Occurrence of non-tuberculous *Mycobacteria* in Guppy (*Poecilia reticulata*) collected from ornamental fish farms in the Western, North Western, and Central Provinces of Sri Lanka

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Introduction and objectives: Mycobacteriosis caused by non-tuberculous mycobacteria (NTM) is among the most common chronic bacterial diseases affecting fish. NTM have increasingly been reported from tropical freshwater aquarium/ornamental fish. NTM associated with ornamental fish poses a significant zoonotic concern. This study was conducted with the aim of examining the occurrence of mycobacteria in apparently healthy guppy (*Poecilia reticulata*) cultured in fish breeding farms in the Central (CP), North Western (NWP) and Western provinces (WP) of Sri Lanka.

Methods: Ten randomly selected ornamental fish farms from each province were visited. A sample of fifteen guppies was collected from each farm. When different strains and life stages (broodstock, grow-out) of guppies were available, samples were collected from each different strain and life stage. Fish were transported to the laboratory, euthanised humanely, and subjected to necropsy. To isolate mycobacteria, kidney, liver, and spleen tissues from guppies from each sample were pooled, decontaminated and cultured on Ogawa egg medium, and incubated for two months at room temperature. Colonies suggestive of mycobacteria were subjected to Ziehl Neelsen stain. Each acid-fast isolate was confirmed as mycobacteria by PCR amplification of *Mycobacterium* genus-specific primers targeting the *rpoB* gene.

Results: A total of 63 guppy samples (4 broodstock, 59 grow-out adults) were collected from 30 farms. No significant gross pathological changes were observed during the necropsy. Of 63 pooled tissue samples cultured, 17 (27%) samples (1 broodstock, 16 grow-out) collected from 12 farms (40%) yielded mycobacteria [12 samples from WP (70.6%); 5 samples from CP (29.4%)]. Mycobacterial isolates were identified based on colony morphology, acid-fast microscopy, and PCR. All isolates were slow growing. The 12 farms from which mycobacteria were identified included 7 farms from the WP and 5 farms from the CP. Mycobacteria were not isolated from any of the sampled farms in the NWP.

Conclusions: The occurrence of NTM in guppy farms may be due to asymptomatic infection or mere colonisation as culture positive fish did not show clinical signs or pathological changes suggestive of mycobacteriosis. However, apparently healthy guppies bearing mycobacteria may serve as a possible source of infection for people via direct contact with these fish through occupational exposure or as a hobby.

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