Session 2

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Emerging, re-emerging and new diseases of tilapia

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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

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

<table>
<thead>
<tr>
<th>Category</th>
<th>Virus Name</th>
<th>Genus</th>
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<tbody>
<tr>
<td>Re-emerging</td>
<td>Lymphocystis disease virus (LCDV)</td>
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<td>Infectious pancreatic necrosis virus (IPNV)</td>
<td>Aquabirnavirus</td>
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<td>Bohle virus</td>
<td>Ranavirus</td>
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<td>Iridovirus-like</td>
<td>Iridoviridae</td>
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<td>Emerging</td>
<td>Viral nervous necrosis (VNN)</td>
<td>Betanodavirus</td>
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<td>Infectious spleen and kidney necrosis virus (ISKNV)</td>
<td>Megalocytivirus</td>
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<td>New/ newly emerging</td>
<td>Tilapia larvae encephalitis virus (TLEV)</td>
<td>Herpesvirus</td>
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<tr>
<td></td>
<td>Tilapia lake virus (TiLV)</td>
<td>Tilapinevirus</td>
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</tbody>
</table>

Case reports with little concern: Bohle virus, Viral nervous necrosis (VNN), Infectious spleen and kidney necrosis virus (ISKNV), Tilapia larvae encephalitis virus (TLEV), Tilapia lake virus (TiLV)

Global concern: Lymphocystis disease virus (LCDV), Infectious pancreatic necrosis virus (IPNV), Iridovirus-like, Megalocytivirus, Herpesvirus
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
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

❖ **Histopathological feature:** Vacuolation was observed in brain, eye and spinal cord of diseased fish

❖ **Detection methods:** PCR methods (OIE disease card)

Keawcharoen et al. JFD 2015, 38, 49-54
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’ TCGTGGGCCTTATCCCGGT 3’)
  TLEV-2 (5’ GAGACCAGAAAGTGCTTCTC 3’)

Lack of investigation in other countries

Tilapia lake virus disease (TiLVD)

Tilapia lake virus: a threat to the global tilapia industry?
Mona Dverdal Jansen¹, Ha Thanh Dong² and Chadag Vishnumurthy Mohan³

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2 Department of Microbiology, Faculty of Science, King Mongkut’s University of Technology Thonburi (KMUUT), 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**
**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

**Zhang et al. 2018**
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

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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 ºC)
Francisellosis of tilapia

Geographical distribution

Adapted from Nguyen et al. 2015 Aquac Res. doi:10.1111/are.12802
Franciscellosis 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.
Franciselllosis 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*
Presumptive diagnosis

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

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

**Edwardsiella ictaluri**

- Edwardsiella ictaluri PH-0744, Ayu/Japan (AB453281)
- Edwardsiella ictaluri 93-146, Channel catfish/USA (CP001600)
- Edwardsiella ictaluri ATCC 33202, Channel catfish/USA (NR024769)
- Edwardsiella ictaluri 2234, Red tilapia/Vietnam
- Edwardsiella ictaluri 2254, Red tilapia/Vietnam
- Edwardsiella ictaluri 2248, Red tilapia/Vietnam
- Edwardsiella ictaluri UK1, Nile tilapia/Western Hemisphere (KM676418)
- Edwardsiella ictaluri T1-1, Striped catfish/Thailand (KR080248)
- Edwardsiella anguillarum ET080813 (NR136429)
- Edwardsiella hoshinae JCM1679 (NR024768)
- Edwardsiella tarda ATCC 15947 (NR024770)
- Serratia marcescens (NR036886)

**Phylogenetic tree based on 16S rRNA**
Edwardsiellosis of tilapia

Challenged experiments fulfilled Koch’s postulates
  - Fish reproduce the same clinical signs
  - 95-100% mortality in 3-9 days (dose-dependent)

Histopathological features of edwardsiellosis in the experimental fish
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*
Aerococcus viridans infection

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 °C)
- Causative agent: unknown

**Senapin et al. Aquaculture (2016) 454:1-7**
Hahella chejuensis is a marine bacteria...occurred in tilapia hatcheries?

Red pigmented bacteria was identified using 16S rRNA

Hahella chejuensis is a marine bacteria

...occurred in tilapia hatcheries?

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

Hahellosis/Red egg disease

- **Hahella-specific probe**
- **Unrelated probe**

**Red egg**

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

**Ovary**

**Testis**

*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

• 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