Original Article

Isolation of potential zoonotic *Mycobacterium* spp. from diseased freshwater angelfish (*Pterophyllum scalare*) from an aquarium

Isolamento de *Mycobacterium* spp. potencialmente zoonótica de peixes anjo de água doce (*Pterophyllum scalare*) doentes de um aquário

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Abstract

Nontuberculous mycobacteria infection is one of the most common chronic bacterial diseases in ornamental aquarium fish and appears to be directly related to stressful husbandry practices. Furthermore, it also represents zoonotic potential. Here we present the isolation and characterization of non-tuberculous mycobacteria from diseased freshwater angelfish (*Pterophyllum scalare*) in São Paulo, Brazil. Nine discarded breeding females with signs of disease were evaluated. The fish exhibited lethargy, loss of appetite, cachexia, skin ulcers, and exophthalmia. At necropsy, four fishes presented macroscopic granulomas in the spleen. *Mycobacterium chelonae, M. fortuitum*, *M. gordonae*, *M. intracellulare* and *M. peregrinum* were isolated and identified by *hsp65* PCR restriction analysis. Histopathological analysis revealed microscopic lesions compatible with mycobacteriosis, and *Mycobacterium* bacillus were observed by Ziehl-Neelsen stain. Notably, all *Mycobacterium* species identified in this study have already been reported in human patients; therefore, diseased animals may be a source of infection for people who handle fish and aquariums.

Keywords: *Mycobacterium*, freshwater angelfish, granuloma, PCR.

Resumo

A infecção por micobactérias não tuberculosas é uma das doenças bacterianas crônicas mais comuns em peixes ornamentais de aquário e parece estar diretamente relacionada a práticas de manejo estressantes. Além disso, também representa potencial zoonótico. Aqui, apresentamos o isolamento e a caracterização de micobactérias não tuberculosas de peixes-anjo de água doce doentes (*Pterophyllum scalare*) em São Paulo, Brasil. Foram avaliadas nove fêmeas reprodutoras descartadas com sinais de doença. Os peixes exibiam letargia, perda de apetite, caquexia, úlceras cutâneas e exoftalmia. Na necropsia, quatro peixes apresentaram granulomas macroscópicos no baço. *Mycobacterium chelonae*, *M. fortuitum*, *M. gordonae*, *M. intracellulare* e *M. peregrinum* foram isolados e identificados por análise de restrição por PCR de *hsp65*. A análise histopatológica revelou lesões microscópicas compatíveis com micobacteriose, e o bacilo de *Mycobacterium* foi observado pela coloração de Ziehl-Neelsen. Notavelmente, todas as espécies de *Mycobacterium* identificadas neste estudo já foram relatadas em pacientes humanos; portanto, animais doentes podem ser uma fonte de infecção para pessoas que manipulam peixes e aquários.

Palavras-chave: *Mycobacterium*, peixe-anjo de água doce, granuloma, PCR.

1. Introduction

Breeding freshwater ornamental fish is a relatively common practice worldwide. However, improper management by fish farmers can create a series of situations including loss of water quality, which lead to stress that affects the environment and the animals. Stress has inhibitory effects on the immune system of animals, making them susceptible to numerous opportunistic pathogens (Essa et al., 2009). Most fish pathogens share the environment with the animals. However, they pose a risk to the fish when present in significant amounts and especially under stressful situations.

Mycobacteriosis is caused by nonturberculous mycobacteria (NTM), which are ubiquitous in the natural environment worldwide and are persistent residents of fish microbiota (Kušar et al., 2017). The main clinical signs of mycobacteriosis in aquarium fish are poor growth,

emaciation, retarded sexual maturation, or decreased reproductive performance. Other lesions can include skeletal deformities and chronic nonhealing shallow to deep ulcers or fin erosion. White nodules may be present on the viscera, especially on the hypertrophic kidney or spleen (Noga, 2010). The diagnosis of mycobacterial disease in aquarium fish is based on bacterial culture, histopathological, and molecular methods.

M. chelonae, M. fortuitum, *M. gordonae*, and *M. peregrinum* have been isolated from several aquarium fish worldwide (Najiah et al., 2011; Novotny et al., 2010; Pate et al., 2005; Sakai et al., 2005; Shukla et al., 2013). In humans, *M. chelonae, M. fortuitum*, *M. gordonae,* and *M. intracellulare* have also been isolated from skin lesions (Mei et al., 2019), patients undergoing hemodialysis (Morgans et al., 2019), chronic abscess due to anabolic application (Silva Neto et al., 2019), cardiac transplant patients (Tebas et al., 1995), and skeletal muscle infection (Napaumpaiporn and Katchamart, 2019), among others (Tortoli, 2009).

Angelfish *Pterophyllum scalare* (Schultze, 1823) is a common fish bred in captivity and traded in aquarium stores in Brazil. Due to the zoonotic potential of fish mycobacteriosis and the increasing reports of opportunistic NTM infections around the world, the aim of this study was to isolate and identify the species of non-tuberculous mycobacteria from diseased freshwater angelfish (*P. scalare*) from a breeder in São Paulo, Brazil.

2. Materials and Methods

2.1. Animals

A total of nine discarded breeding females of freshwater angelfish (*P. scalare*) with signs of disease, obtained from a fish breeder, were used in the study. The fish were maintained in pairs (female and male) in individual aquariums of 0.1 m³ (with individual filtration), fed twice a day *ad libitum* with mixed commercial diet (Tetra® Min flakes and Color bits granules), and without rest between one spawning and another. Water quality parameters were daily measured and maintained at: temperature 28-30°C, dissolved oxygen 5 mg L^{-1}, pH 6.8-7.2, total ammonia <0.1 mgL^{-1} and nitrite < 0.05 mgL^{-1}. The females presenting reduced reproduction rate or clinical signs of disease were separated from the pair and submitted to the Department of Preventive Veterinary Medicine and Animal Health, School of Veterinary Medicine and Animal Science, University of São Paulo, for diagnostic purposes in May 2019.

Fish were sedated with a solution of Eugenol at 75 mg/L (Roubach et al., 2005) - for 2 to 6 min and then euthanized by the spinal cord section technique (Noga, 2010). They were then sanitized with 70% alcohol to remove possible environmental contaminants and necropsied for sample collection. This study was approved by the institutional Ethics Committee (approval number CEUA Nº 6289090519).

2.2. Histopathology

The gill, gut, kidney, liver, skin, stomach, and spleen of three fish were fixed in buffered 10% formalin solution for histopathological analysis. Fragments of lesions were carefully removed, embedded in paraffin, cut into posterior cross sections of 5 µm, and stained with hematoxylin-eosin to observe microscopic lesions and Ziehl Neelsen for *Mycobacterium* bacillus visualization. The slides were examined, and photomicrographs were taken using a DM 5000 B microscope equipped with a differential interference contrast (DIC) system and analyzed in a computerized system (Qwin Lite 3.1, Leica Microsystems).

2.3. Isolation and identification of Mycobacteria

From the nine studied animals, seven fish were too small to separate each structure; therefore, samples of macerated internal organs were prepared. One fish had a splenic granuloma, and another had a splenic granuloma and a skin lesion. Spleen granulomas, livers, and kidneys/gonads were sampled from eight of the fish, and from the nineth the liver, kidneys, splenic granuloma, and skin lesion tissues were analyzed.

For mycobacteria isolation, all samples were decontaminated with 0.75% cetylpyridinium chloride (Ikuta et al., 2016). Thereafter samples were inoculated in duplicate into Stonebrink and Löwenstein-Jensen media and incubated at 25 °C and 37 °C for up to 60 days (CPZ, 1995). Obtained isolates were classified phenotypically according to the colony pigmentation and time required for colonies to be clearly visible to the naked eye (rapid growing < 7 days; slow growing > 7 days) (Kim et al., 2013).

The acid-fast bacilli isolated were selected by phenotypical characteristics in each culture flask and were subjected to DNA extraction as previously described by Boom et al. (1990), with initial lysozyme (100 mg/mL) and proteinase K (20 mg/mL) (US Biological, Swampscott, MA, USA) digestion at 37 ºC for 60 minutes. For molecular identification, PCR restriction analysis (PRA) of the *hsp65* gene was performed according to Telenti et al. (1993) using *BstEII* (Promega) *and HaeIII* (Thermo Scientific) enzymes. Briefly, *hsp65* gene amplification was performed using 20 pmoles of each primer Tb11 and Tb12 (Telenti et al., 1993), 1.5 mM MgCl$_2$, 200 mM of dNTP, 1 U of Taq DNA polymerase (Fermentas Inc., Glen Burnie, MD, USA), 1 X PCR buffer and ultra-pure water. The amplification consisted of 95°C for 15 min followed by 35 cycles of 1 min at 94°C, 58ºC for 1 min, 72ºC for 1 min, and 10 min at 72ºC for final extension. From the amplified product, 10 µL were used for each enzyme digestion in which *BstE*II was incubated at 60ºC for 1 h and *Hae*III at 37ºC for 1.5 h, according to the manufacturer's instructions. Electrophoresis in agarose gel (3.5%), stained with SYBR Safe (Invitrogen®), was performed at 90V for 4 h using the 50 bp DNA ladder (New EnglandBioLabs Inc) as molecular weight marker. The obtained restriction patterns were further analyzed within the PRAsite database (http://app.chuv.ch/prasite/index.html) for species identification.

3. Results

The evaluated fish exhibited clinical signs of lethargy, loss of appetite, cachexia, skin ulcers (Figure 1a-1b), and exophthalmia (Figure 1c). At necropsy, four fishes presented macroscopic granulomas in the spleen (Figure 1d-1f).

Histopathological analysis showed as main lesions the inflammatory process by lymphocytes and macrophages in the brain, gills, gut, liver, muscle, and spleen; degeneration in the brain, gut, liver, muscle, and spleen; eosinophils in the gills and gut; hyperplasia in the gills, gut, and spleen;
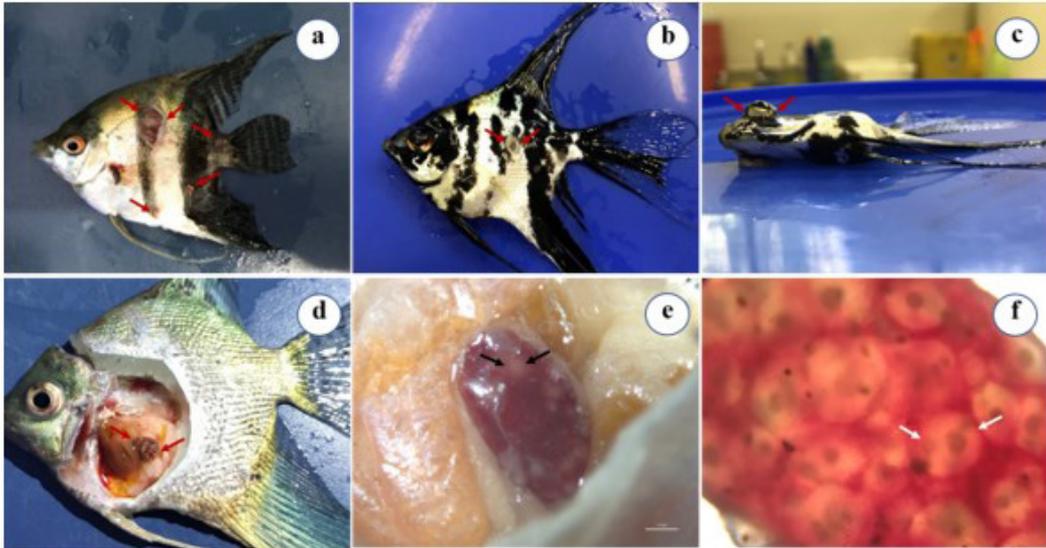
Figure 1. Freshwater angelfish (*Pterophyllum scalare*) with skin ulcers (a, b); exophthalmia (c); macroscopic granulomas of the spleen (d); and granulomas found on an internal wet mount exam of a fish with mycobacteriosis (e). Note the dark brown center with surrounding lighter capsule visible on a wet mount spleen biopsy [4x magnification] (f).
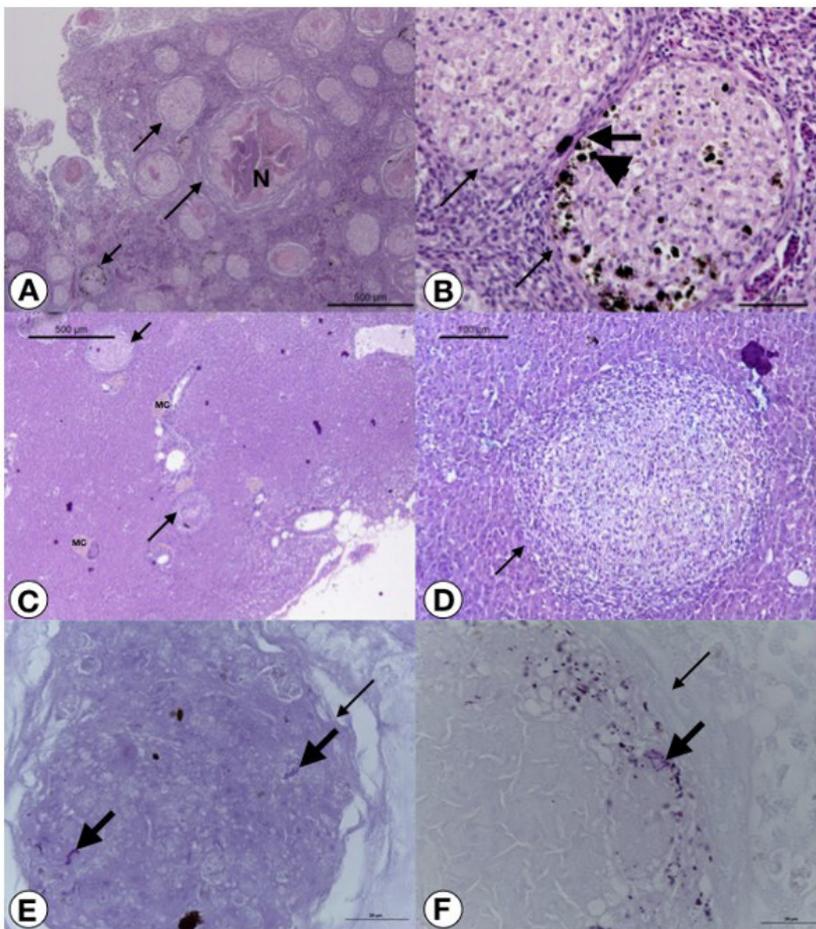
Figure 2. Microscopic lesions in the spleen and liver were stained with hematoxylin-eosin and Ziehl-Neelsen. Presence of numerous fibrous granulomas (thin arrow) in the spleen (A, B, E, F) and liver (C, D) containing a necrotic center (N) in the spleen (A), melanomacrophages (arrow head) in the spleen (B), bacteria (large arrow) in the spleen (B), and melanomacrophages-center (MC) in the liver (C). In the Ziehl-Neelsen stain, acid-fast bacilli (large arrow) were observed in the spleen (E, F).

bacterial colonies in the gills, liver, muscle and spleen; and numerous granulomas in the liver and spleen (Figure 2). In the Ziehl-Neelsen stain, acid-fast bacilli were observed in the spleen.

Mycobacterium spp. was isolated from all examined fish, and the species *M. chelonae, M. fortuitum*, *M. gordonae, M. intracellulare,* and *M. peregrinum* were confirmed by molecular identification. Among three fish (animals 1, 2, and 5), co-infection by more than one species was observed, including *M. chelonae*, *M. fortuitum,* and *M. peregrinum* (Table 1).

4. Discussion

Mycobacteriosis caused by NTM appears to be causally related to stressful husbandry practices. The females of freshwater angelfish (*P. scalare*) in the present study were subjected to high chronic reproduction stress by the breeder, who did not allow time for rest between spawning. As time passed, the reproduction rate of the females decreased, and when the first clinical signs of the disease appeared, the owner discarded the females from the system and replaced them with apparently healthy females. The constant high pressure of stress during reproduction may have induced the females to be more predisposed to infection by environmental *Mycobacterium* spp., leading to the appearance of clinical signs and granulomas in the internal organs.

Due the different infection profiles identified, it was not possible to correlate the presence of one *Mycobacterium* species and lesions observed at studied animals. Nevertheless, the clinical description corroborates previous studies in fishes worldwide (Kušar et al., 2017; Novotny et al., 2010; Shukla et al., 2013; Zanoni et al., 2008). In a previous study in Slovenia, Pate et al. (2005) found *M. chelonae, M. fortuitum*, *M. gordonae,* and *M. peregrinum* in Goldfish (*Carassius auratus*), Guppy (*Poecilia reticulata*), freshwater angelfish (*P. scalare*), and three-spot gourami (*Tricogaster trichopterus*). In addition to these species, we also detected *M. intracellulare* in fish with spleen granulomatosis. Our histopathological findings corroborate those of Lescenko et al. (2003) who observed that more than 60% of granulomatous lesions in infected aquarium fish, from the Czech Republic, were caused by

Table 1. Identified *Mycobacterium* species according to analyzed animals and respective samples.

Animal	Samples	*Mycobacterium* species	Incubation temperature	*Mycobacterium* colonies Phenotype	*Mycobacterium* colonies Genotype	
					*Bst*EII pattern	*Hae*III pattern
1	Internal organs (macerated)	*M. chelonae* type 1	25ºC	Rapidly growing non-pigmented	320.130	200.60.55
		M. fortuitum type 1	37 ºC	Rapidly growing non-pigmented	235.120.85	145.120.60.55
2	Internal organs (macerated)	*M. fortuitum* type 1	25 ºC	Rapidly growing non-pigmented	235.120.85	145.120.60.55
		M. peregrinum type 1	37 ºC	Rapidly growing non-pigmented	235.210	145.140.100.50
3	Internal organs (macerated)	*M. peregrinum* type 1	37 ºC	Rapidly growing non-pigmented	235.210	145.140.100.50
4	Internal organs (macerated)	*M. gordonae* type 10	25 ºC	Slowly growing scotochromogen	235.120.100	130.110.90
5	Internal organs (macerated)	*M. chelonae* type 1	25 ºC	Rapidly growing non-pigmented	320.130	200.60.55
		M. peregrinum type 1	37 ºC	Rapidly growing non-pigmented	235.210	145.140.100.50
6	Internal organs (macerated)/ splenic granuloma	*M. chelonae* type 1	25 ºC	Rapidly growing non-pigmented	320.130	200.60.55
7	Internal organs (macerated)/ splenic granuloma and skin lesion	*M. fortuitum* type 1	37 ºC	Rapidly growing non-pigmented	235.120.85	145.120.60.55
		M. fortuitum type 2		Rapidly growing non-pigmented	235.120.85	140.120.60.55
8	Spleen/splenic granuloma Liver Kidney/gonads	*M. intracellulare* type 2	25 ºC	Slowly growing non-pigmented	235.210	140.110.80
9	Kidney/splenic granuloma and skin lesion Liver/splenic granuloma and skin lesion Skin/splenic granuloma and skin lesion	*M. intracellulare* type 2	25 ºC	Slowly growing non-pigmented	235.210	140.110.80

Mycobacterium. In addition, similar to these authors, we also noted a low quantity of this bacterium in tissues with granulomas stained with Ziehl-Neelsen. This could be due to granulomatous tissue changes through direct microscopy, which could have led to the destruction of the mycobacteria (Zanoni et al., 2008). Rallis and Koumantaki-Mathioudaki (2007) in histopathological examinations, showed that fish infected by *Mycobacterium* had nonspecific inflammatory infiltration of epithelioid cells, lymphocytes, and Langhan's giant cells without caseation, which was also observed in this study.

The NTM infection also represents a public health risk due to its zoonotic potential. Contact of injured areas with contaminated environments may be responsible for infection in humans and animals (Tortoli, 2009). Therefore, it is common for people to become infected while working with sick fish or cleaning aquariums (Yacisin et al., 2017). Despite the fact that NTM is relatively avirulent in healthy hosts, all species identified in the present study have already been reported in human patients (Silva Neto et al., 2019; Napaumpaiporn and Katchamart, 2019; Dodiuk-Gad et al., 2007; Gharbi et al., 2019; Ko et al., 2017). Considering that these mycobacteria are part of the environment as well as of the fish microbiota (Cowman et al., 2018; Kušar et al., 2017).

5. Conclusion

All studied angelfish with chronic reproduction stress were diagnosed with spleen granulomatosis caused by one or more *Mycobacterium* species. The detected *M. chelonae, M. fortuitum*, *M. gordonae*, *M. intracellulare* and *M. peregrinum* species present zoonotic potential, according to the literature, and therefore sick animals may be a potential source of infection for humans who manipulate and /or work with fish and aquariums.

Acknowledgement

References

BOOM, R., SOL, C.J.A., SALIMANS, M.M.M., JANSEN, C.L., WERTHEIM-VAN DILLEN, P.M.E. and VAN DER NOORDAA, J., 1990. Rapid and simple method for purification of nucleic acids. *Journal of Clinical Microbiology*, vol. 28, no. 3, pp. 495-503. http://dx.doi.org/10.1128/jcm.28.3.495-503.1990. PMid:1691208.

CENTRO PANAMERICANO DE ZOONOSIS – CPZ, 1995. *Tuberculosis. Bacteriologia de la tuberculosis: el cultivo del Mycobacterium tuberculosis*. Buenos Aires: CPZ. Nota técnica no. 27.

COWMAN, S.A., JAMES, P., WILSON, R., COOKSON, W.O.C., MOFFATT, M.F. and LOEBINGER, M.R., 2018. Profiling mycobacterial communities in pulmonary nontuberculous mycobacterial

disease. *PLoS One*, vol. 13, no. 12, p. e0208018. http://dx.doi.org/10.1371/journal.pone.0208018. PMid:30533049.

DODIUK-GAD, R., DYACHENKO, P., ZIV, M., SHANI-ADIR, A., OREN, Y., MENDELOVICI, S. SHAFER, J., CHAZAN, B., RAZ, R., KENESS, Y. and ROZENMAN, D., 2007. Nontuberculous mycobacterial infections of the skin: a retrospective study of 25 cases. *Journal of the American Academy of Dermatology*, vol. 57, no. 3, pp. 413-420. http://dx.doi.org/10.1016/j.jaad.2007.01.042. PMid:17368631.

ESSA, M.A.A., KENAWY, A.M. and EL-GAWAD, D.M.A., 2009. Epizootiological and histopathological studies on Mycobacteriosis in some ornamental fishes. *Global Veterinaria*, vol. 3, no. 2, pp. 137-143.

GHARBI, R., MHENNI, B., FRAJ, S.B. and MARDASSI, H., 2019. Nontuberculous mycobacteria isolated from specimens of pulmonary tuberculosis suspects, Northern Tunisia: 2002–2016. *BMC Infectious Diseases*, vol. 19, no. 1, p. 819. http://dx.doi.org/10.1186/s12879-019-4441-1. PMid:31533664.

IKUTA, C.Y., MORATO, F., SOUZA, G.O., HEINEMANN, M.B., AMAKU, M. and FERREIRA NETO, J.S., 2016. Influence of the incubation conditions on culture media to optimize primary isolation of *Mycobacterium bovis. Semina: Ciências Agrárias*, vol. 37, no. 5, suppl. 2, pp. 3693-3700. http://dx.doi.org/10.5433/1679-0359.2016v37n5Supl2p3693.

KIM, C.-J., KIM, N.-H., SONG, K.-H., CHOE, P.-G., KIM, E.-S., PARK, S.-W., KIM, H.-B., KIM, N.-J., KIM, E.-C., PARK, W.-B. and OH, M.-D., 2013. Differentiating rapid- and slow-growing mycobacteria by difference in time to growth detection in liquid media. *Diagnostic Microbiology and Infectious Disease*, vol. 75, no. 1, pp. 73-76. http://dx.doi.org/10.1016/j.diagmicrobio.2012.09.019. PMid:23114094.

KO, J.S., KIM, S.K., YONG, D.E., KIM, T.-I. and KIM, E.K., 2017. Delayed onset *Mycobacterium intracellulare* keratitis after laser in situ keratomileusis. *Medicine*, vol. 96, no. 51, p. e9356. http://dx.doi.org/10.1097/MD.0000000000009356. PMid:29390522.

KUŠAR, D., ZAJC, U., JENIČ, V., OCEPEK, M., HIGGINS, J., ŽOLNIR-DOVČ, M. and PATE, M., 2017. *Mycobacteria* in aquarium fish: results of a 3-year survey indicate caution required in handling pet-shop fish. *Journal of Fish Diseases*, vol. 40, no. 6, pp. 773-784. http://dx.doi.org/10.1111/jfd.12558. PMid:27747884.

LESCENKO, P., MATLOVA, L., DVORSK, L., BARTOS, M., VAVRA, O., NAVRATIL, S., NOVOTNY, L. and PAVLINK, I., 2003. Mycobacterial infection in aquarium fish. *Journal of Veterinary Medicine*, vol. 48, no. 3, pp. 71-78.

MEI, Y., ZHANG, W., SHI, Y., JIANG, H., CHEN, Z., CHOKKAKULA, S. LONG, S., PAN, C. and WANG, H., 2019. Cutaneous tuberculosis and nontuberculous mycobacterial infections at a national specialized hospital in China. *Acta Dermato-Venereologica*, vol. 99, no. 11, pp. 997-1003. http://dx.doi.org/10.2340/00015555-3283. PMid:31396633.

MORGANS, H.A., MALLETT, K.F., VANSICKLE, J.F.S. and WARADY, B.A., 2019. *Mycobacterium fortuitum* infection of a hemodialysis catheter in a pediatric patient. *Hemodialysis International*, vol. 23, no. 3, pp. E93-E96. http://dx.doi.org/10.1111/hdi.12731. PMid:30746835.

NAJIAH, M., LEE, K.L., NOORASIKIN, H., NADIRAH, M. and LEE, S.W., 2011. Phenotypic and genotypic characteristics of *Mycobacterium* isolates from fighting fish *Betta* spp. in Malaysia. *Research in Veterinary Science*, vol. 91, no. 3, pp. 342-345. http://dx.doi.org/10.1016/j.rvsc.2010.09.010. PMid:20971487.

NAPAUMPAIPORN, C. and KATCHAMART, W., 2019. Clinical manifestations and outcomes of musculoskeletal nontuberculous mycobacterial infections. *Rheumatology International*, vol. 39,

no. 10, pp. 1783-1787. http://dx.doi.org/10.1007/s00296-019-04392-8. PMid:31352560.

NOGA, E.J., 2010. *Fish disease: diagnosis and treatment.* Ames: Blackwell Publishing, 519 p. http://dx.doi.org/10.1002/9781118786758.

NOVOTNY, L., HALOUZKA, R., MATLOVA, L., VAVRA, O., BARTOSOVA, L., SLANY, M. and PAVLIK, I., 2010. Morphology and distribution of granulomatous inflammation in freshwater ornamental fish infected with mycobacteria. *Journal of Fish Diseases*, vol. 33, no. 12, pp. 947-955. http://dx.doi.org/10.1111/j.1365-2761.2010.01202.x. PMid:21091722.

PATE, M., JENČIČ, V., ŽOLNIR-DOVČ, M. and OCEPEK, M., 2005. Detection of mycobacteria in aquarium fish in Slovenia by culture and molecular methods. *Diseases of Aquatic Organisms*, vol. 64, no. 1, pp. 29-35. http://dx.doi.org/10.3354/dao064029. PMid:15900685.

RALLIS, E. and KOUMANTAKI-MATHIOUDAKI, E., 2007. Treatment of *Mycobacterium marinum* cutaneous infections. *Expert Opinion on Pharmacotherapy*, vol. 8, no. 17, pp. 2965-2978. http://dx.doi.org/10.1517/14656566.8.17.2965. PMid:18001256.

ROUBACH, R., GOMES, L.C., FONSECA, F.A.L. and VAL, A.L., 2005. Eugenol as an efficacious anaesthetic for tambaqui, *Colossoma macropomum* (Cuvier). *Aquaculture Research*, vol. 36, no. 11, pp. 1056-1061. http://dx.doi.org/10.1111/j.1365-2109.2005.01319.x.

SAKAI, M., KONO, T., TASSAKKA, A.C.M.A.R., PONPORNPISIT, A., AREECHON, N., KATAGIRI, T. and ENDO, M., 2005. Characterization of a *Mycobacterium* sp. isolated from guppy *Poecilia reticulata*, using 16S ribosomal RNA and its internal transcribed spacer sequences. *Bulletin of the European Association of Fish Pathologists*, vol. 25, no. 2, pp. 64-69.

SHUKLA, S., SHARMA, R. and SHUKLA, S.K., 2013. Detection and identification of globally distributed mycobacterial fish pathogens in some ornamental fish in India. *Folia Microbiologica*, vol. 58, no. 5, pp. 429-436. http://dx.doi.org/10.1007/s12223-013-0225-y. PMid:23358914.

SILVA NETO, J.P., MACHADO, K.N.C. and ROISMAN, L., 2019. Choroidal granuloma caused by *Mycobacterium fortuitum. International Journal of Retina and Vitreous*, vol. 5, p. 37. http://dx.doi.org/10.1186/s40942-019-0185-8. PMid:31636998.

TEBAS, P., SULTAN, F., WALLACE JUNIOR, R.J. and FRASER, V., 1995. Rapid development of resistance to clarithromycin following monotherapy for disseminated *Mycobacterium chelonae* infection in a heart transplant patient. *Clinical Infectious Diseases*, vol. 20, no. 2, pp. 443-444. http://dx.doi.org/10.1093/clinids/20.2.443. PMid:7742453.

TELENTI, A., MARCHESI, F., BALZ, M., BALLY, F., BOTTGER, E.C. and BODMER, T., 1993. Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis. *Journal of Clinical Microbiology*, vol. 31, no. 2, pp. 175-178. http://dx.doi.org/10.1128/jcm.31.2.175-178.1993. PMid:8381805.

TORTOLI, E., 2009. Clinical manifestations of nontuberculous mycobacteria infections. *Clinical Microbiology and Infection*, vol. 15, no. 10, pp. 906-910. http://dx.doi.org/10.1111/j.1469-0691.2009.03014.x. PMid:19845702.

YACISIN, K., HSIEH, J.L., WEISS, D., ACKELSBERG, J., LEE, E., JONES, L. LEUNG, Y.L., LI, L., YUNG, J., SLAVINSKI, S., HANSON, H., RIDPATH, A., KORNBLUM, J., LIN, Y., ROBBE-AUSTERMAN, S., RAKEMAN, J., SIEMETZKI-KAPOOR, U., STUBER, T. and GREENE, S.K., 2017. Outbreak of non-tuberculous mycobacteria skin or soft tissue infections associated with handling fish – New York City, 2013–2014. *Epidemiology and Infection*, vol. 145, no. 11, pp. 2269-2279. http://dx.doi.org/10.1017/S0950268817001066. PMid:28629484.

ZANONI, R.G., FLORIO, D., FIORAVANTI, M.L., ROSSI, M. and PREARO, M., 2008. Occurrence of *Mycobacterium* spp. in ornamental fish in Italy. *Journal of Fish Diseases*, vol. 31, no. 6, pp. 433-441. http://dx.doi.org/10.1111/j.1365-2761.2008.00924.x. PMid:18471099.