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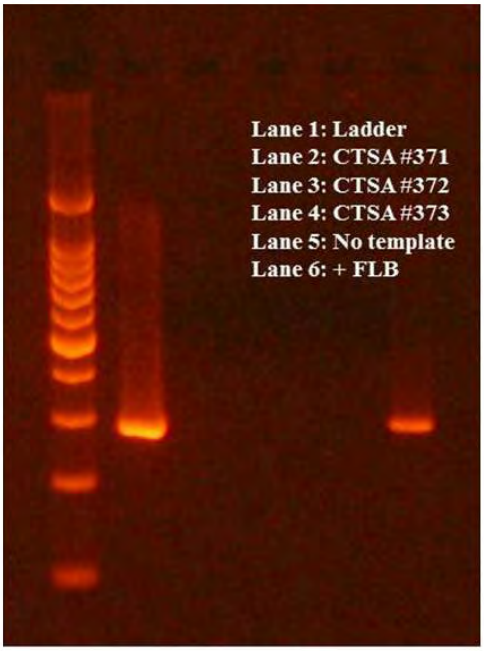
Francisellosis in Tilapia

PCR Test Results: *Francisella* Primers



RuthEllen Klinger-Bowen, Clyde S. Tamaru, Kathleen McGovern-Hopkins, and Bradley K. Fox
Department of Molecular Biology and BioEngineering, College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa.

Nathene Lynn Antonio and James Brock
Moana Technologies, LLC

Esteban Soto
Ross University- School of Veterinary Medicine, Basseterre, St. Kitts, West Indies

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RuthEllen Klinger-Bowen, Clyde S. Tamaru, Kathleen McGovern-Hopkins, and Bradley K. Fox
Department of Molecular Biology and BioEngineering, College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa.

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Introduction

In the winter of 1994, tilapia mortalities occurred around Oahu both in the wild as well as in culture systems[1]. At that time, the Aquaculture Development Program veterinarian, Dr. James Brock and colleagues identified the causative agent as a piscirickettsia-like organism. This disease organism was familiar to other species worldwide, but had previously been predominantly reported in salmonids. Recently, largely thanks to molecular techniques, this organism has been classified as a bacterium, placed in the genus *Francisella*. Worldwide in distribution, it occurs in cultured and wild populations of freshwater and marine fish as well as invertebrates[2]. This fact sheet serves to familiarize you with the most updated information of *Francisella* and its effect on Hawaii's tilapia populations.

What is *Francisella*?

Members of the genus *Francisella* are bacteria that has been known to cause disease in mammals (including humans), fish (fresh water and marine), and invertebrates. Two species within the genus are notable and the first is *Francisella tularensis*, the causative organism of tularemia, a possible fatal disease in humans and a potential biological warfare agent. The second species is *Francisella noatunensis*, which is an emerging fish pathogen causing significant losses in both wild and farmed fish. The *Francisella* bacteria in fish are subdivided into several subspecies, depending primarily on whether the diseases occur in tropical (*Francisella noatunensis* subsp. *orientalis*) or temperate (*F. noatunensis* subsp. *noatunensis*) environments[3]. Fortunately for fish culturists, the *Francisella* bacteria found in fish are not known to be zoonotic, meaning they do not cause disease in humans. This organism is classified as facultative intracellular bacteria, which means it can survive and replicate in the fish's cells as well as in the environment. Research by Dr. Esteban Soto has shown that as little as 23 *Francisella* bacteria need be present to start infecting fish and causing disease[4]; making this bacterium one of the most pathogenic bacteria for tilapia.

What to look for

In Hawaii, disease outbreaks attributed to *Francisella* are most prevalent in the cooler winter months, when temperatures are 77ºF (25°C) or lower. The first signs of an infection are when fish appear sluggish, refuse to feed, but otherwise appear normal. As the disease progresses, fish may be observed at the water's surface or near aeration, gasping. There may also be skin lesions and frayed fins. When the body cavity is opened to expose the internal organs, the spleen is often enlarged, containing grainy to large white nodules (Figure 1).

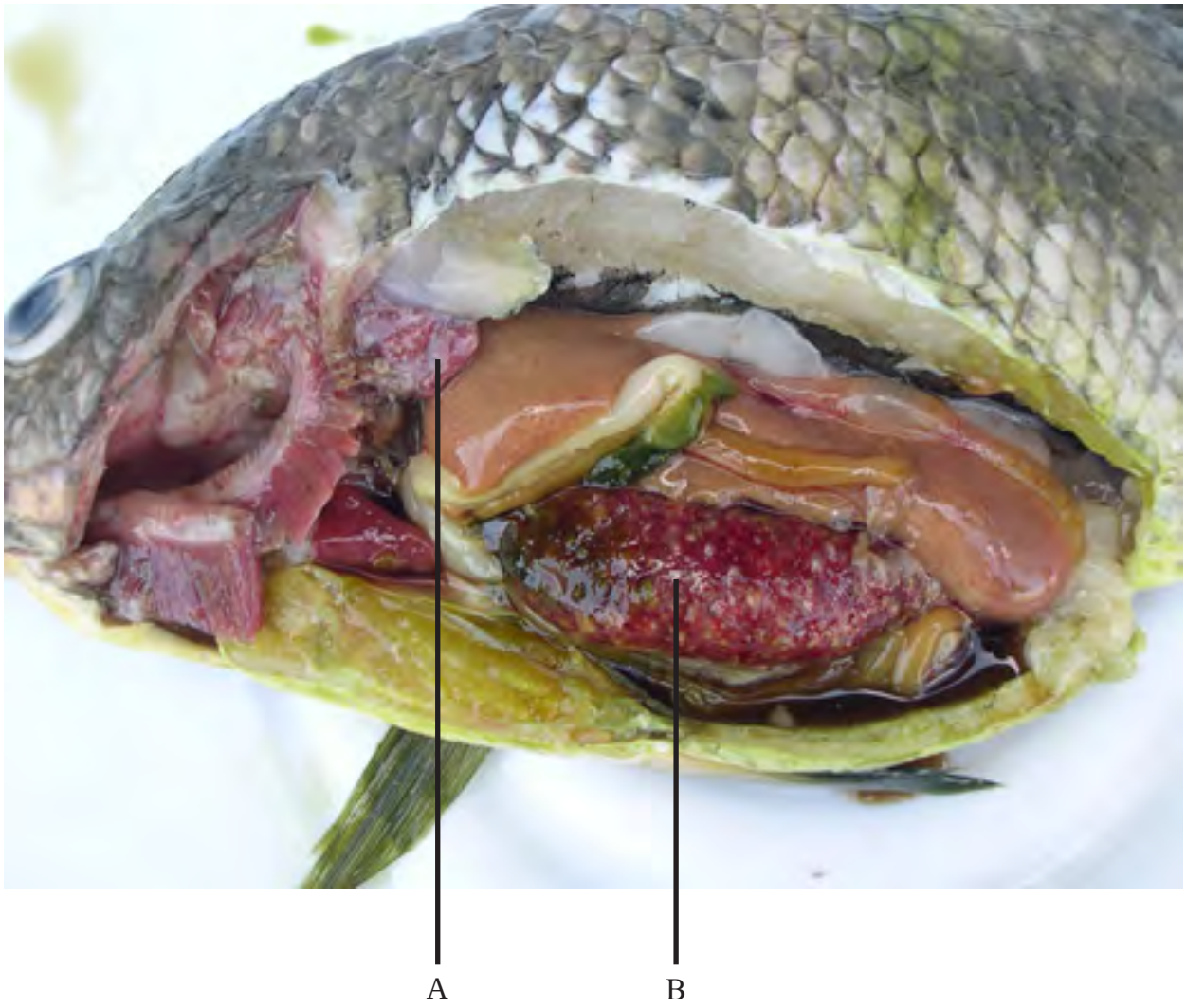

Figure 1. Adult tilapia with an enlarged head kidney (A) and spleen (B). Note large numbers of white nodules in both organs. Photo courtesy of Dr. Juan Morales, Universidad Nacional of Costa Rica.

The spleen and anterior (i.e., head) kidney may also be pale and enlarged with white nodules. These nodules when examined with light microscopy are known as granulomas, which is the fish's immune system's attempt to encase and rid the foreign organism (Figure 2).

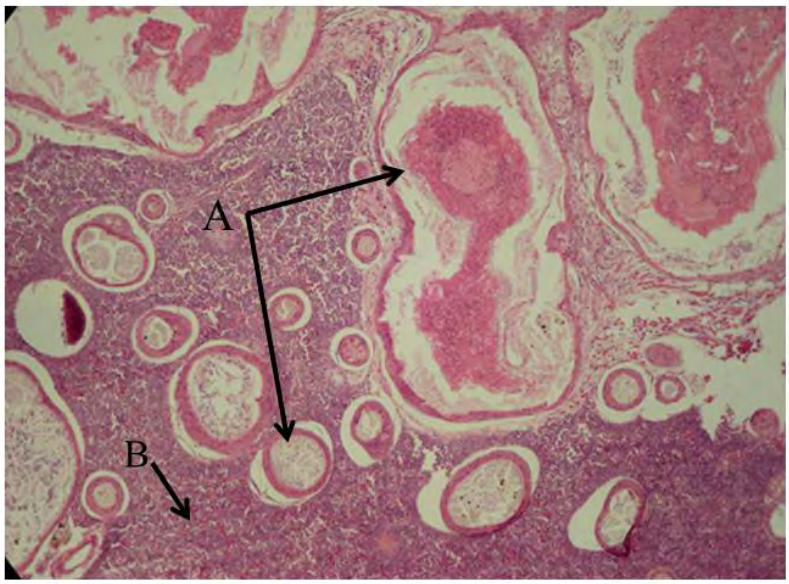

Figure 2. Histological section of spleen with granulomas (A) and normal spleen tissue (B). Photo courtesy of Dr. Juan Morales, Universidad Nacional of Costa Rica

It should be noted that granulomas can be present in many different kinds of disease outbreaks, and this along with the above clinical signs does not confirm that your fish has Francisellosis. At present, the definitive test for the *Francisella* bacterial infection is with detection using the Polymerase Chain Reaction (PCR) technique. The cost for this test can be relatively high, ranging between $55 - 75/sample, but it will verify if your fish population is infected with *Francisella.*

Control and Treatment

Francisella is difficult to treat once it is present in your fish. There is promising research for a tilapia *Francisella* vaccine; however, at present there are only three FDA approved antibiotics, florfenicol (Aquaflor) oxytetracycline and sulfadimethoxine/ormetoprim (Romet-30), available for use in food fish[5]. Of the three, florfenicol and oxytetracycline hold the most promise. Experimental trials have demonstrated that florfenicol given in medicated feed at a dosage of 15 mg/kg of fish weight for a period of 10 days can treat fish challenged with *Francisella*[6]. The FDA approved oxytetracyline is labeled Terramycin 200® and can be administered as an immersion bath at 200 – 700 ppm for six hours, typically administered to younger (smaller) individuals. The recommended feed dose is 2.5 – 3.5 g Terramycin 200®/100g feed per day for ten days. Since infected fish typically do not eat, it may be a better prophylactic treatment (i.e., feed just before cooler temperatures occur) in anticipation of a possible infection. Remember that oxytetracycline has a 21 day withdrawal period, meaning that fish cannot be sold or consumed for 21 days after the last treatment.

The best way to avoid *Francisella* outbreaks is to always quarantine new fish and not mix stocks until you are confident they do not have the bacteria via PCR technology. If your fish become sick, we encourage you to contact your fish health specialist for a thorough examination and confirmation of a causative agent.

Francisella update in Hawaii

Funding from the Center for Tropical and Subtropical Aquaculture (CTSA) has allowed researchers from the University of Hawaii College of Tropical Agriculture and Human Resources (CTAHR) to study *Francisella's* prevalence in Hawaii's aquacultured and feral populations of tilapia. To date, it has been found that *Francisella*:

- Has been detected in tilapia in cultured (aquaculture and aquaponics) systems and in the wild (Black Chin Tilapia, *Sarotherodon melanotheron*).
- There may be strain differences in severity and response of the host fish. Initial results suggest the golden tilapia strain (*Oreochromis mossambicus*) is more susceptible to the disease than the two other common Hawaii strains, "koilapia" (*O. honorum*), named because of its mottled color pattern, and the blue tilapia (*O. aureus*). (see Figures 3 – 6).
- Individuals can survive an outbreak but become asymptomatic (no outward signs) for the disease. While these fish will look normal and eat well, they still harbor the *Francisella* bacteria internally.

Figure 3. Golden tilapia (Oreochromis mossambicus)

Figure 4. "Koilapia" (O. honorum)

Figure 5. Blue tilapia (O. aureus)

Figure 6. Black chin tilapia (Sarotherodon melanotheron)

Future studies

Many questions remain regarding the epidemiology of *Francisella,* and future research will hopefully provide the science based-answers that still need to be addressed, such as:

- Can asymptomatic fish (ones that do not show clinical signs but test PCR positive for the bacteria) infect introduced naive fish?
- Do fish that survive become immune to the disease?
- Transmission is known to occur fish to fish. However, can the parent(s) pass the bacteria to the progeny?
- Are the bacteria able to persist in the water and/or plants (in the case of aquaponic systems)?
- How long does the pathogen remain after fish are removed?
- *Francisella* has been found on Oahu. Is it present in culture or in the wild on the neighbor islands in Hawaii?
- Is it present on other Pacific Islands?
- What other species are affected by *Francisella* in Hawaii and the region?
- Is it beneficial for fish to be treated prior to or after infection has occurred?

Summary

Francisella bacteria have caused disease and mortalities in Oahu tilapia primarily in the cooler winter months when temperatures are 77°F (25°C) or lower. Recent information suggests that disease outbreaks do not occur above 86°F (30°C). The only definitive test for *Francisella* is with PCR technology. Terramycin 200® and florfenicol have been used as a treatment with varying success. However, the best prevention of *Francisella* is to quarantine any new stock (i.e., keep all nets, buckets, etc. separate in your quarantine system to avoid contamination into your established system(s)).

There are a myriad of questions still to be answered about the epidemiology of this bacteria and researchers in Hawaii and abroad are working to elucidate how the bacteria spreads and ultimately how to prevent it from causing disease.

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Additional information

Tilapia: Asymptomatic Carriers of Francisella. http://www.ctsa.org/files/publications/July_11_Regional_e-Notes.pdf