Update on Francisellosis in Tilapia in Hawaii

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Aquaponics in the Classroom
October 13, 2012
Windward Community College
AKNOWLEDGMENTS

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- Mitigating The Diseases Of Freshwater Cultured Fish Species In Hawaii And The Pacific Region
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Aknowledgements

- Shermiah Iaea
- Ilima-Ho Lastimosa
- Robert Lastimosa
- Stan Kodama
- Ron Koza
Rationale for Project

• In 1994, wild and farm-raised tilapia die of an unknown disease.

• Department of Agriculture issues PQ policy 98-09, Section 150A-8, HRS effective November 5, 1998

Enlarged spleen with granulomas typical of an active infection

Histological section of spleen with granulomas (A) and normal spleen tissue (B). Photo courtesy of Dr. Juan Morales, Universidad Nacional of Costa Rica
What is the first thing that comes to your mind when Tilapia is mentioned?
Rationale for Ongoing Research

Continued expansion and diversification of tilapia in Hawaii’s aquaculture industry
Conventional Polymerase Chain Reaction (PCR)

And

Real Time PCR
DNA Sequence

10-87 (+TRLO)
GGATCTACTGCGTTGGATAGCTAGTTGGGTGGGGTAA
GGGCCTACCAAGGCTACGATCCATAGCTGATTTGAG
AGGATGATCAGCCACATTTGGGACTGAGACACCGGC
CACAATTCTACGGGAGGCAGCAGTGGGGAATATTG
GACAATGGGGGGAAACCCTGATCCAGCAATGCCATGT
GTGTGAAGAAGGCTCTAGGGTTGTAAAGCACTTTTAG
TTGGGGAGGAAAGCCTGTGAGTAATAGCTTTTCAGG
AA

Basic Local Alignment Search Tool, BLAST
Program Result = Francisella noatunensis
subspecies orientalis 99%
Update

• The pathogen, *Francisella noatunensis* subsp. *orientalis* (syn. *F. asiatica*) or Fno

• Causative agent for mortalities in several tilapia species in Hawaii, the continental United States, Taiwan, Latin America and Japan.

• To avoid confusion RLO, TRLO, FLB and Fno are referring to the same pathogen.
Distribution of Positive Cases on Oahu
Dec 2010 – Current
Distribution of FLB Among Tilapia Species

- **S. melanotheron**
- **O. mossambicus**
- **O. honorum**
- **O. aureus**
- **O. niloticus**

Positive (%) distribution among fish species:

<table>
<thead>
<tr>
<th>Species</th>
<th>Positive (%)</th>
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<tbody>
<tr>
<td>S. melanotheron</td>
<td>(30)</td>
</tr>
<tr>
<td>O. mossambicus</td>
<td>(146)</td>
</tr>
<tr>
<td>O. honorum</td>
<td>(175)</td>
</tr>
<tr>
<td>O. aureus</td>
<td>(47)</td>
</tr>
<tr>
<td>O. niloticus</td>
<td>(20)</td>
</tr>
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</table>

Tilapia Species

Images of fish species.
Tissue distribution during an active Fno infection
Change in Tissue Distribution of FLB-DNA During and After An Active Infection

N=10  N=10
N=8   N=8
N=8   N=8
Blue Tilapia Challenge Test

FLB POSITIVE (%)

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<th>DATE</th>
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<th>N=5</th>
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Introduce *O. aureus*
Temporal Change in FLB-DNA Positive Individuals in a Tilapia Population
Blue Tilapia Challenge Test, Maris Garden (ongoing)
Species and tissue distribution of FLB during an active infection

![Image of fish and plants]

**Graph:**
- **Gill (CLIP)**: N=4
- **Gill (FTA)**: N=4
- **Spleen**: N=4

**Legend:**
- **Golden**
- **Koilapia**

Vials T79 – T120 03/02/2011
Temporal Change in Positive Individuals for FLB-DNA After De-population and Restocking

Restocked 4/11/2011
Distribution of FLB-DNA among gonadal tissues

<table>
<thead>
<tr>
<th>Individual</th>
<th>Spleen</th>
<th>Ovary</th>
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</thead>
<tbody>
<tr>
<td>Female – 1</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Female – 2</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Female – 3</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Female – 4</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Female – 5</td>
<td>Negative</td>
<td>Negative</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Individual</th>
<th>Spleen</th>
<th>Testes</th>
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<tbody>
<tr>
<td>Male – 1</td>
<td>Positive</td>
<td>Negative</td>
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<tr>
<td>Male – 2</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Male – 3</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Male – 4</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Male – 5</td>
<td>Positive</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Fry being mouth brooded by Female-1 negative for FLB
Can other fishes have Fno?

*Sphyraena barracuda*  
*Clarias fuscus*
Summary

- Delineation of the pathogen as *Francisella noatunensis* subsp. *orientalis*.
- Demonstration of the existence of asymptomatic carriers of Fno-DNA that can persist for at least a year after a clinical outbreak.
- Other cultured and wild fish species can carry Fno DNA.
- Wild tilapia (S. melanotheron) populations around Oahu are positive (≈ 60% prevalence) for Fno-DNA.
Summary - Continued

- Significant differences in prevalence of Fno among various tilapia species.
- Preliminary data suggest that Fno is not vertically transmitted.
- Depopulation and restocking of clean stocks maybe an effective means mitigating the disease
- Opportunities do exist to establish Fno-free centers
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