Bacterial Diseases of Tilapia

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Bacterial Diseases in Farmed Tilapia

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

• Causative agents
  • *Streptococcus agalactiae*
  • *Streptococcus iniae*
  • *Streptococcus dysagalactiae*
  • *Lactococcus garviae*
  • *Aerococcus viridans* (?)

- 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)
Streptococcossis

❖ Causative agents

Intracellular living style of *S. agalactiae*

Liver

Brain

HT Dong 2009
Streptococciosis

- **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
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 °C for 24-48 hrs. → pinpoint colonies
  - Gram positive, oval/round shape, catalase (-) → *Streptococcus*
  - API 20 strep kit

*S. agalactiae* on NA
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 °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

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 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 on 16S rRNA sequences

**Genomovar I & I/II**
- Cold water fish
- Flavobacterium columnare ATCC 49512
- Flavobacterium columnare ATCC 23463 clone GI-1
- Flavobacterium columnare F10-HK-A clone GI/II-1
- Flavobacterium columnare IAM 14301
- Flavobacterium columnare FK 401
- Flavobacterium columnare ATCC 49513
- Flavobacterium columnare LP8
- Flavobacterium columnare EK28
- Flavobacterium columnare LV339-01
- Flavobacterium columnare Ga-6-93
- Flavobacterium columnare ALG-00-530 clone GII-18
- Flavobacterium columnare PT-14-00-151 clone GII-B-1

**Genomovar II & II-B**
- Warm water fish
- Striped Catfish isolates (n=8)
- Tilapia isolates (n=50)

**Genomovar III**
- Flavobacterium columnare CUVET1215
- Flavobacterium columnare AU-98-24
- Flavobacterium columnare PH-97028
- Flavobacterium columnare GA-02-14 clone GIII-1
- Flavobacterium columnare ARS-1 clone GIA-1
- Flavobacterium johnsoniae ATCC 23107
- Flavobacterium psychrophilum strain ATCC 49418
- Flavobacterium branchiophilum NBRC 15030.
**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?

F. columnare
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

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 °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)

<table>
<thead>
<tr>
<th>diseased fish</th>
<th>normal fish</th>
</tr>
</thead>
</table>

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Francisellosis of tilapia

Diagnostics (Level 2)

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

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

ISH using specific probe revealed location of Fno in the spleen

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)

[Image: Genus-specific PCR (Forsman et al. 1994) and Duplex PCR (Dong et al. 2016)]

Francisella

Fno
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

- USA 2001; 2010; 2013
- Mexico 2016
- Hawaii 2005
- Costa Rica 2007
- China 2015
- Taiwan 2001
- Thailand 2013
- Indonesia 2004
- Japan 2005
- Brazil 2012
- Chile 2006
- North Sea 1988
- Celtic Sea 2009
- UK 2010
- Sweden 2004
- Norway 2004

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°C)
- Very chronic, disease outbreaks depend on environmental factors

Prevention/treatment
- Use Fno-free fish
- Antibiotics have been used for treatment
- Temperature >30°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

**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

- **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)

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*

- Gram negative bacteria
- Rod or oval shaped
- Non-spore forming
- Oxidase (+), catalase (+)
Hemorrhagic septicemia caused by motile aeromonads

- **Clinical signs (Level 1)**

  [Images of fish with hemorrhagic septicemia symptoms]

  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
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

16S rRNA (Dong et al. 2017)

MLST (Martino et al. 2011)
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](image1.png)

![Red eggs](image2.png)

Hahellosis/Red egg disease

Diagnostics (Level 2)
• Histology
• Bacterial isolation

Bacterial isolation using TSA

Red egg

Normal egg

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

Rod bacterial cells
Hahellosis/Red egg disease

**Diagnostics (Level 3)**

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

Specific PCR methods were developed targeting 16S rRNA

Red pigmented bacteria was identified using 16S rRNA

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 °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
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

Miscellaneous Disease in Tilapia

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](https://doi.org/10.1111/jfd.12493) | Cited by: 9

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

Miscellaneous Disease in Tilapia

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

*F. columnare* and *Saprolegnia* sp.
Thank you for your kind attention