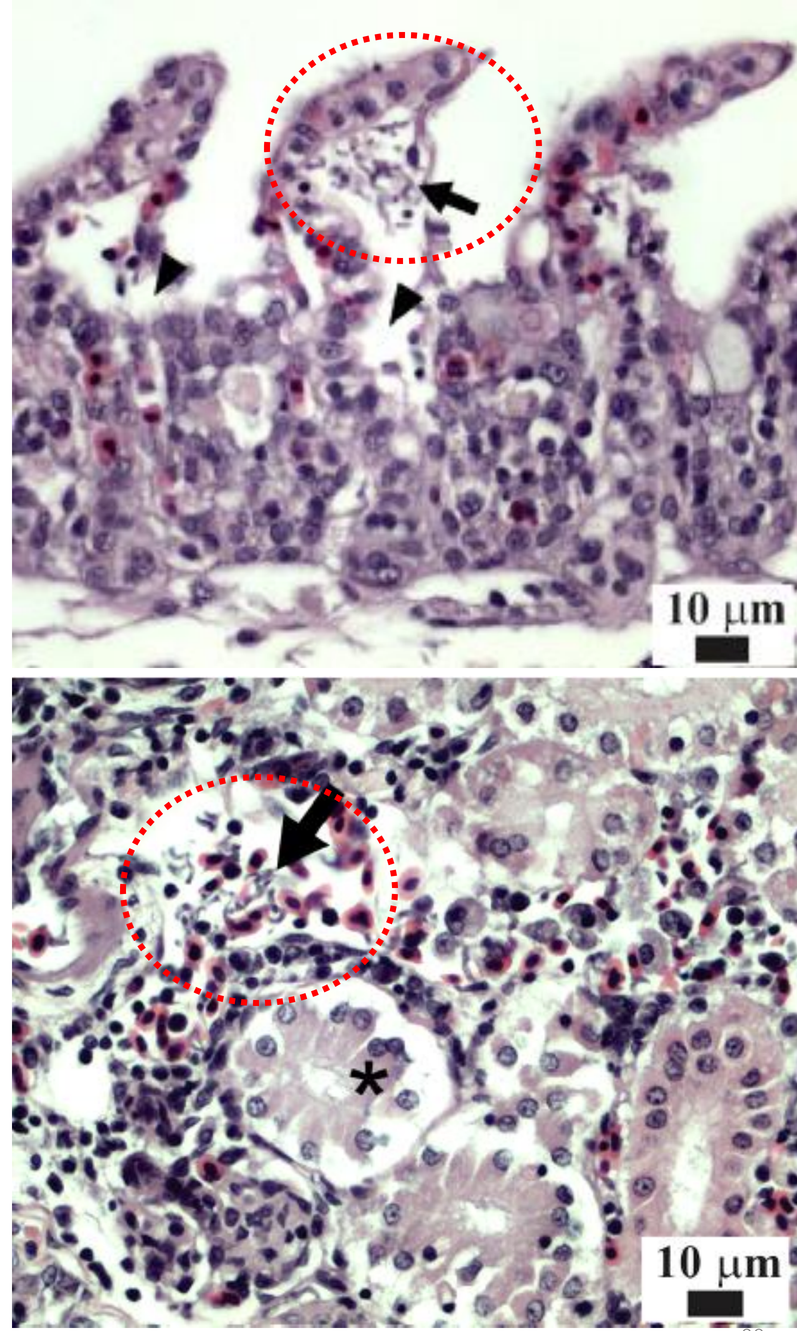
Raphael Barbetta de Jesus, Sílvia Umeda Gallani, Gustavo Moraes Ramos Valladão, Gabriela Pala, Thiago Fernandes Alves da Silva, Jaqueline Custódio da Costa, Suzana Kotzent, Fabiana Pilarski*

São Paulo State University (UNESP), Aquaculture Center of Unesp (CAUNESP), Microbiology and Parasitology Laboratory of Aquatic Organisms, Jaboticabal, São Paulo, Brazil

- The first outbreak of *Trypanosoma* in Nile tilapia (~460 g) in South America
- Unspecific signs such as anorexia, skin darkening and gill paleness

Trypanosomiasis

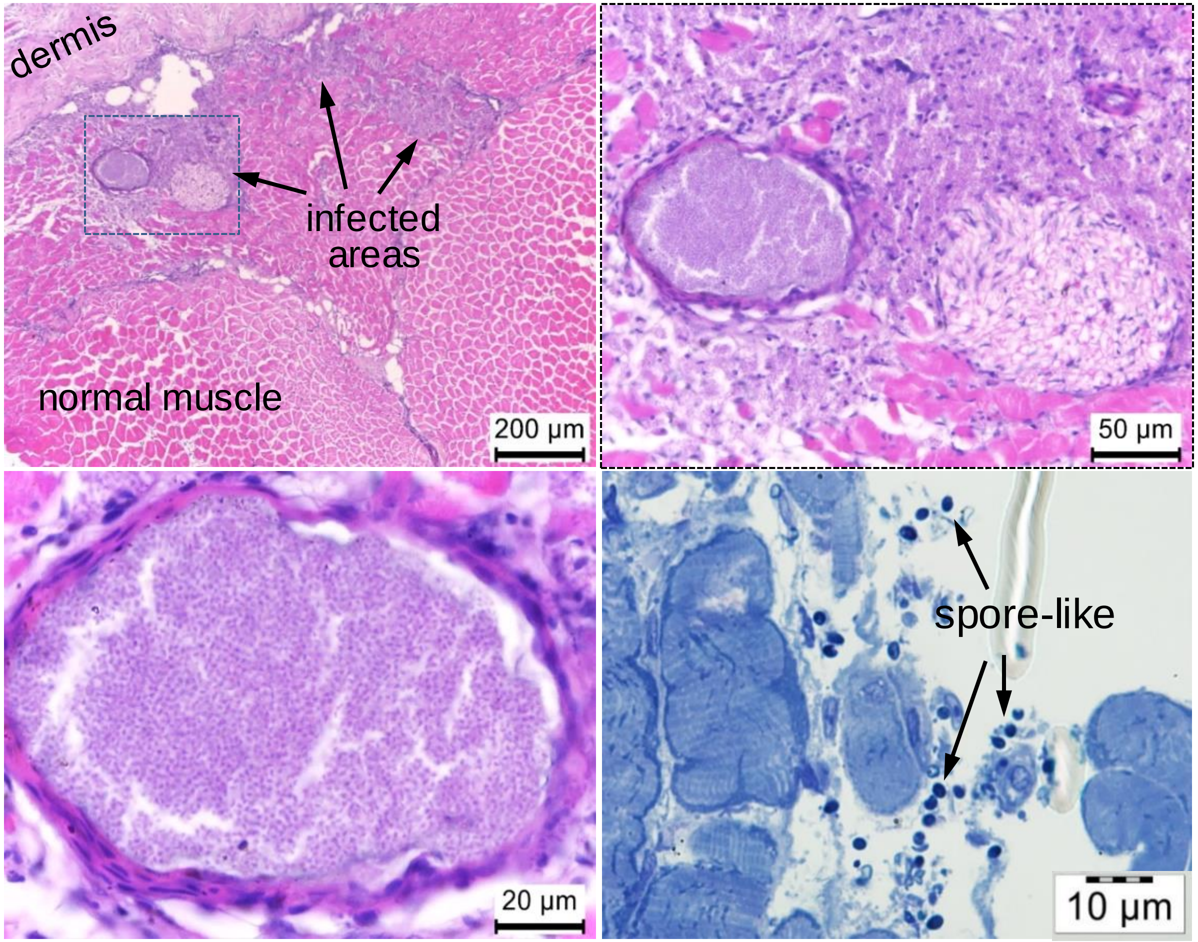




- *Trypanosoma* sp. (combined morphology & molecular analysis)
- 18S rDNA showed 95-98% identity to *Trypanosoma* sp.

de Jesus et al. 2018 Aquaculture 491: 169-176

Emerging unknown pathogen



50% fish (n=10) in a TiLV-positive cage showed a novel histopathological change (microsporidian-like?)

Comments

- ❖ Emerging diseases are never ending threats in aquaculture industry
- ❖ Preparedness for rapid response to emerging diseases should be encouraged
- ❖ Rapid pathogen discovery and early diagnosis will limit its spread and reduce negative impact
- ❖ SPF and autogenous inactivated vaccine programs should be promoted for long-term development

Acknowledgments

Colleagues/collaborators

KMUTT King Mongkut's University of Technology Thonburi

Mahidol University Centex Shrimp BIOTEC-NSTDA

BIOTEC a member of NSTDA

NTU ĐẠI HỌC NHA TRANG 1959

Asian Institute of Technology 1959

Kasetsart University 1943

USDA United States Department of Agriculture

WorldFish

Veterinærinstituttet Norwegian Veterinary Institute

Nam Sai Farms