

MYCOBACTERIUM FORTUITUM ISOLATED FROM THREE SPECIES OF FISH IN SOUTH AFRICA

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ABSTRACT

BRAGG, R. R., HUCHZERMAYER, HILDEGARD F. A. K. & HANISCH, MONICA A. M., 1990. *Mycobacterium fortuitum* isolated from three species of fish in South Africa. *Onderstepoort Journal of Veterinary Research*, 57, 101-102 (1990).

Mycobacterium fortuitum was isolated from 3 species of freshwater fish in South Africa for the first time. The bacterium was isolated from oscars, guppies and discus fish from different sources. Heavy mortalities as a result of infection with this bacterium were reported from a guppy farm and the multi-resistance of this isolate to antimicrobials rules out the treatment of infected fish.

INTRODUCTION

Mycobacterial infections in fish are common and have been well documented. *Mycobacterium* spp. have been isolated from 151 different species of fish from 40 different families (Nigrelli & Vogel, 1963). The two most important mycobacterial species involved in diseases of fish are *M. fortuitum* and *M. marinum*.

Mycobacteriosis in fish was first described in France from carp held there in a pond which was contaminated by sputa and excreta from tuberculous persons (Bataillon, Dubard & Terre, 1897, as cited by Parisot, 1958). This isolate was identified as *M. piscium* (Bataillon *et al.*, 1902, as cited by Parisot, 1958). A bacterium isolated from neon tetra (Nigrelli, 1953) was identified as *M. fortuitum* (Ross & Brancato, 1959), and this was regarded as the first isolation of *M. fortuitum* from fish. A *Mycobacterium* sp. isolated from the family salmonidae in the USA, was termed *M. salmoniphilum* (Ross, 1960). Thoen & Schliesser (1984) stated that *M. piscium* and *M. salmoniphilum* were not valid species names and these isolates have been reclassified as *M. fortuitum*.

Symptoms of mycobacteriosis in fish vary from species to species, but generally the fish are emaciated and show inflammation of the skin, exophthalmia, open lesions and ulcerations. Internally, whitish granulomas may be seen on some organs, particularly the liver, kidney, heart and spleen (Dulin, 1979; Van Duijn, 1981).

The severity of mycobacteriosis in fish may be related to the age of the fish, its nutritional state, oxygen tension and stocking densities. Skin lesions, caused by handling or parasitic infestation, may facilitate infection. Aquatic fauna, such as water fleas, snails and turtles may act as reservoirs of the organisms (Theon & Schliesser, 1984). Feeding on uncooked fish or viscera may also be responsible for a number of outbreaks of mycobacteriosis in fish (Nigrelli & Vogel, 1963).

The incidence of mycobacteriosis in wild fish can be high. Aberinethy & Lund (1978) found an 8 % prevalence of mycobacteriosis in fish from a river in Washington, USA. An even higher prevalence has been reported amongst aquarium-kept fish which may vary from 10-15 % in some cases (Wolke & Stroud, 1978).

There has been a number of documented cases of humans contracting mycobacterial infections from fish, 2 cases of which are from South Africa (Mousdicas & Saxe, 1987; McGregor, 1976). Skin granulomas, associated with *M. marinum* aquired from fish tanks and swimming pools, have been reported.

This is the first report of the isolation of *M. fortuitum* from freshwater fish in South Africa.

MATERIALS AND METHODS

Fish specimens and symptoms

Two oscars (*Apistogramma ocellatus*), one from the Natinal Zoological Gardens of South Africa and the other from a hobbyist were submitted from Pretoria. Both fish had skin lesions on the head which varied in size from 5-15 mm in diameter. There were no internal lesions in these fish and no water samples were submitted.

Two guppies (*Lebistes reticulatus*) were submitted from a commercial fish farm in Natal. These fish were emaciated, had severe fin destruction, swam with a jerky motion and crowded into the corners of the pools. Heavy mortalities were reported in these fish. A hobbyist from the Eastern Transvaal submitted a guppy which swam in circles and showed nervous symptoms. A hobbyist from Johannesburg submitted an emaciated discus fish (*Symphysodon aequifasciatus*) which had recently been bought from a pet shop. This fish showed severe fin lesions.

Acid-fast staining

Smears were made from the head lesions of the oscars and from homogenized material of the guppies and discus. The smears were stained by the Ziehl-Neelsen method and examined microscopically for the presence of acid-fast rods.

Isolation

Methods for isolation and identification of the bacterium and the methods for drug susceptibility testing were those described by Nel, Kleeberg & Gatner (1980).

Lesions from the oscar that yielded acid-fast rods were excised and homogenized. The whole body of the guppies and discus were homogenized. The homogenized tissue was divided into 2 parts, 1 part being decontaminated with 2 % HCl, while the other was decontaminated with 4 % NaOH. The samples were left at room temperature for 15 min, then centrifuged for 10 min at 1650 × g. The supernatant fluid was discarded, the sediments washed by centrifugation and inoculated onto each of two tubes of Löwenstein-Jensen (LJ) egg medium with glycerine, LJ medium without glycerine, LJ medium with 0,5 % pyruvate and Herold's egg yolk medium containing mycobactin (Nel *et al.*, 1980). The tubes were incubated at 27 °C and 37 °C and observed for growth of acid-fast colonies at weekly intervals for 4 weeks.

Identification

As soon as colonies of acid-fast bacteria were observed, the isolates were subcultured onto the same

medium which supported the growth of the isolates. The isolates were then identified by biochemical tests and characterization of biological properties (Nel *et al.*, 1980).

Drug susceptibility testing

Drug susceptibility tests were done according to Nel *et al.*, (1980) on LJ medium using standardised inocula and concentrations of antimicrobials. The antimicrobials used were thiophene-2-carboxylic-acid-hydrazide (1,0 µg/ml), isoniazid (0,2 µg/ml and 1,0 µg/ml), streptomycin (5,0 µg/ml), rifampicin (30,0 µg/ml), p-aminosalicylate (1,0 µg/ml), thiacetazone (1,0 µg/ml), ethionamide (20,0 µg/ml) and ethambutol (2,0 µg/ml).

RESULTS

Smears made from the head lesions of the oscars revealed acid-fast rods. No acid fast rods were seen in the smears from the guppy and discus material.

Colonies of acid-fast bacteria were found on all samples between 1 and 2 weeks after inoculation. Colonies were a cream colour, smooth, non-chromogenic and rapid-growing. Better growth was obtained from specimens decontaminated with HCl than from those decontaminated with NaOH. Biochemical results are listed in Table 1. From the results in Table 1 the organisms were identified as *Mycobacterium fortuitum* according to Wayne & Doubek, 1968.

TABLE 1 Cultural and biochemical characteristics of the acid-fast bacteria isolated from three species of fish in South Africa

Tests	All strains isolated
Growth: 27°C	R
rate : 37°C	R
: 45°C	R
Pigmentation	-
Niacin (nicotinamide) production	-
Nitrate reduction	+
Thiophene -2-carboxylic-acid-hydrazide	Re
NaCl (5%) tolerance	+
Arylsulfatase production at 3 days	+
Tween hydrolysis	-
MacConkey agar (growth)	+

R = rapid (1-6 days)
 - = negative-absence
 + = positive
 Re = Resistant

All the isolates were found to be resistant to all the antimicrobials tested.

DISCUSSION

The fact that no acid-fast bacteria could be seen in the homogenates of the guppies and discus fish, while isolates were made from these specimens indicates the necessity for culturing from suspect cases. Extensive *post mortem* examinations were undertaken on samples of guppies and no parasitic infestations were seen. Attempts to isolate bacteria from these fish on blood tryptose agar (BTA) plates were unsuccessful. Investigations of the symptoms reported by the producers, i.e. emaciation, crowding in the corners of the tanks and a jerky, erratic swimming motion, revealed similarities to symptoms of

fish mycobacteriosis described by Amlacher (1961). For this reason samples were subjected to the Ziehl-Neelsen staining technique and utilized for mycobacterial cultures.

This outbreak of mycobacteriosis on the guppy farm resulted in high mortalities. The resistance of *M. fortuitum* to all the antimicrobials tested made the treatment of the infection on the farm difficult and it was recommended that the producer destroy all infected stock and disinfect the site before restocking.

The isolation of *M. fortuitum* from this guppy farm is significant because it is the first record of isolation of this organism from a commercial fish farm in South Africa.

The isolation of this organism from various freshwater species of ornamental fish in South Africa is significant in the light of the possibility of humans contracting skin granulomas from handling *M. fortuitum* infected fish or from the water of aquaria housing infected fish.

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