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A CASE REPORT ON FISH TUBERCULOSIS ("FISH HANDLERS' DISEASE") IN RAINBOWFISH (FAM. MELANOTAENIIDAE) AND ROSY BARB (*PETHIA CONCHONIUS*)

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Abstract

Fish mycobacteriosis is caused by atypical Mycobacterium species, some of which can be transmitted to humans. Often, M. marinum, M. fortuitum and M. ulcerans have been described as with the potential to cause fish-borne zoonoses. M. fortuitum and M. marinum are most commonly reported in tropical fish. These are Gram-positive, aerobic, non-motile rods. Humans may become infected by contact with the diseased or infected fish or with contaminated aquarium water, via lacerated or abraded skin. We were called by a client to see some ornamental fish about which the client complained of being off feed for several days, lethargic, in poor condition, showing dropsy, scale loss and abnormal swimming. We asked about the history of these fish, we inspected the fish and tested the water. Three fish (two rosy barbs and one rainbowfish) showing overt signs of the disease, were retained for further investigations. Following euthanasia of the fish, we carried out post-mortem and histopathological examinations. Following the investigation the fish were confirmed infected with atypical Mycobacterium spp.

Key words: *fish tuberculosis, rainbowfish, rosy barb.*

INTRODUCTION

The ornamental fish market is a big industry, involving over 120 exporting and/or importing countries all over the world. It is valued at around US$15 - 30 billion per year. Over 2,500 freshwater and marine fish species are involved in this commercial activity, with over 60% freshwater fish traded globally (Dey, 2016). The United States is the world's largest importing market of ornamental fish, with about 10 million marine fish species imported annually (Biondo & Burki, 2020; Rhyne et al., 2017), of US$ 42.9 million worth (Dey, 2016). Singapore, Indonesia, Thailand, Hong Kong, the Philippines, Malaysia, Japan, Colombia, Peru and Brazil are catered to this market. With over 160 million ornamental fishes kept by hobbyists in the United States [Dey, 2016], ornamental fish is one of the most common pets in the country. With US$ 29.5 million worth imports, in 2014 (Dey, 2016), the United Kingdom was the second world's importer of ornamental fish. Other importing countries holding stable shares in the market are Germany, Singapore, Japan, China and Hong Kong, France, the Netherlands, Italy, Malaysia, Canada and Belgium. Like Singapore, Germany, Hong Kong, Malaysia and the Netherlands are important trading hubs, re-exporting a major portion of their imports (Dey, 2016).

One of the problems associated with this trade is the lack of appropriate disease monitoring and reporting systems, such as those implemented for the terrestrial livestock trade and husbandry. Humans act as the main connecting interface between domestic and wild habitats facilitating the introduction of pathogens existing in the wild, but new to domestic animal populations, including of pathogens with zoonotic potential (Wobeser, 2006). Compared to their terrestrial counterparts, aquatic animals seem to be involved in a smaller number of zoonotic reported cases. As in the case of zoonoses from terrestrial animals, zoonotic pathogens of fish can transmit to humans through ingestion of, or contact with these pathogens (i.e. via broken, abraded or chapped skin), which may be present on the skin, fins, gills of the infected fish, as well as through contact with, or consumption of the infected or contaminated fish (food-borne zoonoses), (Evans et al., 2009; Haenen et al., 2013). Some diseases can also be contacted through accidental ingestion of the water contaminated with faeces, skin mucus or other physiological products of the infected aquatic animals.

Fish mycobacteriosis is caused by atypical *Mycobacterium* species. These are Gram-positive, acid-fast, aerobic, non-motile rods, which can be cultured on blood agar or specialized Löwenstein-Jensen or Middlebrook media (Stephenson et al., 2019; Radomski et al., 2010; Griffith et al., 2007; CLSI, 2008; Saitoh et al., 2000). Growth of these mycobacteria is slow. It may take between 2 weeks and several months for the colonies to develop.

Mycobacterium marinum, *M. fortuitum* and several other *Mycobacterium* species are the cause of common, chronic, severe granulomatous systemic disease in ornamental fish and other aquatic animal species.

Any species of fish, amphibians, reptiles, birds, and mammals, can be considered susceptible. These *Mycobacterium* spp. mildly psychrophilic and mesophilic organisms and typically cause infections in the extremities of man, where temperature is generally lower than 33°C.

Other *Mycobacterium* spp. are described as nontuberculous mycobacteria (NTM) and exclude *Mycobacterium tuberculosis* complex and *Mycobacterium leprae* from the group. There are approximately 150 species of NTM (Bi et al., 2015), with *M. marinum*, *M. fortuitum*, and *M. ulcerans* discussed most commonly as fish-borne zoonoses (Boylan, 2011).

These pathogens have proven a zoonotic potential, causing granulomatous nodules at the site of entry, on hands and arms (hence the disease' names, "fish handlers' disease" or "fish tank granuloma"). Humans are typically infected by contamination of lacerated or abraded skin with aquarium water or fish contact. Other sources of infection are spine punctures, hand scratches on fish tanks, mouth-siphoning fish tanks, splinters from fish net handles, etc. The localized granulomatous nodule usually forms at the site of infection, most commonly, on hands or fingers, approximately 6-8 weeks after exposure to the organism.

In healthy children, lymphadenitis caused by the infection with *M. haemophilum* has been also reported in some cases involving zebrafish (Lindeboom, 2011; Whipps, 2007). In immuno-compromised patients, atypical mycobacteria can cause systematic disease, pneumonia and osteomyelitis.

In tropical fish, the most commonly found mycobacteria are *M. fortuitum* and *M. marinum*. In fish, the infection may be clinical (acute or chronic) or sub-clinical (inapparent), the latter being more prevalent in these fish.

Although any species of fish, can be infected with the bacteria, only those more susceptible to the disease will develop the clinical form. Many aquarium fish and other species may have the infection with no clinical signs. There are also fish which develop the chronic form, remaining carriers for long time. In all these instances, the bacteria is shed into the water, where it can resist for long time, due to its thick walls and capacity to stick to the biofilm.

Stress and overpopulation are usually favouring factors for the bacteria to spread into an aquatic system (Alexander et al., 2021). There are no effective clinical tests to identify the infected fish, nor are there effective treatments or vaccines available.

Fish with mycobacteriosis may present with multiple and varied clinical signs, such as exophthalmia, lethargy, scale loss, abdominal distention, poor body condition, and skin ulcers.

Those fish with sub-clinical (inapparent) mycobacteriosis shed the bacteria without overt disease signs, for several weeks or months. Other fish may undergo acute infections, shedding the bacteria prior to the onset of the clinical symptoms. The faecal-oral route is the main route of transmission among fish. Fish may get infected also via ingestion of infected tissues.

However, release of infectious organisms from infected gill tissue or ulcerated skin lesions are also potential dissemination routes, through which the *Mycobacterium* spreads within the water (Niemeyer-Corbellini et al., 2017).

Poor body condition and abnormal swimming, as well as scale loss, skin ulcer, pigment changes and dropsy are commonly seen in those highly sensitive fish.

Since mycobacteria can resist for months in aquarium water, and because usual disinfect-tants, such as dilute sodium hypochlorite and quaternary ammonia compounds are not effective in the case of atypical mycobacteria,

contaminated water remains the most common source of infection for both susceptible fish and humans (Boylan, 2011).
Biofilms protect the atypical mycobacteria making it resistant to these disinfectants (Boylan, 2011). To properly disinfect an aquatic system containing the mycobacteria it priorly requires removal of the biofilm by mechanical scrubbing, to allow disinfectants to effectively kill the mycobacteria. In the case, the most effective disinfectants are phenols, high concentrations of alcohol, and strong sodium chlorite solutions. Safety measures for disposal of contaminated aquarium water should also be accounted for.
As nontuberculous mycobacteria can be found in freshwater fish and their products, it requires appropriate handling and treatment before consumption (Lorencova et al., 2013).
This paper will describe a case of fish tuberculosis (fish mycobacteriosis) in a multi-species freshwater tropical fish tank.

MATERIALS AND METHODS

The fish were being kept in a well tank (Figure 1) of 600 liters. In the tank, there were around 70 to 80 fishes. The fish species present in the aquatic system were: rummynose tetra, zebra Danio, silver dollar, rainbowfish, harlequin rasbora, rosy barb, black neon tetra, neon tetra, black phantom tetra, serape tetra and silver tip tetra.

Figure 1. The fish tank

To test the water tank we used the Sera test kit. The water was tested for pH, ammonia, nitrite, nitrate, KH and temperature. CO_2 was derived from tables.
To euthanize the sampled fish, we used immersion of the fish in a bucket of water with iso-eugenol (Aqui-S®). Ten minutes after the respiratory (opercula) movements stopped, we proceeded to the necropsy and collected samples for histo-pathology. For the latter stage, we used light microscopy.

RESULTS AND DISCUSSIONS

One rosy barb had been put by the owner in a bucket with water, for a closer inspection.

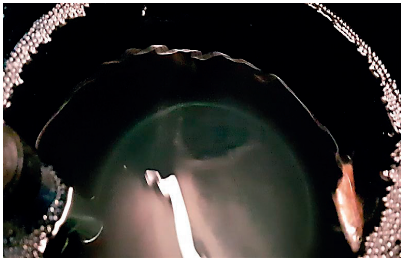

Figure 2. Rosy barb, disoriented

In that fish, the owner reported incoordination of swimming (flashing) and loss of appetite (Figure 2). We further noticed membranous faecal string (diarrhoea) and dyspnoea in that fish Water testing (Figure 3) indicated that the water parameters were close to optimal (Figure 4).

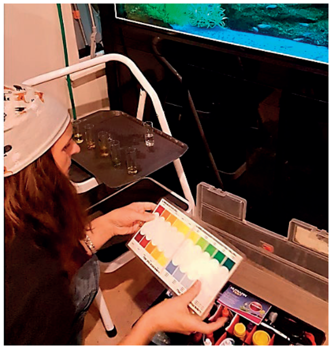

Figure 3. Testing of the tank water

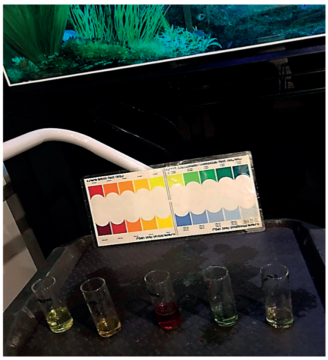

Figure 4. Water testing -the main parameters tested were close to optimum levels

Following inspection of the aquasystem, we noticed a few other rosy barbs and rainbow fishes showing more or less overt signs as those displayed by the previously inspected fish. In addition to these signs, the affected fish were also showing abdominal distention, loss of scales, skin ulcers and haemorrhages. The bucket fish and two additional diseased fishes from the tank (one rosy barb and one rainbow fish), were captured, euthanized, and were then subjected anatomo-pathological and histo-pathological examinations (Figure 5).

Figure 5. Rosy barb, abdominal distention, loss of scales and haemorrhage

The granulomatous inflammation of various organs was consistently seen in all the euthanized fish. The clinical signs previously seen in these fish correlated with the organ most severely affected. The kidney and heart of the fish with dropsy were most affected by the nodules. In the fish with spiraling swimming (the rosy barb in the bucket, Figure 2) the granulomatous lesions were also present in the brain. The other rosy barb, sampled from the fish tank, was having systemic mycobacteriosis, with the nodules affecting most organs, including the intestines (Figure 6).

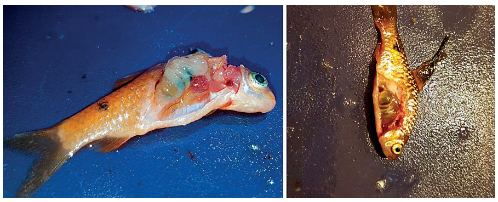

Figure 6. Mycobacteriosis-like granulomatous nodules in rosy barb

The histo-pathological exam revealed the presence of multiple granulomatous inflammatory reactions in the skin, spleen, kidney, heart and brain (Figures 7-9).

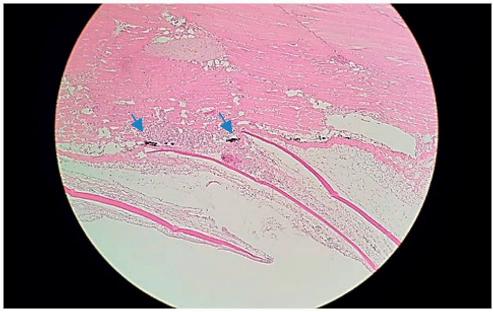

Figure 7. Ulcerative dermatitis and granulomatous inflammation, infection with Mycobacterium (rosy barb)

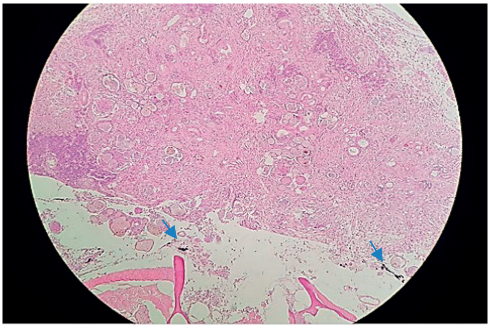

Figure 8. Granulomatous inflammation, infection with Mycobacterium in kidney (rainbowfish)

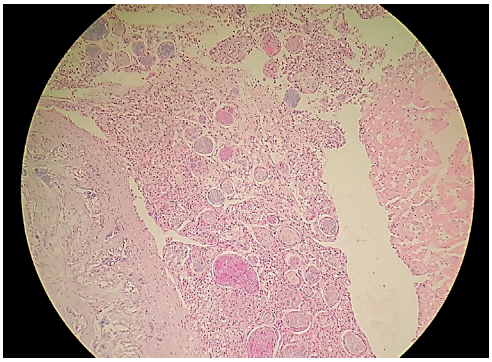

Figure 9. Granulomatous inflammation, infection with Mycobacterium in heart (rosy barb)

CONCLUSIONS

Since from the eleven species kept in the tank only rainbowfish and rosy barb (sub-tropical species) were showing clinical signs, it can be concluded that these two species were more susceptible to the disease than the tropical fish with which they shared the tank, i.e. rummynose tetra, zebra Danio, silver dollar, harlequin rasbora, black neon tetra, neon tetra, black phantom tetra, serape tetra and silver tip tetra. It is also likely that the fish were immuno-supressed due the water temperature, which had been set to 30°C to accommodate the requirements for the tropical fish in the tank, but which was higher than normal for them.

The owner was advised to do frequent water changes, gradually reduce the water temperature from 30°C to 24°C, avoid as much as possible stress in the fish, and reduce the density of the fish in that tank.

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