Occurrence of Piscirickettsiosis-like syndrome in tilapia in the continental United States

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Abstract. From 2001 to 2003, tilapia (Oreochromis sp.) farms in Florida, California, and South Carolina experienced epizootics of a systemic disease causing mortality. The fish exhibited lethargy, occasional exophthalmia, and skin petechia. The gills were often necrotic, with a patchy white and red appearance. Grossly, the spleen and kidneys were granular with whitish irregular nodules throughout. Granulomatous infiltrates were observed in kidney, spleen, testes, and ovary tissues, but not in the liver. The granulomas contained pleomorphic cocccoid bacteria, measuring 0.57 ± 0.1 × 0.8 ± 0.2 μm, that were Giemsa-positive, acid-fast-negative, and Gram-negative. The bacteria had a double cell wall, variable electron-dense and -lucent areas, and were present in the cytoplasm and within phagolysosomes. The syndrome was associated with cold stress and poor water conditions. These findings are consistent with an infectious process caused by a Piscirickettsia-like bacterium described previously in tilapia in Taiwan and Hawaii. This report involves the first identified cases of a piscirickettsiosis-like syndrome affecting tilapia in the continental United States.

Key words: Piscirickettsiosis; Piscirickettsia-like organisms; piscirickettsiosis-like syndrome; Tilapia.

Since the identification of Piscirickettsia salmonis as the first rickettsia-like organism recognized as a fish pathogen,9 a number of Piscirickettsia-like organisms (PLOs) have been observed microscopically or isolated in cell culture from a variety of marine and fresh-water piscine species from locations worldwide.5,11,12,16 Piscirickettsiosis or piscirickettiosis-like syndromes can be severe, resulting in high mortality and significant economic losses.

The number of reports of piscirickettsiosis, with significant associated economic losses, have been increasing for the last 5–10 years in British Columbia,2,8 Ireland,16 and Scotland.10 PLOs have also been observed in and isolated from seabass in Europe1,7 and California3 during epizootics characterized by high mortality and severe economic losses. Tilapia in Taiwan4,6 and Hawaii15 have also been severely affected by PLO epizootics. Here we report on the first identified cases of PLO epizootics in tilapia in the continental United States.

Between 2001 and 2003, 3 facilities in the continental United States (California, South Carolina, and Florida) reported epizootics with signs consistent with a piscirickettiosis-like syndrome. All 3 facilities had increased mortalities during times of fish stress due to lowered temperature or water quality. The fish appeared lethargic, with darkened color, and would crowd toward the middle of the pond or tank. Occasionally, petechia were observed on the sides of the fish. Fins had light fraying, some exophthalmia was observed, and gills were necrotic, with a patchy white and red appearance. Internally, the spleen and kidneys were extremely granular with whitish irregular nodules throughout. The liver was a pale reddish tan with patchy lighter areas.

In hematoxylin and eosin (HE) sections, the spleens had extensive necrosis and multifocal to confluent granulomatous inflammatory infiltrates (Fig. 1). Occasional melanophages were associated with the infiltrates. Splenic parenchyma was replaced by multiple and coalescing granulomas. The granulomas were characterized by central areas of necrosis and/or vacuolated macrophages mixed with very few neutrophils surrounded by large (but less vacuolated) macrophages and fibrosis. Occasionally, a fine, fibrous capsule encompassed the granulomas. The gills had mild blunting and the tips were minimally hypercellular, with increased secretory mucus cells. Granulomas at the base of the gills and the gill filaments were multifocally and mildly expanded by a mixed population of inflammatory cells. The cytoplasmic vacuoles of vacuolated macrophages contained numerous small pleomorphic cocccoid bacteria (Fig. 2). The bacteria were Gram-negative, stained Giemsa positive, and acid-fast negative. This inflammatory process nearly replaced the entire normal splenic architecture.

In the winter of 2001, tilapia (Oreochromis mossambicus × Oreochromis hornorum) from an intensive recirculating aquaculture facility in southern California were observed swimming slowly and crowding in the middle of the ponds. The farmer recorded increased mortality of 5–10 times above normal for 2 weeks. The water quality was as follows: temperature 22°C, ammonia > 5 mg/liter, pH > 8.2, salinity 5 ppt, alkalinity 450 ppm, hardness 270 ppm. Pond water was filtered by passing through four sedimentation earth ponds, and 50% of the water per day was replaced after filtering. Fish were fed 3 times daily and the ponds were harvested by size selection every 3 weeks. To reduce external and gill parasites (Ambiphrya, Epistylis, Cleidodiscus), the water was treated with potassium permanganate every 2 weeks.
The increased mortality observed by the farmer was a continuing problem, with the facility having heavier losses in winter (5–10-fold greater in winter than summer) but losses were also observed in summer, when the fish were stressed by poor water quality. The mortalities increased when the temperature was below 25°C. The facility reported that the majority of the mortalities were observed in the juvenile tilapia ranging in weight from 20 to 90 g.

Facility personnel observed white nodules in the viscera of the tilapia. Spleens submitted in neutral buffered 10% formalin were embedded in paraffin and sectioned. The sections were stained with H & E, Giemsa, and acid-fast stain. The splenic lesions were consistent with *Piscirickettsia*-like bacterial infections observed in tilapia in Hawaii. *Piscirickettsia salmonis* and the Hawaiian PLO infections have previously been treated with tetracycline and oxytetracycline. When tilapia were fed oxytetracycline-medicated feed, mortalities were reduced to near 0 by the fifth day.

In the winter of 2002–2003, tilapia mortalities at a research facility in South Carolina were observed with nodular lesions in the gills and viscera (Fig. 3). Formalin-fixed spleen tissue and dead fish were submitted for examination. Water-quality data were not submitted. Fish were submitted in 2 groups, based on pen location. In both groups, white nodules were observed grossly and necrogranulomatous inflammation observed microscopically in the gills, spleen, and kidney. The liver and remaining organs were unremarkable in group 1 (3 fish); however, in group 2 (3 fish), there was severe necrogranulomatous inflammation throughout the liver, multifocally throughout the skeletal muscle, bone, mildly in the subcutis, epicardium (1 fish), surrounding the spinal cord (1 fish), and infiltrating the choroid gland.
In December 2002, tilapia (O. niloticus × O. aureus) from a grow-out facility in Florida were submitted for diagnosis. Ten fish were collected for examination but only 3 survived for the 1-hour transport time to the laboratory. The fish were 4–6 months of age, 5–20 cm long, and 70,000 fish had been stocked in a 260,000-liter tank 3.5 months earlier. Water was supplied from a well and the water, tested from several locations within the system, had the following conditions: pH 7.5–8.0, salinity 11 ppt, nitrite 0.03–0.11 ppm, total ammonia nitrogen 5.0–6.0 ppm, total alkalinity 136.8 ppm, total hardness > 855 ppm.

Grossly, 2 of the 10 fish had petechia on one side and some fish in the system were observed to be bloated. Some fish with frayed fins and some with exophthalmia were observed. The gills were necrotic, with a patchy white and red appearance. Skin had excess mucus and microscopic examination revealed Trichodina, Ambiphrya, and gyrodactylids on the skin, fins, and gills. There was little food present in the stomach or intestines and a clear fluid was observed in the abdomen of 2 out of the 10 fish. Cultures of brain and kidney tissues on TSA+blood were negative. A Lowenstein–Jensen culture with spleen tissue was negative for mycobacterium.

Tissue samples from both the South Carolina and Florida cases were routinely processed for transmission electron microscopy and prokaryotic organisms typical of PLOs were observed within large vacuoles that compressed the host cell cytoplasm and displaced the host nucleus to one side. The pleomorphic coccoid bacteria measured 0.57 × 0.1–0.8 × 0.2 μm. The bacteria had a double cell wall, variable electron-dense and -lucent areas, and occurred free in the cytoplasm and within phagolysosomes. PLOs condensed to various degrees were also present in small and large phagolysosomes that did not appear to be located in the same vacuoles as the other PLOs (Fig. 4).

Splenic tissues from both the South Carolina and Florida cases tested negative for the presence of P. salmonis by P. salmonis-specific polymerase chain reaction (PCR) and P. salmonis-specific fluorescent antibody assays. The tilapia disease agent (0.57 × 0.8 μm) is smaller than the reported...
size of *P. salmonis* (0.5 × 1.5 μm). The combination of characteristics—different host species, smaller cell size, no response to *P. salmonis*-specific PCR and *P. salmonis*-specific fluorescent antibody assays—indicates that the bacterium causing epizootics in tilapia in these cases is not *P. salmonis*.

All of the above cases presented disease signs consistent with piscirickettsiosis-like syndrome. From the cases described here, we conclude that a *Piscirickettsia*-like organism is now present in the continental United States. This has implications for the transport of tilapia and would suggest the need for gross and microscopic observation of tilapia splenic tissue prior to transfer, as well as close attention by aquaculturists to production water-quality conditions, as this syndrome is expressed during periods of fish stress.

**References**

Infection of the human central nervous system (CNS) with free-living amoebae was first described in 1966 and is now recognized worldwide. There are 4 genera of amoebae that cause CNS disease in mammals, namely Acanthamoeba (several species), Naegleria fowleri, Balamuthia mandrillaris, and the recently described Sappinia diploidea. Acanthamoeba and Naegleria are ubiquitous in soil and fresh water, including lakes, streams, and hot springs. These amoebae have also been isolated from various artificial water sources, such as swimming pools, tap water, heating and ventilation units, air conditioners, cooling water, sewage, contaminated cell cultures, and contact lens-storing fluid. Their cysts have even been demonstrated in dust during dust storms. The habitat of Balamuthia is not known but is believed to be similar to that of Naegleria and Acanthamoeba.

Primary amoebic meningoencephalitis (PAM) is the term for the human disease caused by N. fowleri. It is an acute, usually fatal, necrotizing, and hemorrhagic meningoencephalitis. As of 2001, 200 cases of PAM have been reported in humans. About half of these were reported in the United States (G. S. Visvesvara, personal communication). Although CNS infections due to Acanthamoeba and Balamuthia have been recorded in animals, such as dogs, sheep, cattle, primates, and horses, there has been only 1 report of naturally acquired PAM in animals, namely in a South American tapir at a zoo in Arizona. Primary amoebic meningoencephalitis has been experimentally induced in mice, sheep, and monkeys.

Naegleria fowleri is thermophilic and tolerates temperatures of up to 45°C; hence, the frequent association of PAM with a history of contact with naturally warm or artificially heated waters. Most human cases occur in healthy young individuals after swimming or bathing in pools, ponds, hot springs, canals, or lakes, although some cases have been associated with tap water or inhalation of cysts during dust storms. The portal of entry is the olfactory mucosa. The disease rapidly progresses and usually culminates in death within 5–7 days.

The life cycle of N. fowleri includes a trophozoite stage (amoebic form), a temporary flagellate stage, and, in unfa-