Bacterial Diseases of Tilapia

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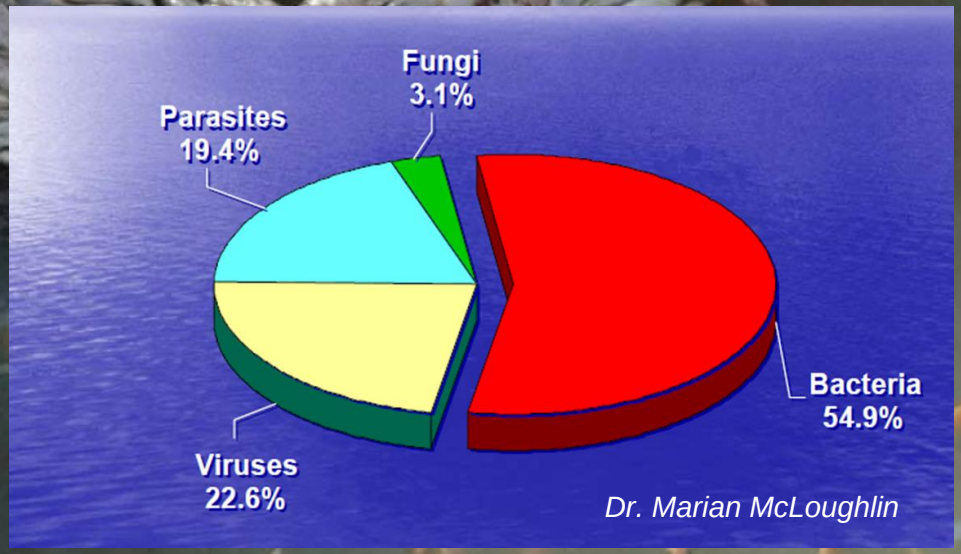

Fungi
3.1%
Parasites
19.4%
Bacteria
54.9%
Viruses
22.6%
Dr. Marian McLoughlin

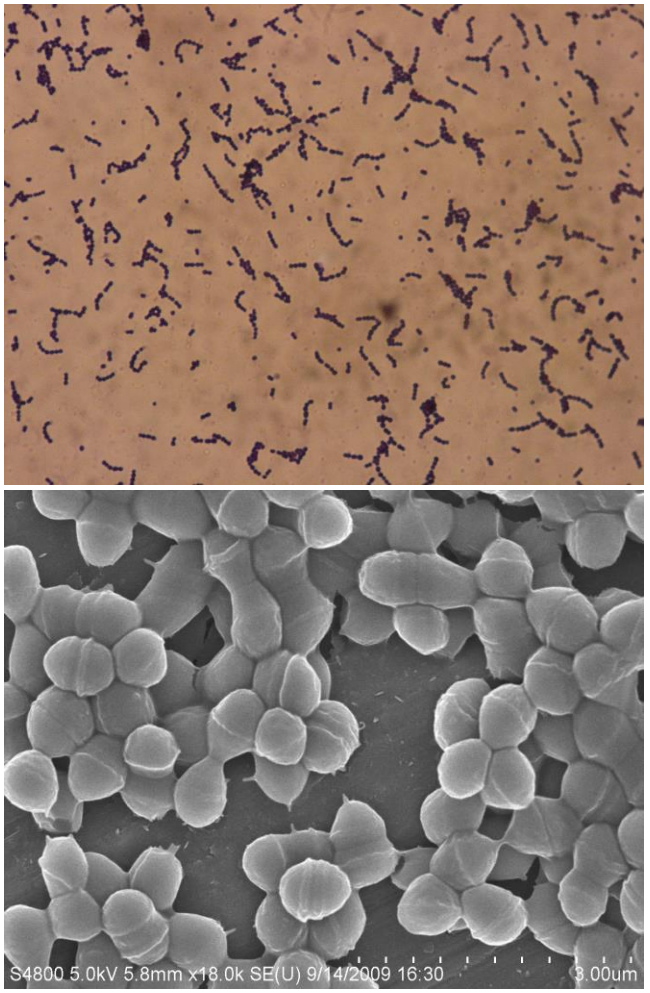
Bacterial Diseases in Farmed Tilapia

- Streptococcosis
- Columnaris
- Francisellosis
- Edwardsiellosis
- Hemorrhagic septicemia caused by motile aeromonads
- Hahellosis (red egg disease)
- Epitheliocystis
- Miscellaneous disease

Streptococcosis

❖ **Causative agents**

- *Streptococcus agalactiae*
- *Streptococcus iniae*
- *Streptococcus dysagalactiae*
- *Lactococcus garviae*
- *Aerococcus viridans* (?)



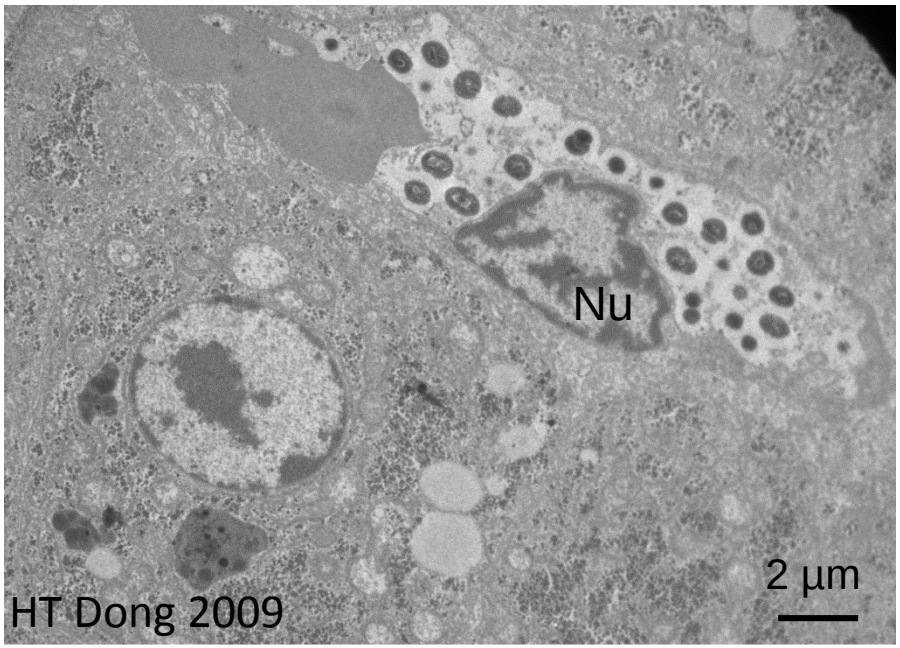
Streptococcus agalactiae

- Gram positive bacteria
- GBS have been classified to 10 serotypes (Ia, Ib, II–IX)
- In aquatic animals: 4 serotypes Ia, Ib, II and III
- Serotype IX is newly reported in tilapia (China)

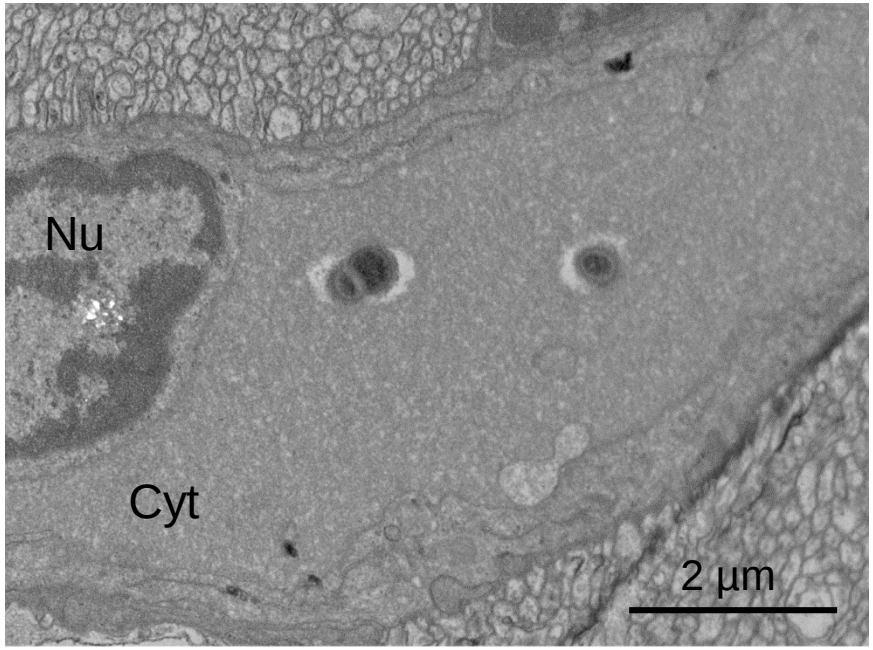
Streptococcosis

❖ Causative agents

Intracellular living style of *S. agalactiae*



Liver



Brain

Streptococcosis

❖ **Clinical signs** (Level 1)

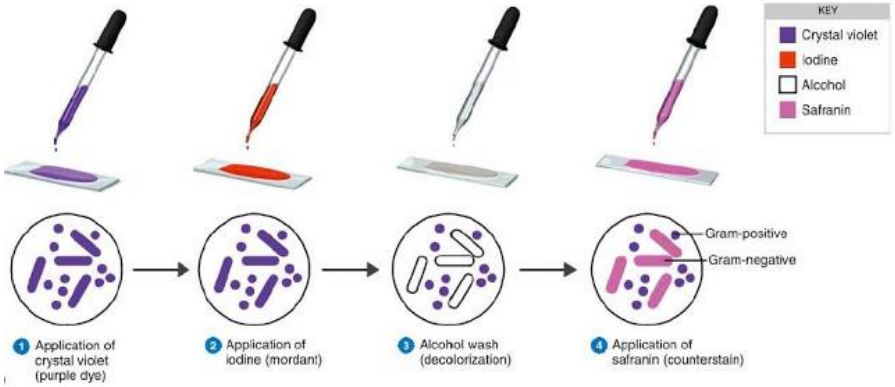
- erratic swimming
- pop eyes (exophthalmia)
- darkening
- lost of appetite

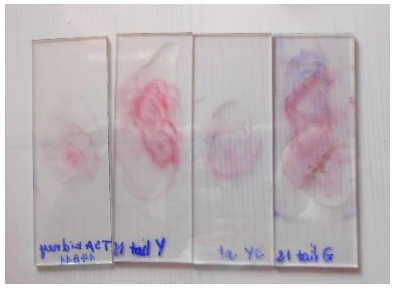
- swollen gallbladder
- brown/dark areas in the liver
- ascites

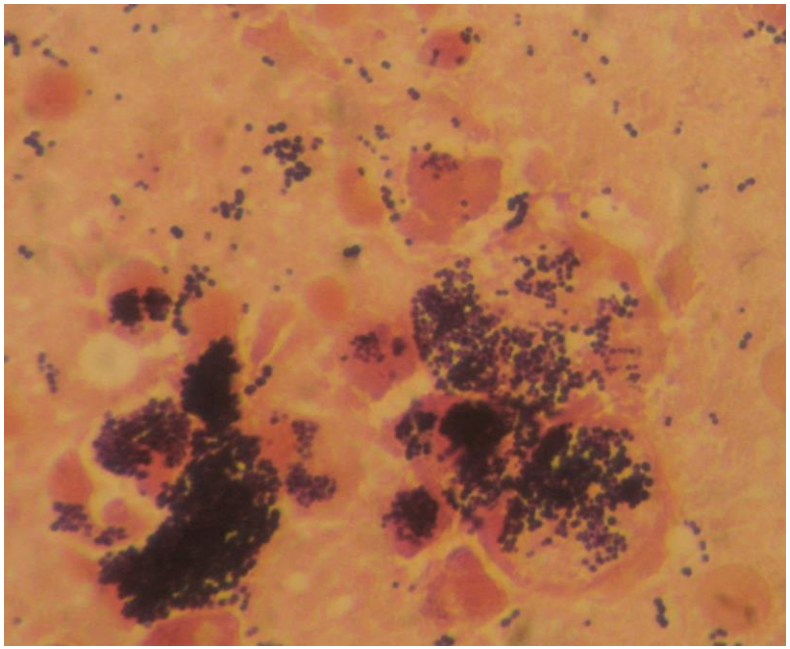
Streptococcosis

Diagnostics (Level 2)

- Rapid Gram staining of smeared tissue e.g. blood, kidney, liver, brain





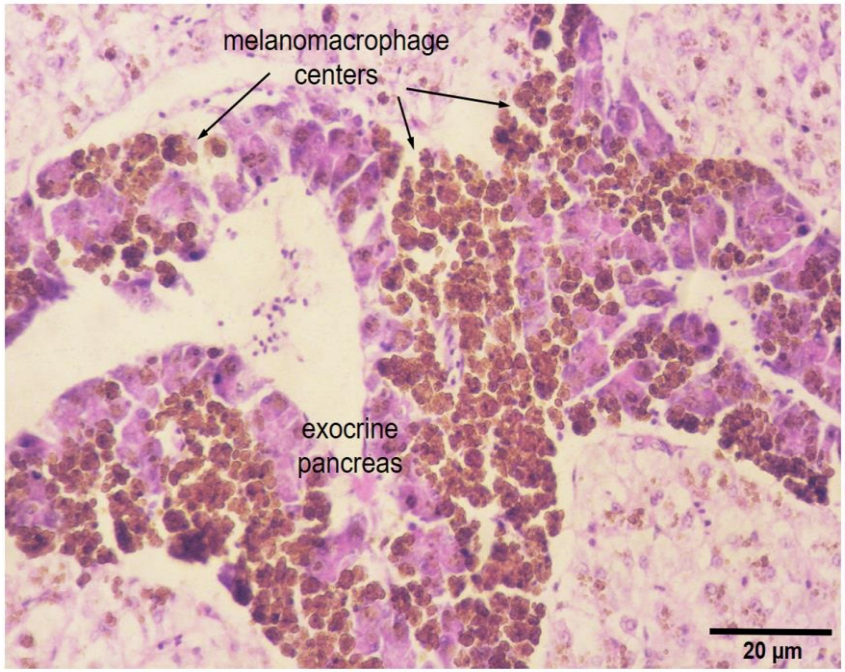


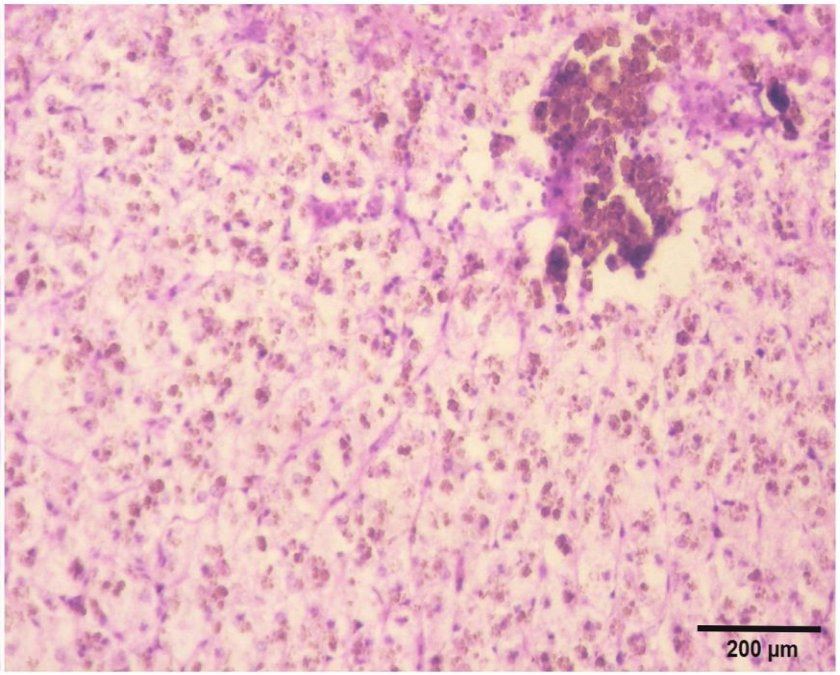
Gram staining of smeared kidney

Streptococcosis

Diagnostics (Level 2)

- histopathology



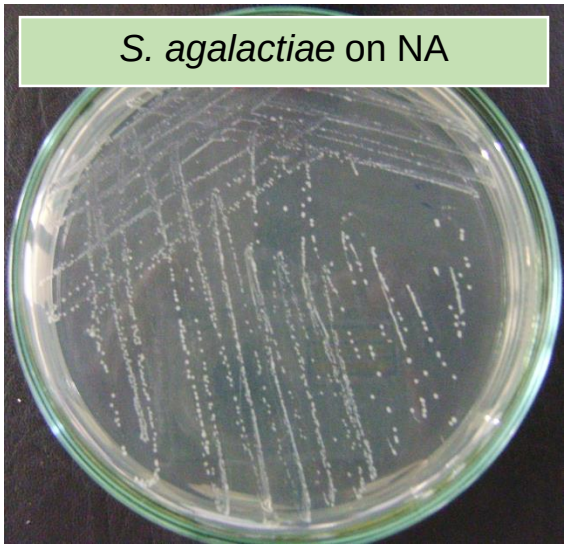


Liver, Nile tilapia infected with *S. agalactiae* showing hyperactivation of melanomacrophage centers with overloaded melanophores in the pancreas, severe hepatocyte degeneration and accumulation of melanophores.

Streptococcosis

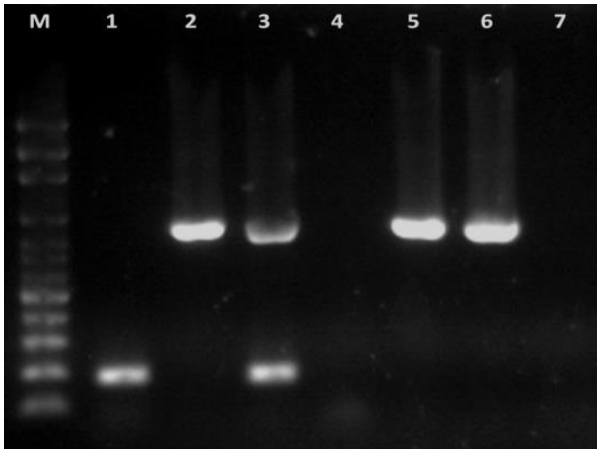
Diagnostics (Level 2)

- Bacterial culture + biochemical tests
  - culture: e.g. TSA, NA, blood agar
  - incubate at 28-33 ^{o}C for 24-48 hrs. → pinpoint colonies
  - Gram positive, oval/round shape, catalase (-) → *Streptococcus*
  - API 20 strep kit

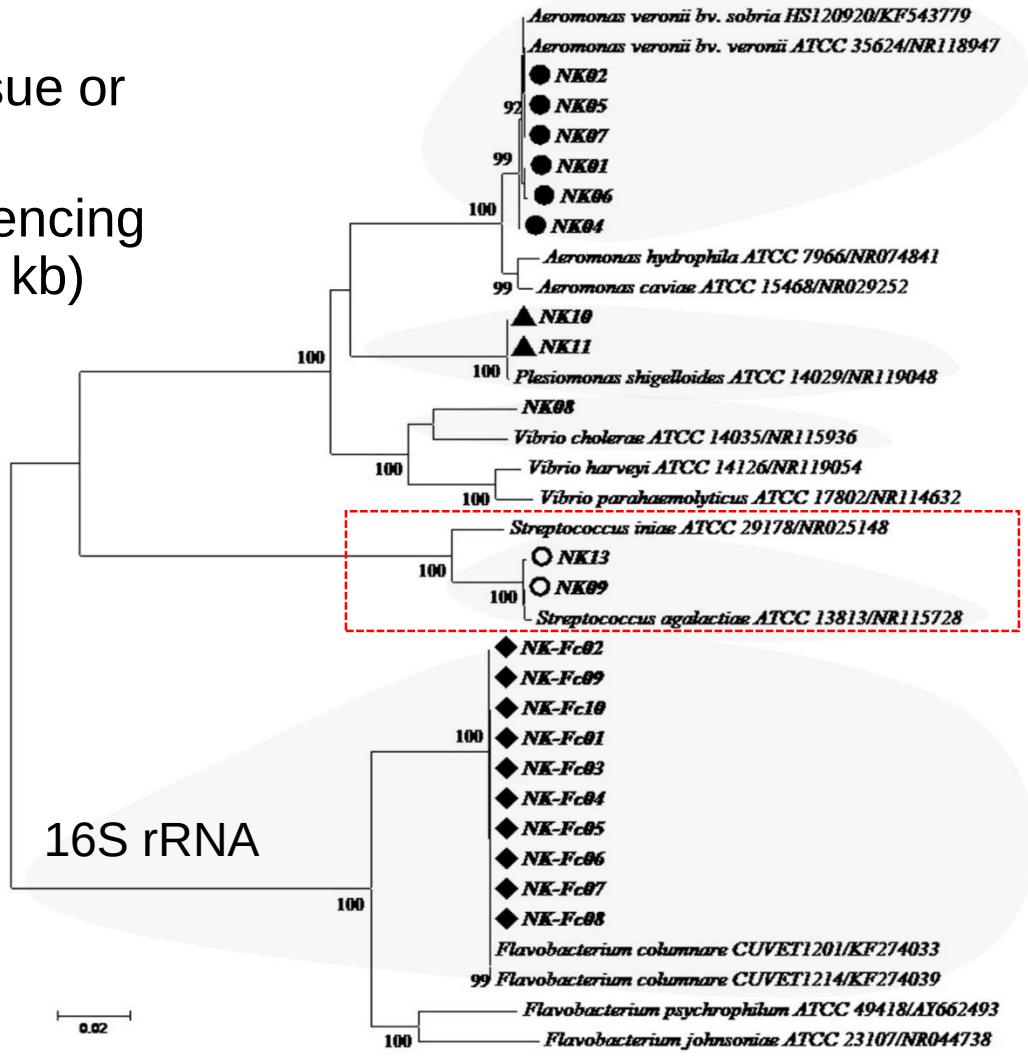


Streptococcosis

- **Diagnostics** (Level 3)
  - Specific PCR (either tissue or pure culture)
  - Bacterial culture + sequencing of 16S rRNA gene (>1.3 kb)



Duplex PCR for detection of *S. agalactiae* & *S. iniae* (Rodkhum et al. 2012)



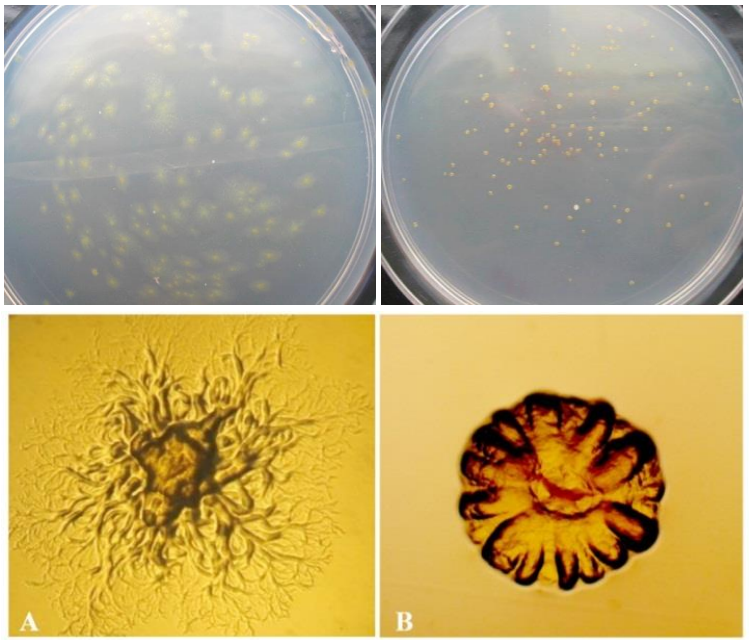
Streptococcosis

- **Mortality**: variable, up to 90%
- **Geographical distribution:** worldwide
- **Risk factors**: high temperature (31-33 ^{o}C), poor water quality
- **Prevention**
  - Treat water with disinfectants
  - Control water temperature (e.g. increased water level in the pond)
  - Supply more oxygen (e.g. increased aeration)
  - Improve fish immunity (e.g. vitamin C, immulostimulants)
  - **Vaccine is available**
    - Injectable vaccine (e.g. MSD, Dr. Nontawith's team at KU, Thailand)
    - Immersion vaccine (China)
    - Vaccine incorporated with feed for oral route (DOF, Malaysia)

Columnaris

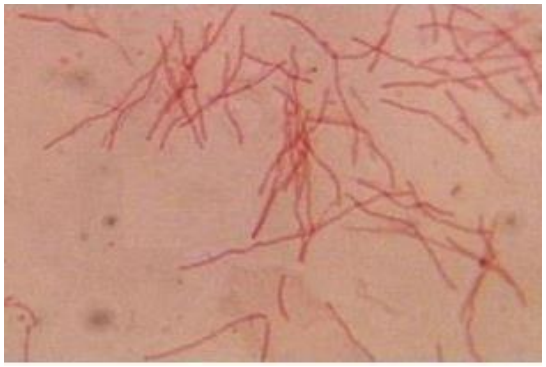
Columnaris

- **Causative agent**
  - *Flavobacterium columnare*
  - Gram negative, long rod-shaped bacterium
  - Gliding motility
  - Form strong biofilm
  - Rhizoid morphotype: pathogenic
  - Non-Rz morphotype: non-pathogenic

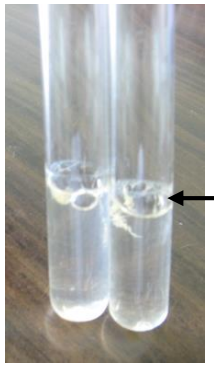


Rhizoid morphotype

Non-Rhizoid morphotype



Gram negative, long rod-shape bacterium



Biofilm formation

Dong et al. J Fish Dis (2015) 38:901-913

Columnaris

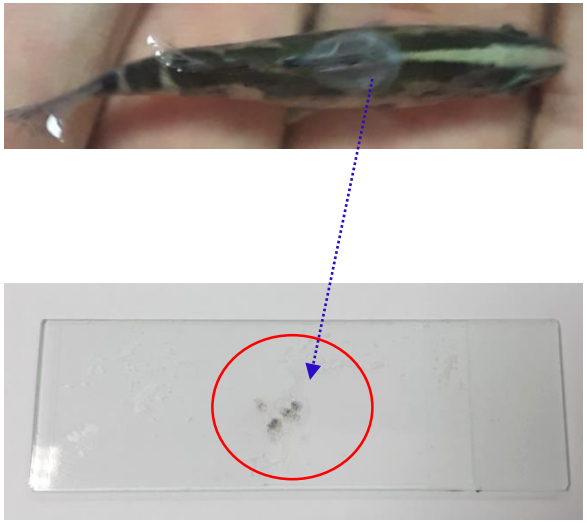
- **Clinical signs** (Level 1)
  - fin rot
  - necrotic gills
  - muscle necrosis
  - “saddle back” lesion
  - pale skin

“saddle back”

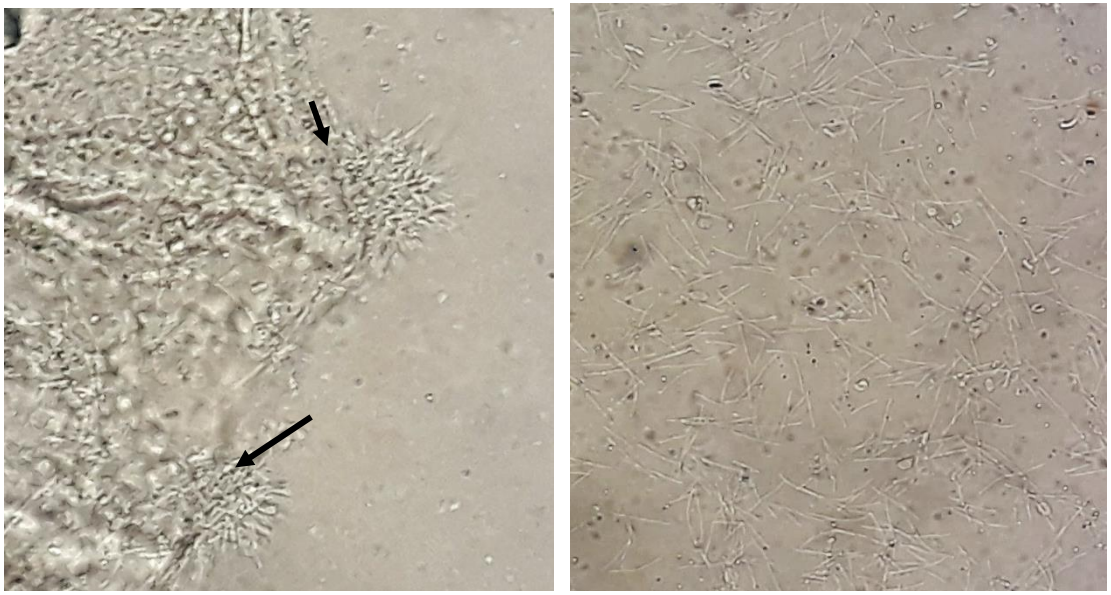
Columnaris

Diagnostics (Level 2)

- wet-mount



Wet mount

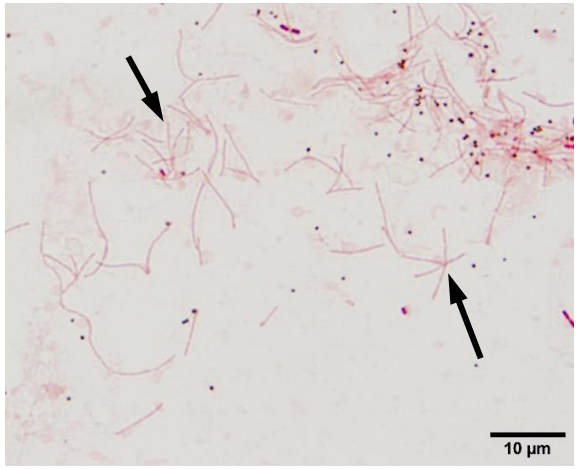


Wet mount of the saddle-back lesion revealed clumps of long rod-shaped bacteria

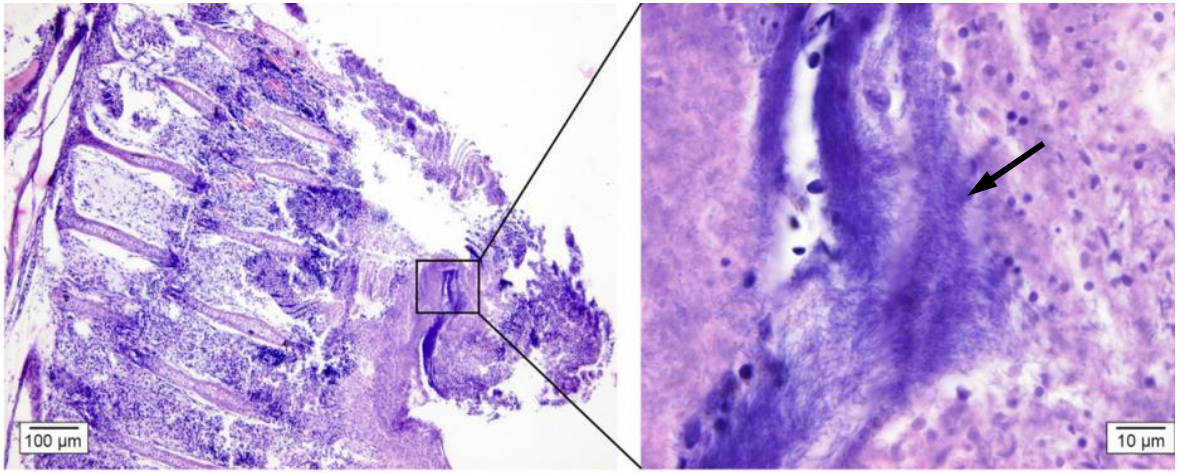
Columnaris

Diagnostics (Level 2)

- Rapid Gram staining of smeared tissue e.g. gills, skin lesion
- Histopathology



Gram staining of smeared tissue

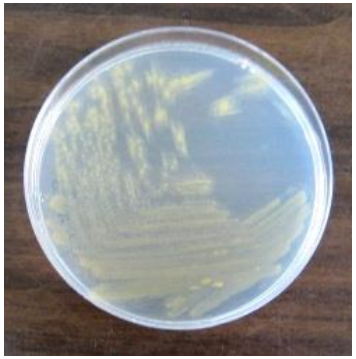


Histopathology of fish gills infected with F. columnare (Declercq et al. Vet Res 2013, 44:27)

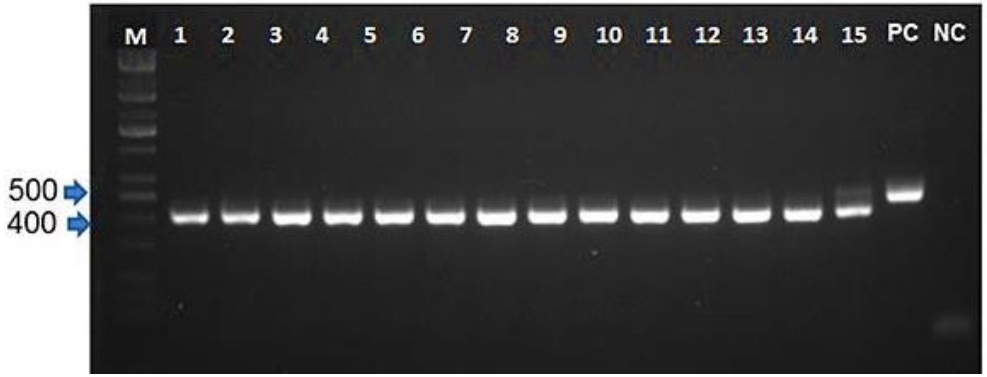
Columnaris

Diagnostics (Level 3)

- Specific PCR (for either fish tissue or pure isolate)
  - ✓Welker et al. 2005
  - ✓Triyanto et al. (1999)
  - ✓Darwish et al. (2004)
- Bacterial culture + sequencing of 16S rRNA
  - Anaker and Ordal's medium (AOA)
  - Modified Shield medium



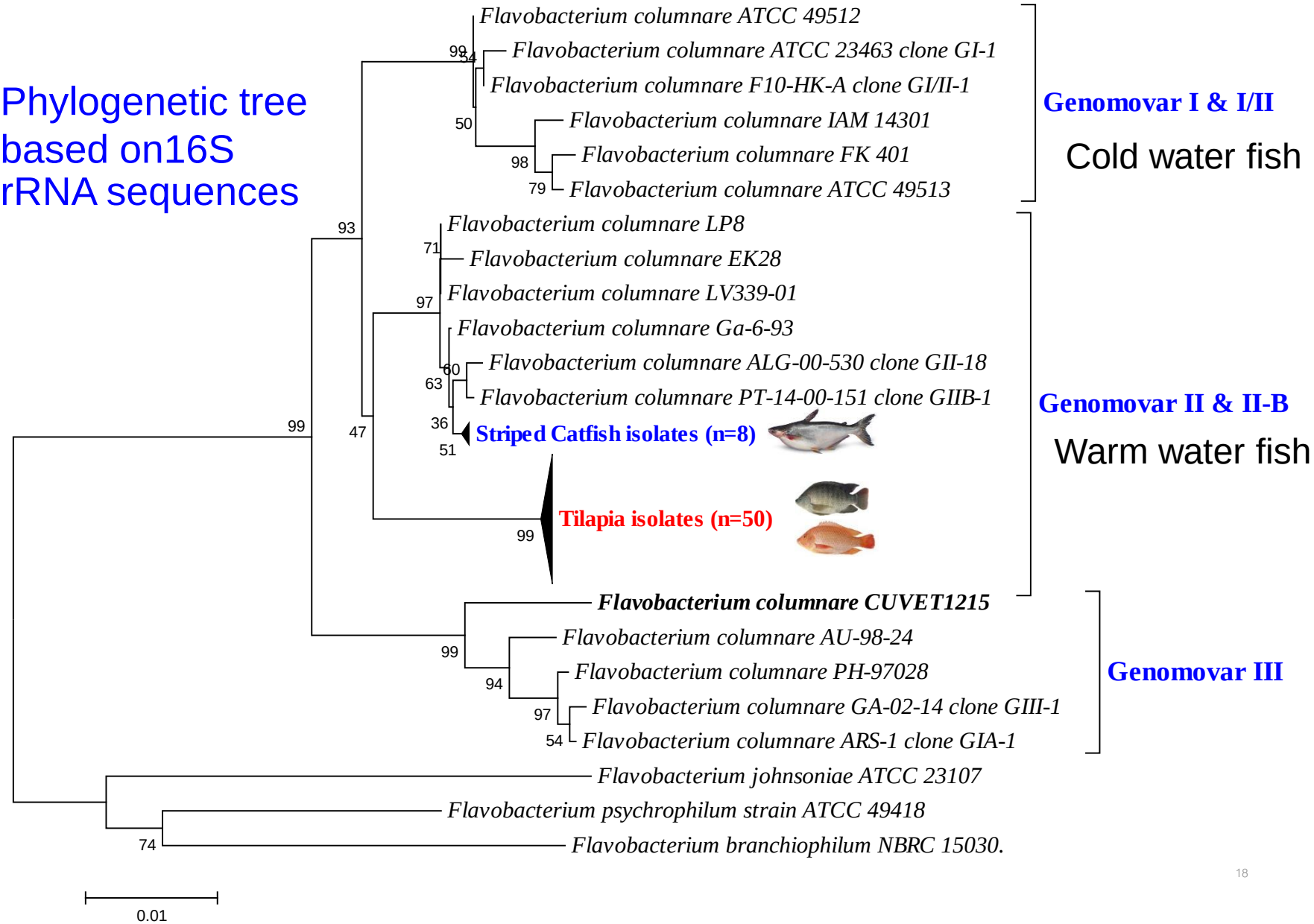
F. columnare on AOA medium



F. columnare-specific PCR according to Welker et al. 2005

Columnaris

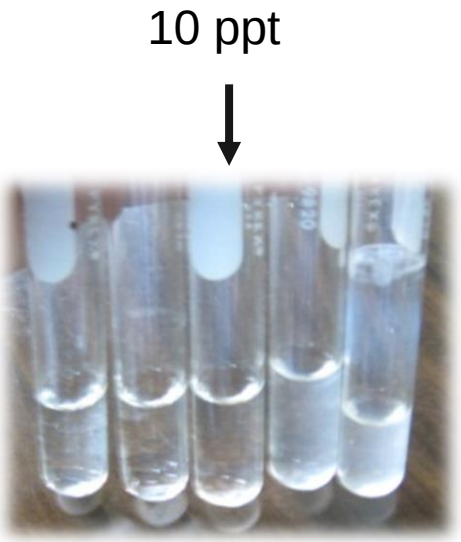
Phylogenetic tree based on16S rRNA sequences



Columnaris

Mortality: variable, reached up to 100% in challenge experiments

Geographical Distribution: worldwide



Risk factors

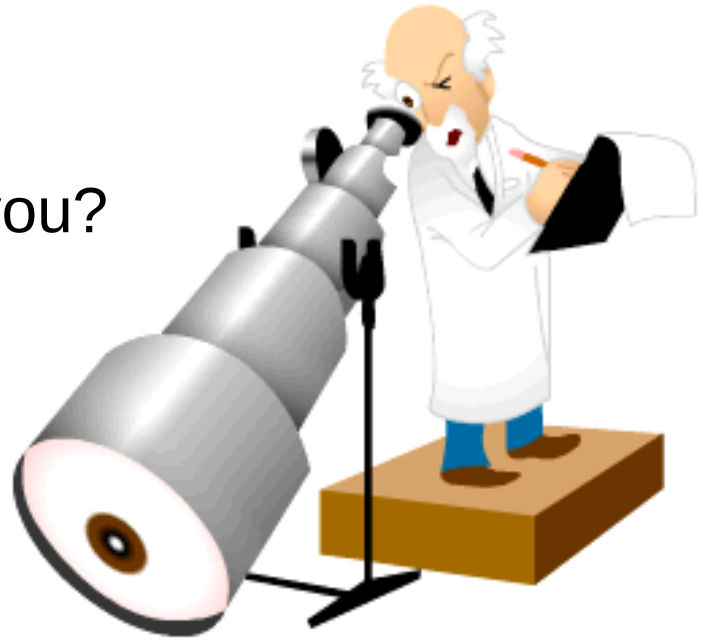
- Disease usually occurs after transportation
- Cage culture is more susceptible than pond culture

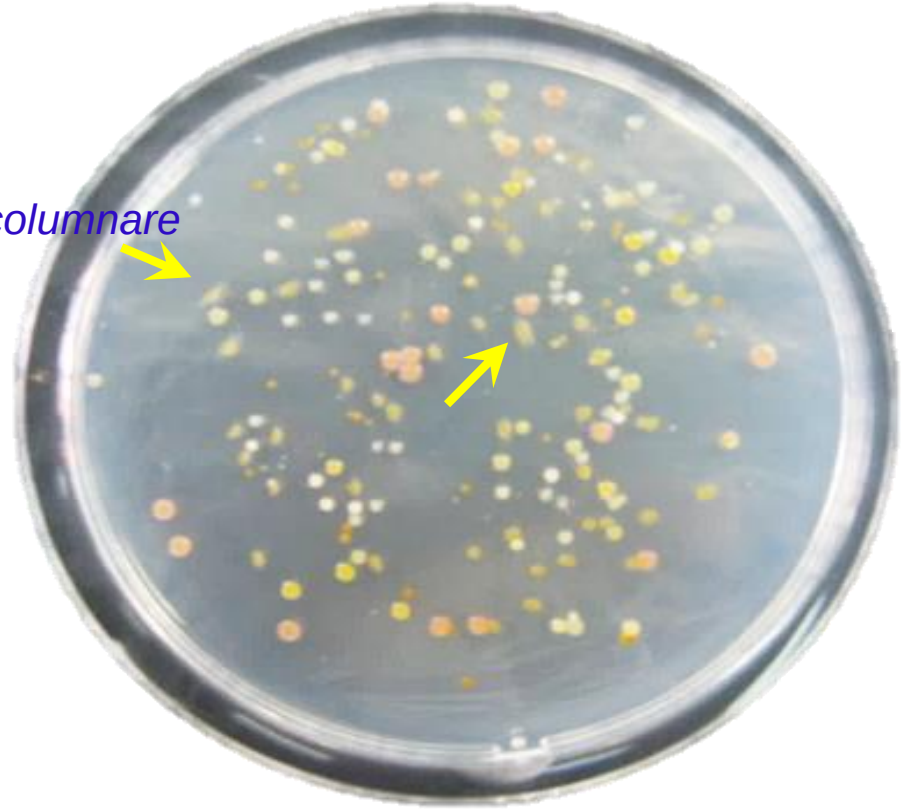
Prevention

- Sodium chloride 10 ppt can prevent/control the disease
- No commercial vaccine available for tilapia

Ignore OR Find Answer?

Who are you?

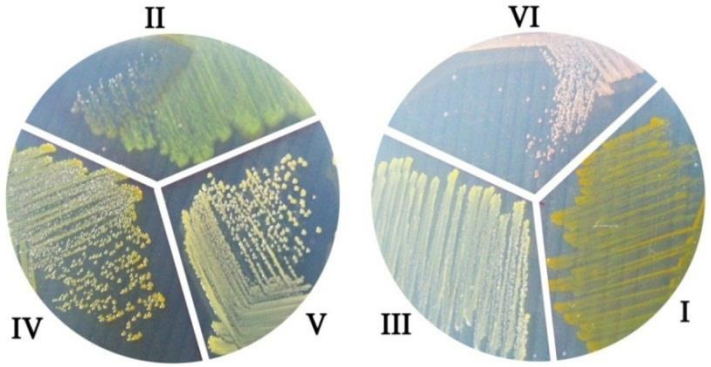




Columnaris

Diversity of *Non-F. columnare* associated with Columnaris diseased fish

Phylogenetic analysis based on 16S rRNA



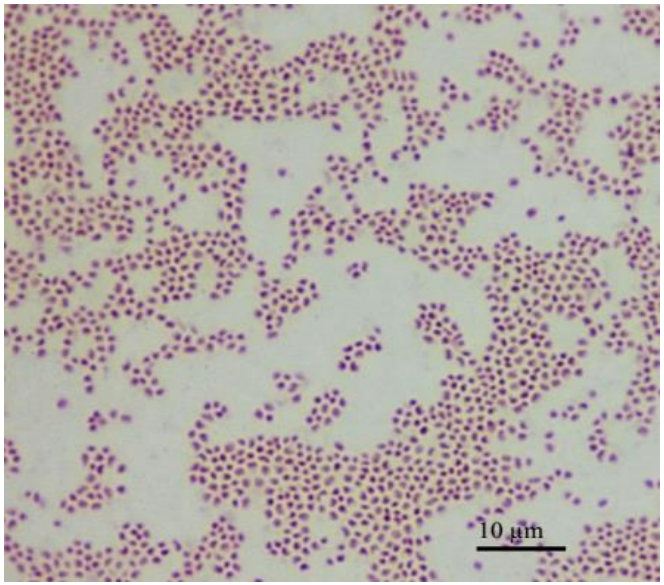
- ✓ Most bacteria first found in tilapia
- ✓ Experimental challenge (I.M.) showed 0-20% mortality
- ✓ May serve as opportunistic pathogens

Dong et al. Thai J Vet Med (2016) 46:251-259

Francisellosis

Francisellosis of tilapia

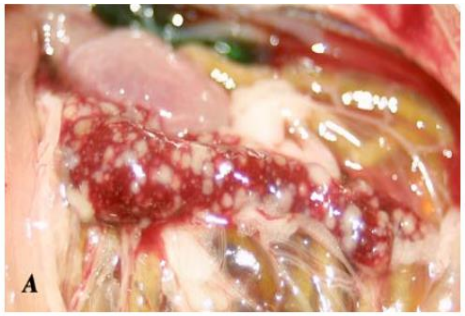
- **Causative agent**
  - *Francisella noatunensis* subsp. *orientalis* (*Fno*)
  - Previously known as Rickettsia-like organism, RLO or Piscirickettsia-like organism
  - Fastidious intracellular bacterium
  - Gram negative, oval shape
  - Require cysteine for growth
  - Optimum temperature for *Fno* (25-28 ^{o}C)



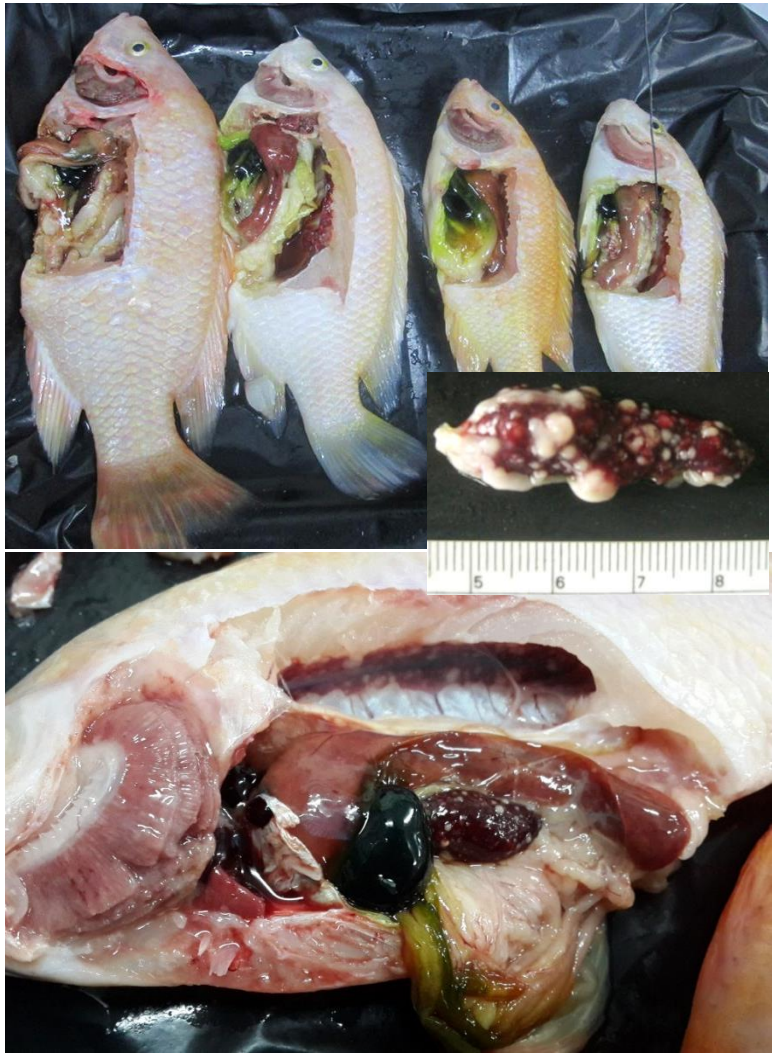
Fno, Gram staining (Photo: VV Nguyen)

Francisellosis of tilapia

- **Clinical signs** (Level 1)
  - Pale body
  - White spots/white nodules on the spleen, head kidney, trunk kidney, gills
  - Lost of appetites



Images by Michael J. Mauel

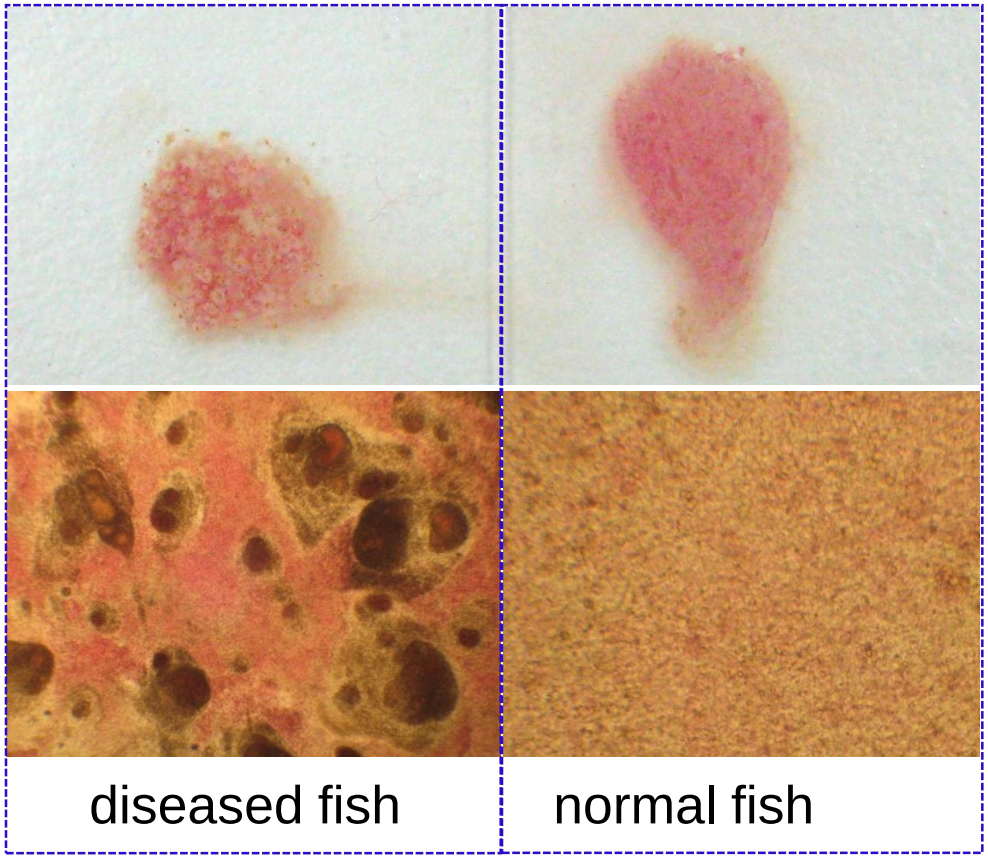


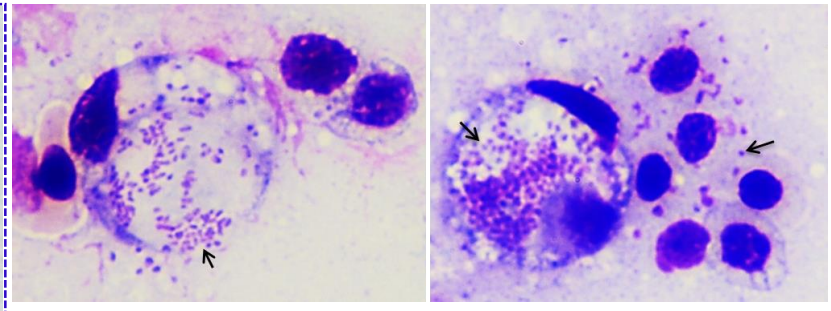
Photographs were taken in conjunction with the outbreaks described in Nguyen et al. 2015. Aquac Res.

Francisellosis of tilapia

Diagnostics (Level 2)

- Wet-mount (e.g. spleen, gills)
- Rapid staining



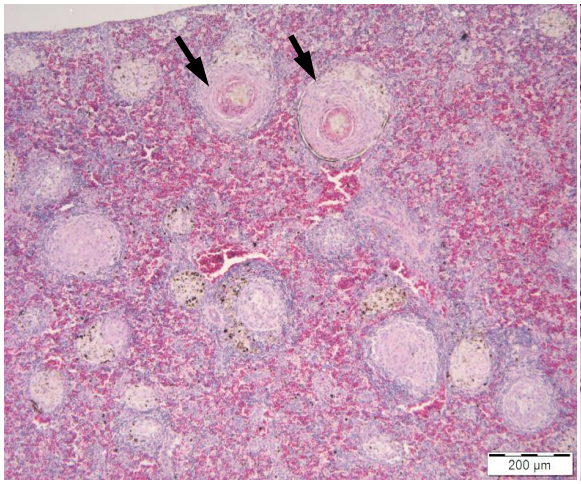


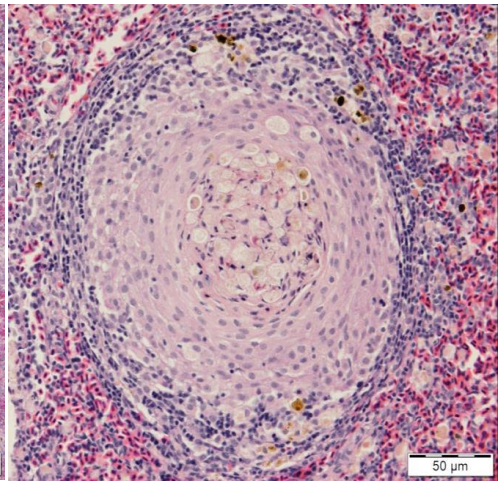
Rapid staining of smeared-head kidney with Giemsa revealed presence of both intra- and extra-cellular bacteria (Nguyen et al. 2015)

Francisellosis of tilapia

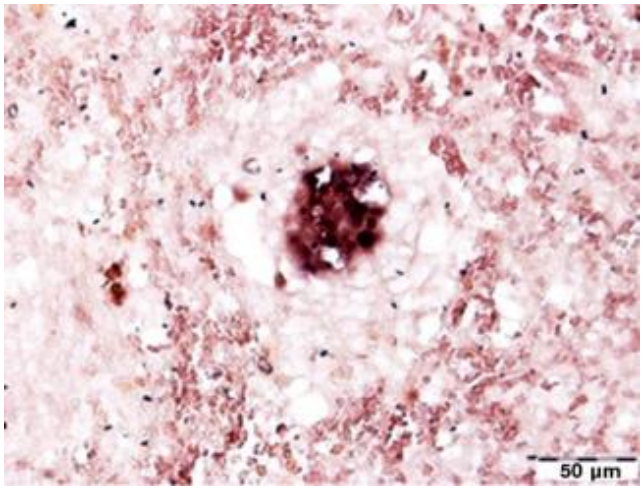
Diagnostics (Level 2)

- Histopathology: presence of granulomas in multiple internal organs
- *In situ* hybridization (ISH) using Fno-specific proble





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



ISH using specific probe revealed location of Fno in the spleen

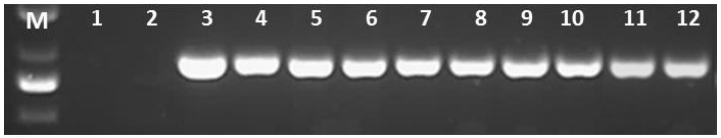
Dong et al. 2016. Dis Aquat Org.

Francisellosis of tilapia

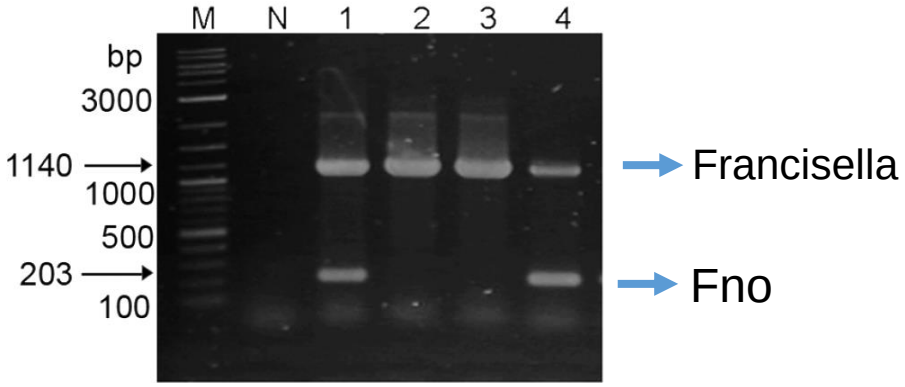
Diagnostics (Level 3)

❖ **Detect Fno from fish tissue**

- 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)



Genus-specific PCR (Forsman et al. 1994)



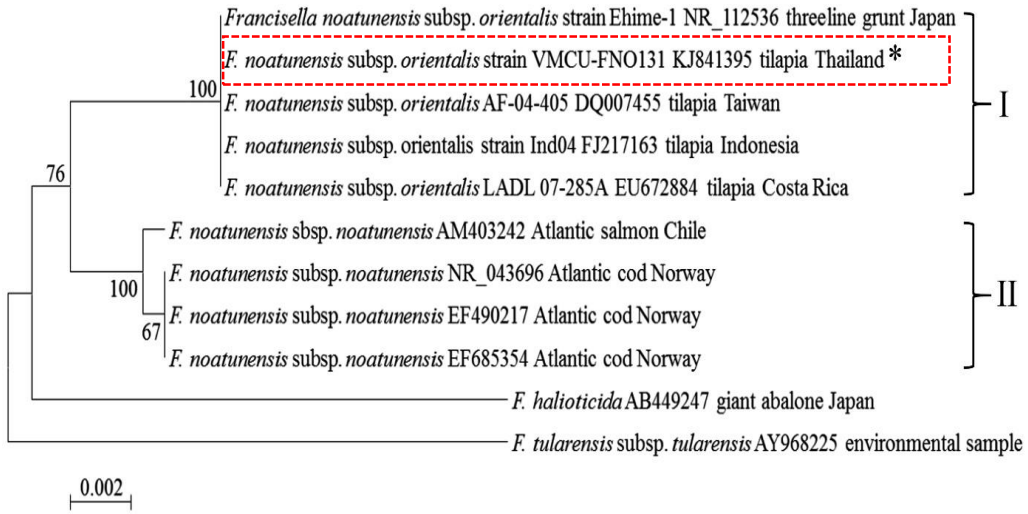
Duplex PCR (Dong et al. 2016)

Francisellosis of tilapia

Diagnostics (Level 3)

- Culture + specific PCR
- Culture + sequencing of 16S rRNA

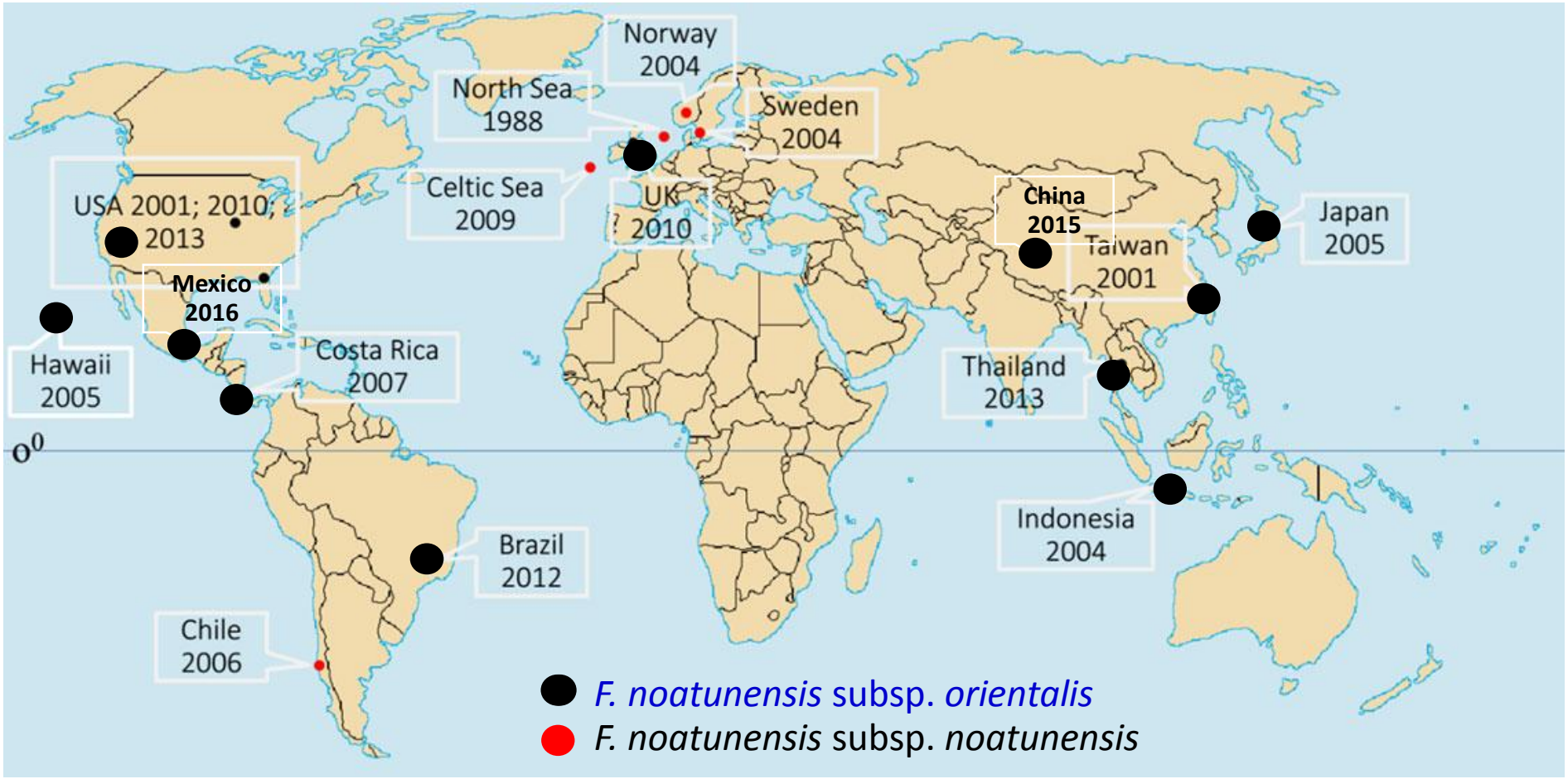
Fno on Cysteine heart agar (CHA)
(Photo: VV Nguyen)



Nguyen et al. 2015. Aquac Res.

Francisellosis of tilapia

Geographical distribution



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

Francisellosis of tilapia

Mortality: 40-50%

Risk factors:

- Disease outbreaks were associated with cool season (<28^0 C)
- Very chronic, disease outbreaks depend on environmental factors

Prevention/treatment

- ❖ Use Fno-free fish
- ❖Antibiotics have been used for treatment
- ❖ Temperature >30^0 C, no mortality occur
- ❖ Commercial vaccine is NOT available

Edwardsiellosis of tilapia

Edwardsiellosis of tilapia

Causative agent:

- *Edwardsiella ictaluri*
- *Edwardsiella tarda*
- *Edwardsiella anguillarum*
- *Edwardsiella piscicida* (?)

❑ Pinpoint colonies on culture media
❑ Rod-shaped Gram negative
❑ Oxidase negative

Rod-shaped Gram negative

Edwardsiellosis caused by *E. ictaluri*

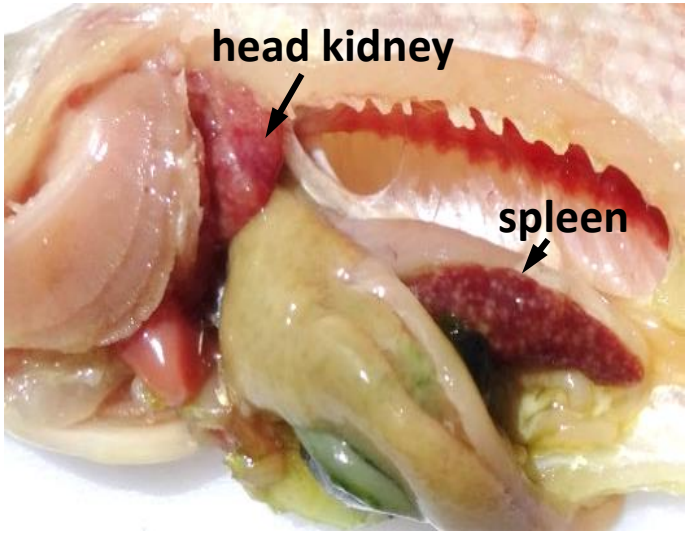
- Common in catfish
- Not common in non-catfish
- Does not kill tilapia in striped catfish ponds (personal observation)
- 2012: first report of E. ictaluri in Nile tilapia in Western Hemisphere (Soto et al. 2012)

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 (Level 1): Francisellosis
- PCR negative for *Fno*

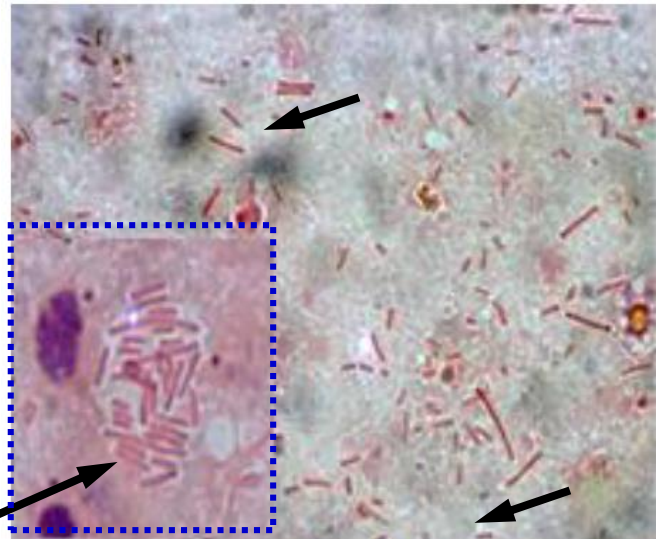


Dong et al. 2019 Aquaculture 499: 17-23

Edwardsiellosis of tilapia

Presumptive diagnosis
(Level 2)

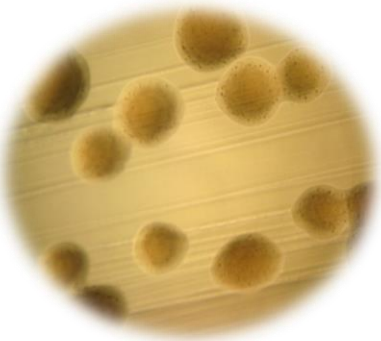
- Gram stained tissue smear revealed numerous Gram negative, rod-shaped bacteria



Intracellular living style

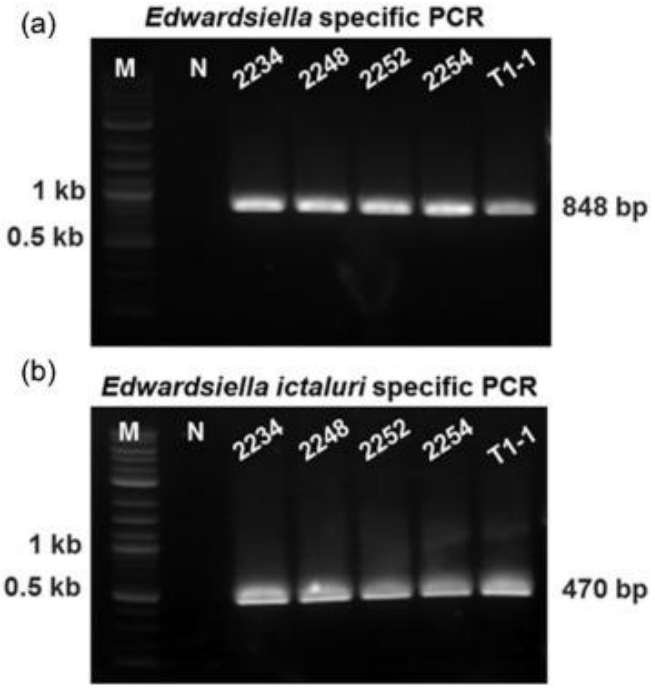
Gram staining of tissue smear

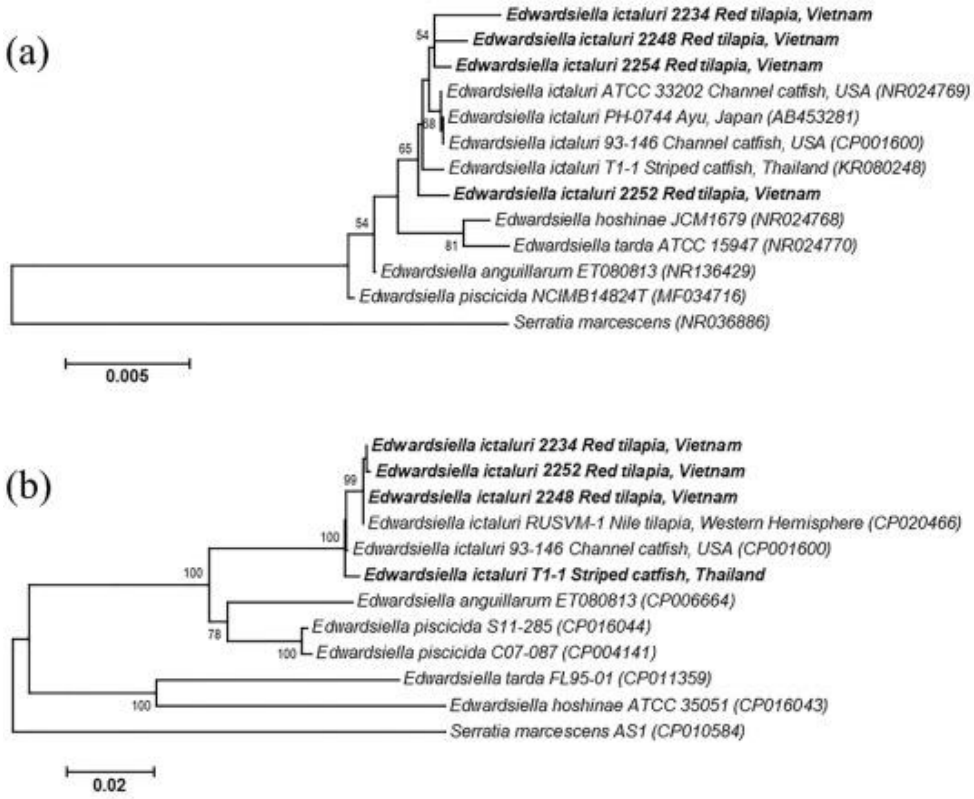
- **Bacterial isolation**: pure pinpoint colonies on TSA or NA
- Gram negative, rod-shaped bacteria



Edwardsiellosis of tilapia

❖ **Confirmed diagnosis** (Level 3)





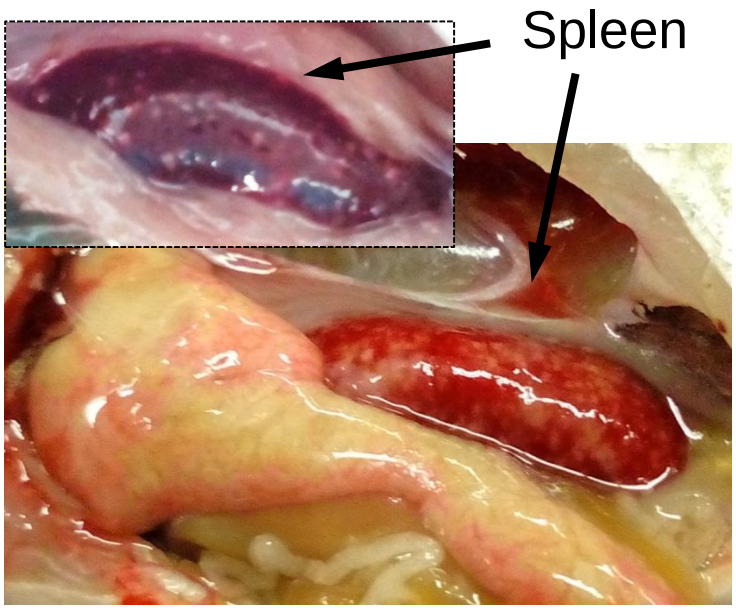
Phylogenetic tree based on 16S rRNA (a) and *gyB* (b)

Dong et al. 2019 Aquaculture 499: 17-23

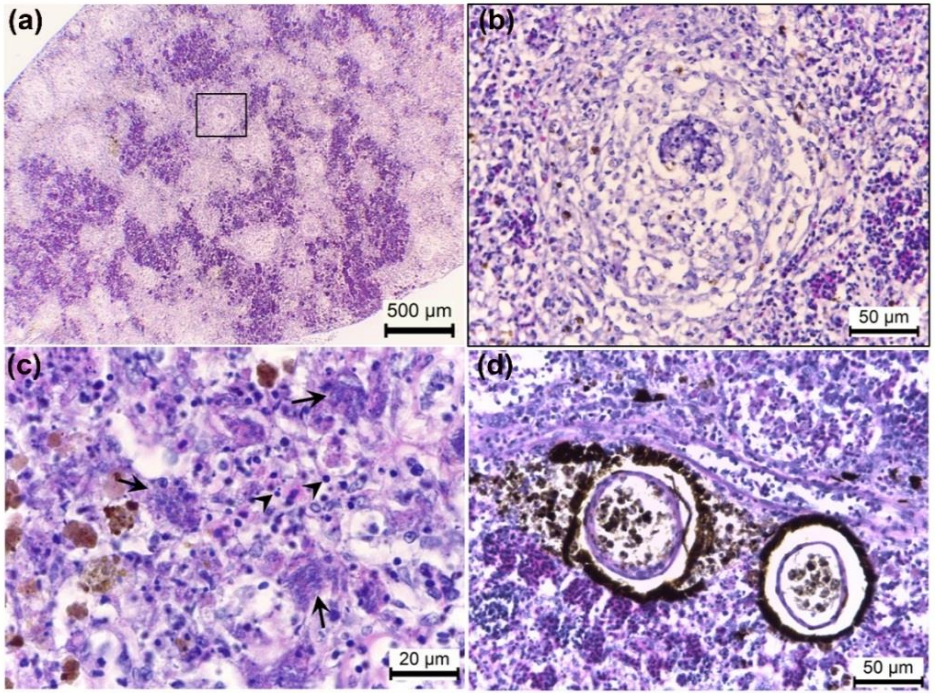
Edwardsiellosis of tilapia

Challenged experiments fulfilled Koch's postulates

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



Experimental fish



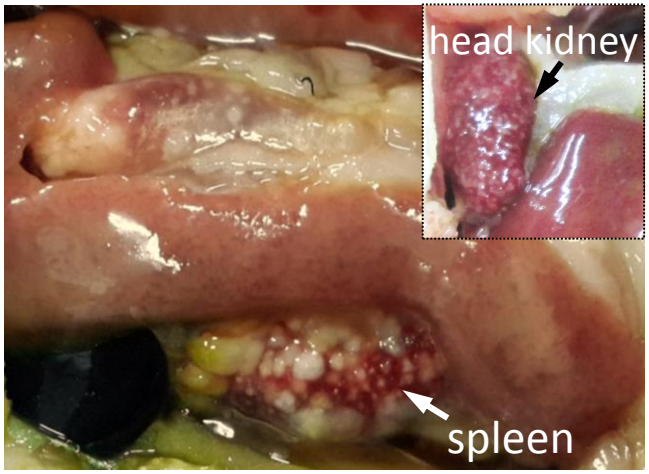
Histopathological features of edwardsiellosis in the experimental fish

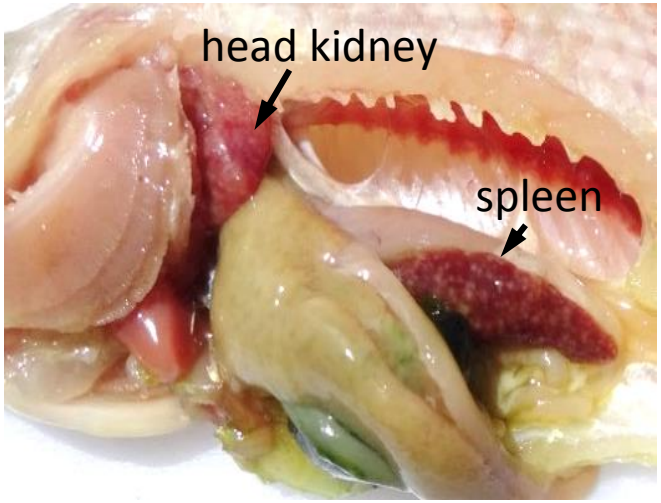
Dong et al. 2019 Aquaculture 499: 17-23

Edwardsiellosis of tilapia

Comments

- 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 in tilapia farming countries

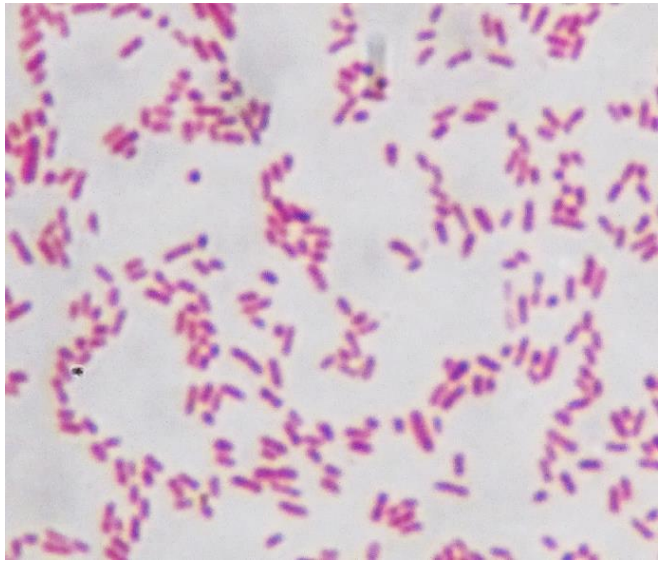




Same same…but different…

Hemorrhagic septicemia caused by motile aeromonads

- **Causative agent**
  - *Aeromonas hydrophila*
  - *Aeromonas veronii*
  - *Aeromonas jandaei*
  - *Aeromonas shuberti*
  - *Aeromonas dhakensis*

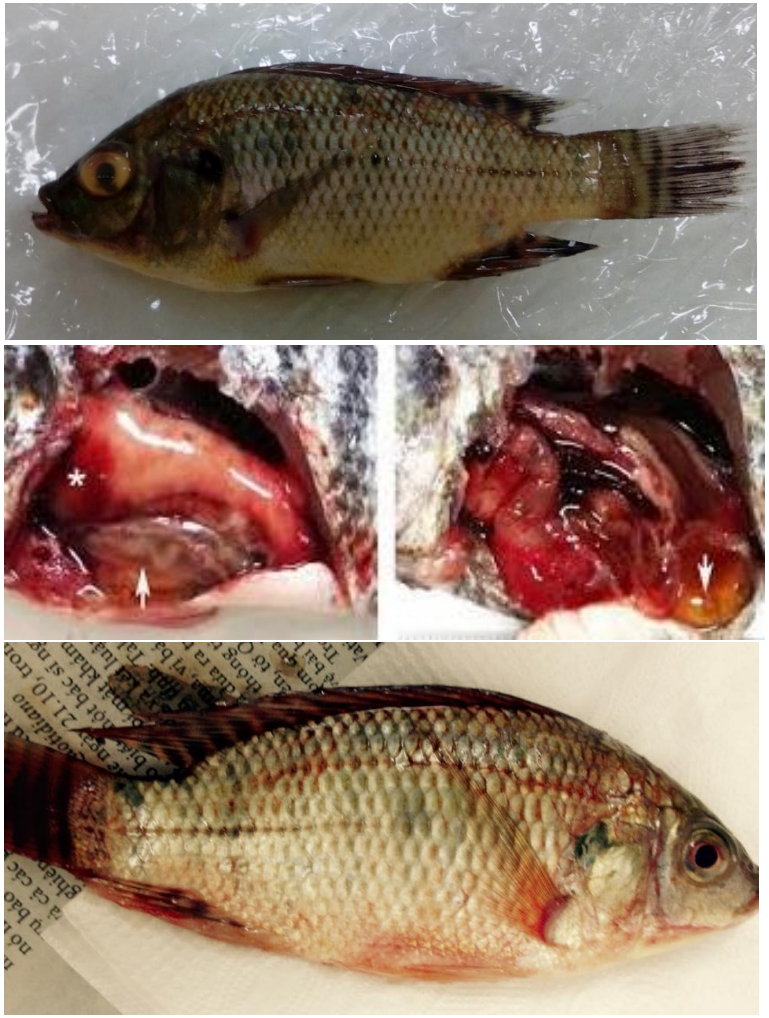


Aeromonas sp. (Gram stain)

- Gram negative bacteria
- Rod or oval shaped
- Non-spore forming
- Oxidase (+), catalase (+)

Hemorrhagic septicemia caused by motile aeromonads

- **Clinical signs** (Level 1)

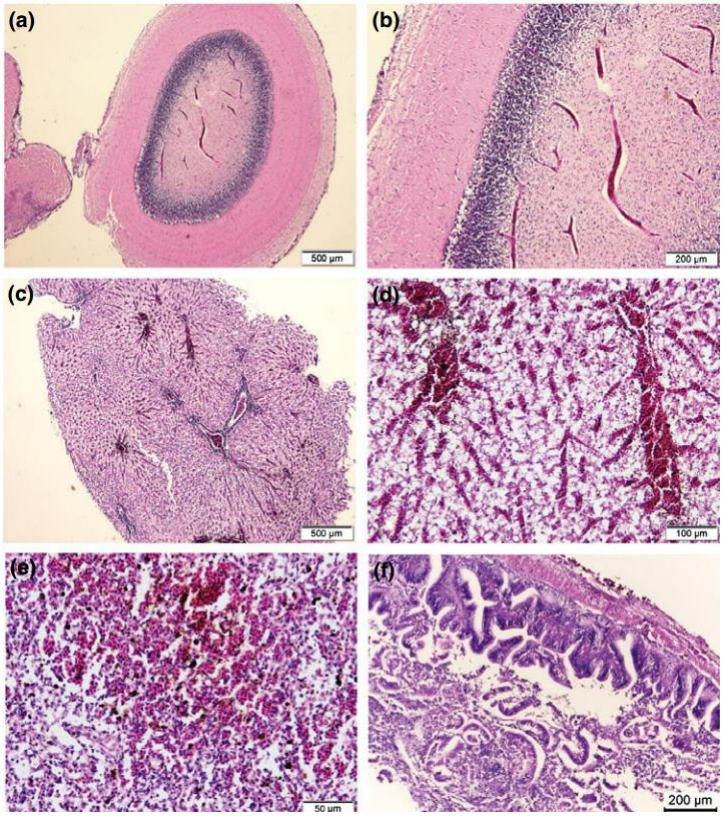


Pictures by Wassif et al. 2018; Dong et al. 2017; Mohamed et al. 2017

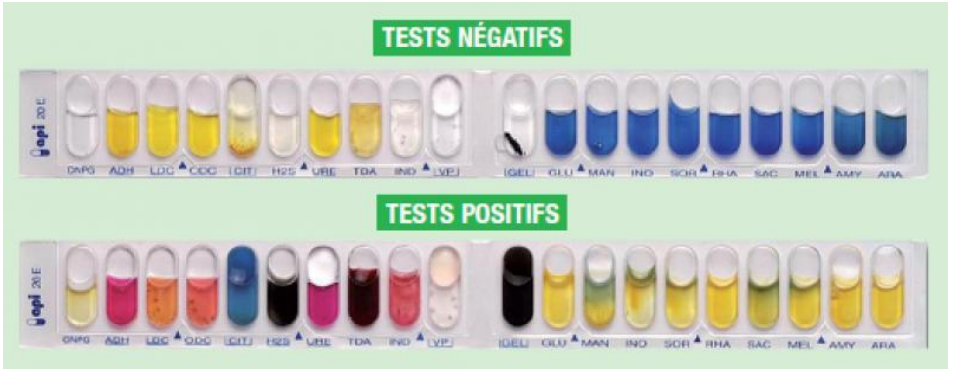
Hemorrhagic septicemia caused by motile aeromonads

Diagnostics (Level 2)

- Histopathology
- Bacterial culture + biochemical tests



Dong et al. 2017 J Fish Dis 40:1395–1403

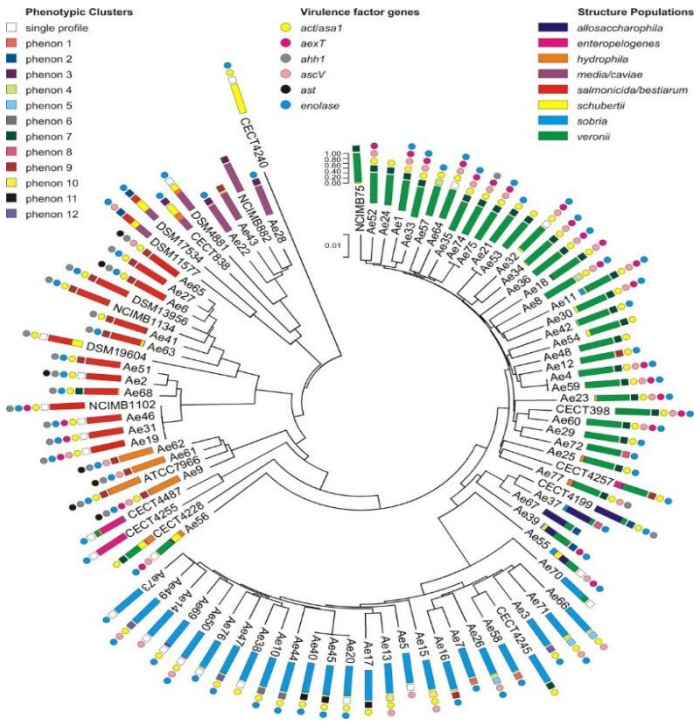


API 20E kit

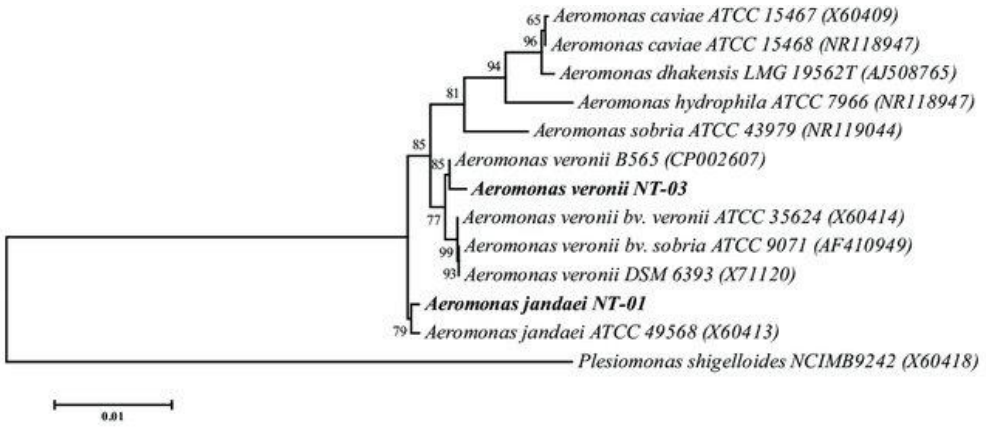
Hemorrhagic septicemia caused by motile aeromonads

Diagnostics (Level 3)

- ❖ Specific PCR (for either fish tissue or pure isolate)
  - Misidentification is common in Aeromonas group
  - Bacterial culture + sequencing of 16S rRNA or MLST is highly recommended



MLST (Martino et al. 2011)



16S rRNA (Dong et al. 2017)

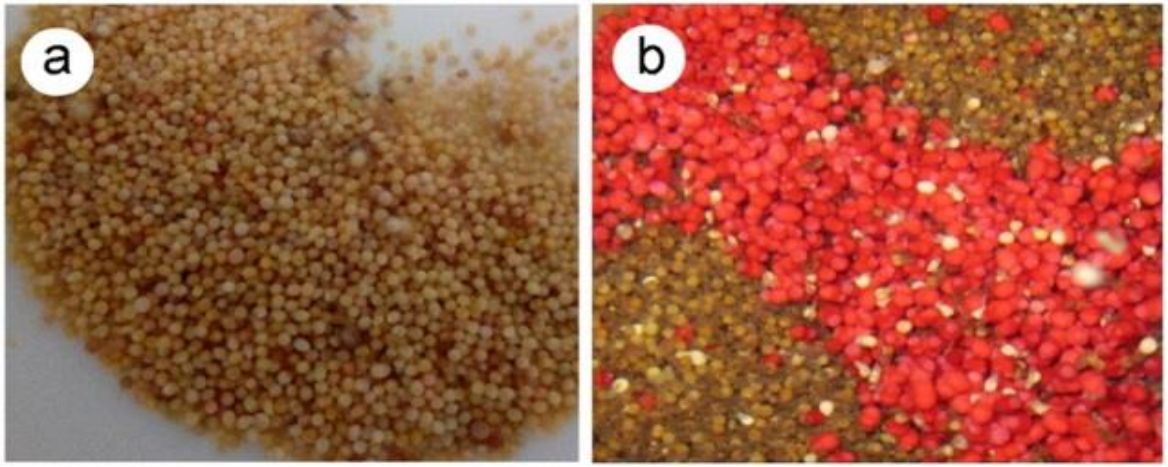
Hahellosis (red egg disease)

Hahellosis/Red egg disease

Causative agent

- *Hahella chejuensis*
- Gram negative, rod-shaped, red pigmented bacteria
- Marine bacterium

Diagnostics (Level 1)



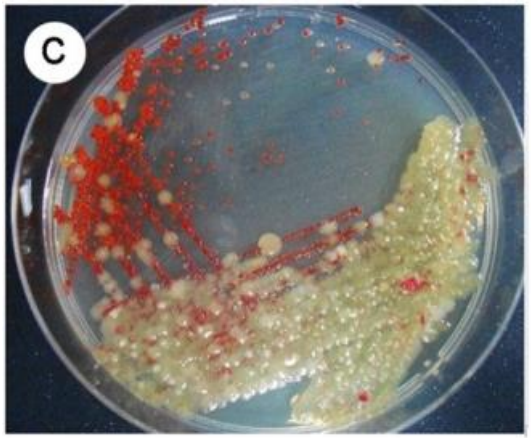
Normal eggs Red eggs

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

Hahellosis/Red egg disease

Diagnostics (Level 2)

- Histology
- Bacterial isolation

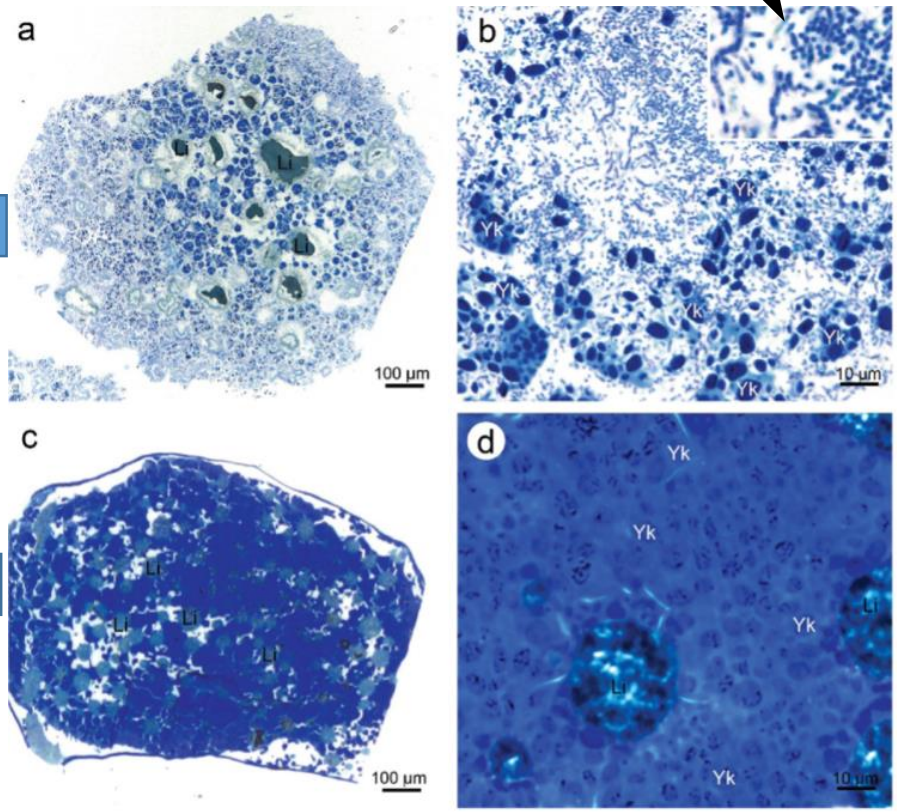


Bacterial isolation using TSA

Red egg

Normal egg

Rod bacterial cells

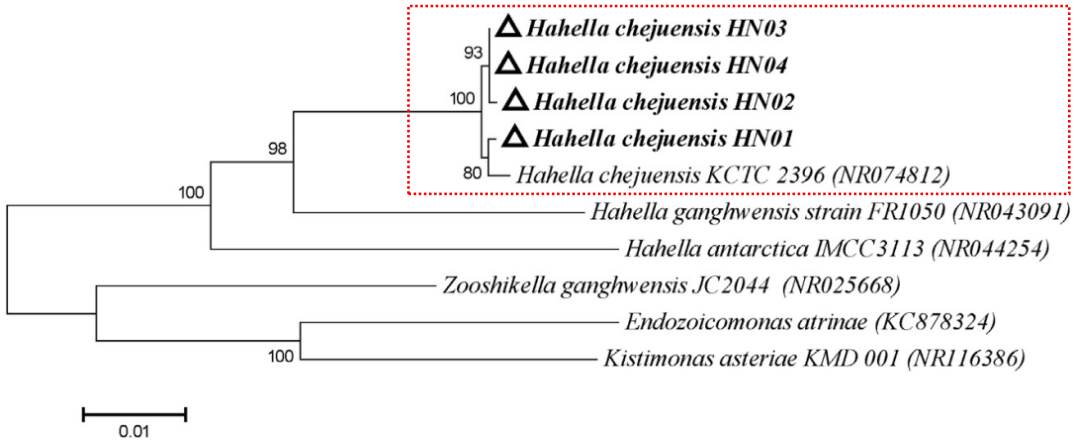


Semi-thin section (1 µm), stained with toluidine blue

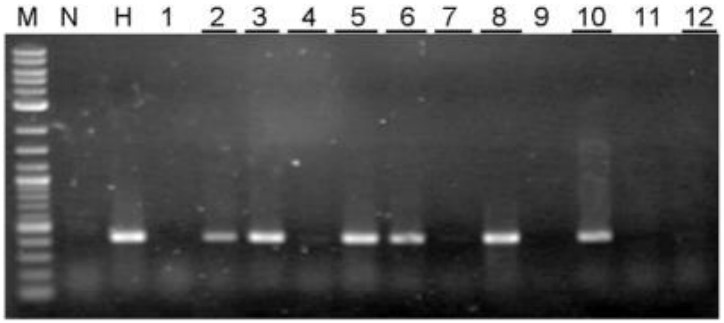
Hahellosis/Red egg disease

Diagnostics (Level 3)

- ✓ Sequencing of 16S rRNA
- ✓ Genus & species specific PCR (Senapin et al. 2016)



Red pigmented bacteria was identified using 16S rRNA



Specific PCR methods were developed targeting 16S rRNA

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

Hahellosis/Red egg disease

Geographical Distribution

✓Tilapia hatcheries in Thailand since 2010

✓Recently found in rabbitfish in Vietnam (unreported)

Mortality: 10-50%

Risk factors:

✓Occur during cold season (<24 ^{0}C)

✓Occurred in hatcheries using 7 ppt NaCl water

Hahellosis/Red egg disease

Disease control

- ✓ 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%)

Epitheliocystis Disease

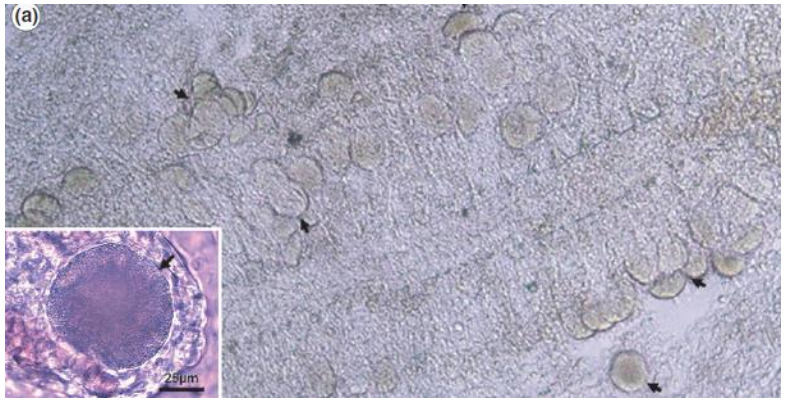
- Causative agent: *Chlamydia-like* organisms (CLOs)
- Affect mainly gills
- Sometime associated with mortality in tilapia fry and fingerlings
- Diagnostics: wet-mount of gills filaments or histology

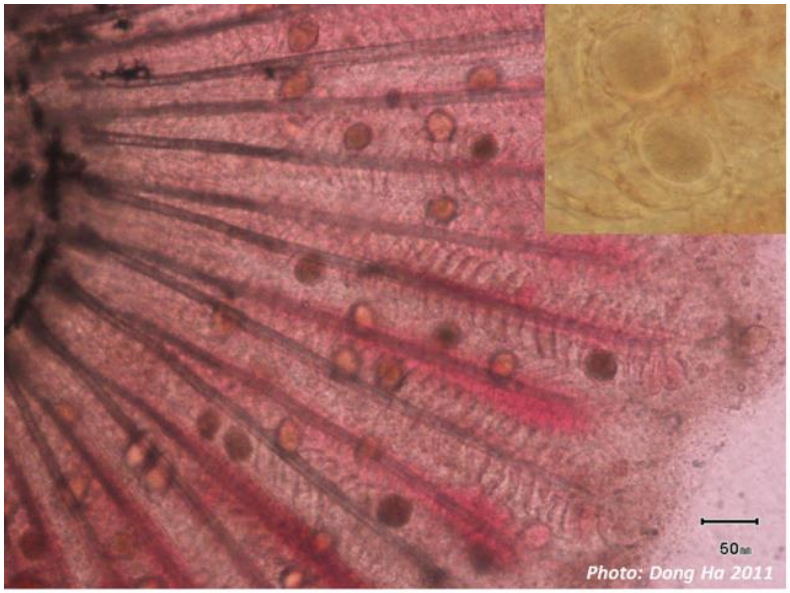
Journal of Applied Ichthyology
J. Appl. Ichthyol. 31 (2015), 927–930
© 2015 Blackwell Verlag GmbH
ISSN 0175–8659
Received: December 15, 2014
Accepted: January 16, 2015
doi: 10.1111/jai.12840

Short communication

A survey of epitheliocystis disease in farmed Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) in Brazil

By S. B. Pádua[1], R. N. Menezes-Filho[1], M. L. Martins[2], M. A. A. Belo[3], M. M. Ishikawa[4], C. A. Nascimento[5], K. C. Saturnino[6] and J. R. Carrijo-Mauad[7]

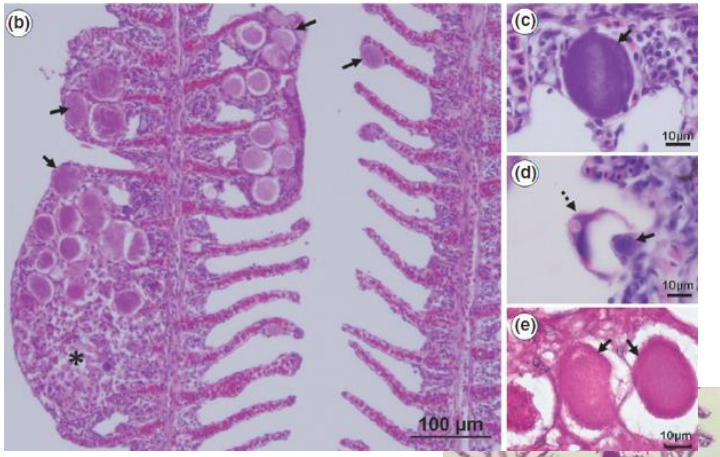




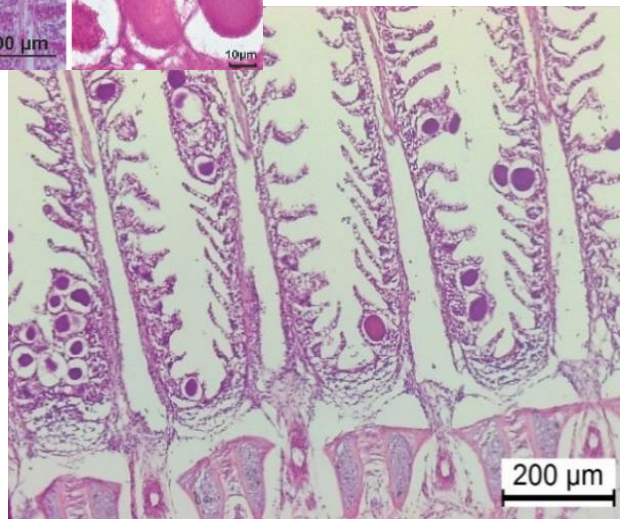
Fresh mount showing heavy infection of CLOs

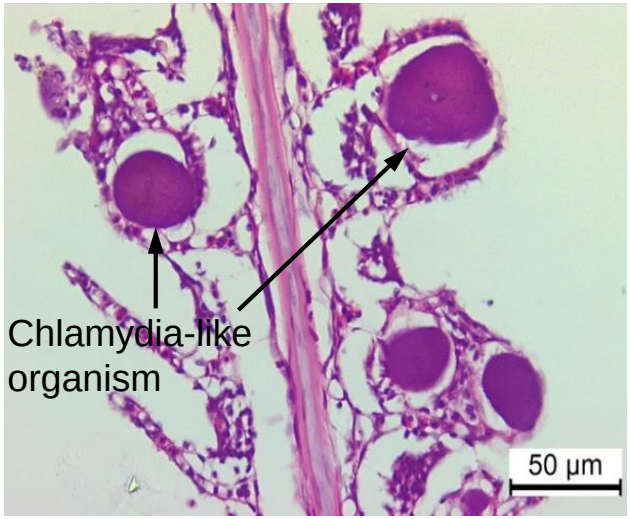
Epitheliocystis Disease

- Diagnostics: histopathology



Nile tilapia, Brazil
(Padua et al. 2014)

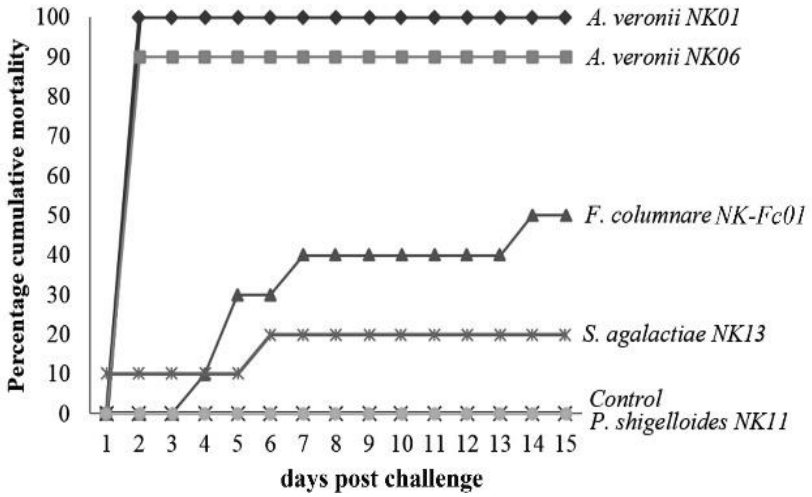




Red tilapia, Thailand (HT Dong)

Single infection vs. Multiple infections

Miscellaneous Disease in Tilapia



- ✓ Concurrent infections of 5 bacteria and a virus in cultured tilapia farms
- ✓ Each fish was infected with 2-4 pathogens
- ✓ ***A. veronii*** **and** ***F. columnare*** were most dominant and exhibited high virulence

Dong et al. Aquaculture (2015) 448:427-435

Miscellaneous Disease in Tilapia

JOURNAL OF
FISH DISEASES

Original Article

Natural coinfection by *Streptococcus agalactiae* and *Francisella noatunensis* subsp. *orientalis* in farmed Nile tilapia (*Oreochromis niloticus* L.)

G B N Assis, G C Tavares, F L Pereira, H C P Figueiredo, C A G Leal

First published: 04 May 2016 | https://doi.org/10.1111/jfd.12493 | Cited by: 9

ELSEVIER

Aquaculture
Volume 485, 2 February 2018, Pages 12-16

Short communication

A case of natural co-infection of Tilapia Lake Virus and *Aeromonas veronii* in a Malaysian red hybrid tilapia (*Oreochromis niloticus* × *O. mossambicus*) farm experiencing high mortality

M.N.A. Amal [a, f], C.B. Koh [b], M. Nurliyana [a], M. Suhaiba [a], Z. Nor-Amalina [c], S. Santha [c], K.P. Diyana-Nadhirah [c], M.T. Yusof [d], M.Y. Ina-Salwany [c, f], M. Zamri-Saad [e, f]

Show more

Miscellaneous Disease in Tilapia

Coinfections of *Flavobacterium columnare* and *Francisella noatunensis* subsp. *orientalis*

F. columnare and *Saprolegnia* sp.

Thank you for your kind attention