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Importation of mycobacteriosis with ornamental fish: Medico-legal implications

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Summary
Mycobacterium fortuitum, as well as *Mycobacterium marinum* and *Mycobacterium chelonae*, are the etiological agents of fish Mycobacterioses. Mycobacteriosis has been reported to affect a wide range of freshwater and marine fish species, suggesting an ubiquitous distribution, and can cause zoonotic infections (known as ''fish tank granuloma'' or ''swimming pool granuloma'') in humans exposed to fish and contaminated water. Infection in human consists of nodular cutaneous lesions that can progress to tenosynovitis, arthritis, and osteomyelitis, depending on the immunological status. Authors describe some cases observed during routinary diagnostic activity in aquarium fish. Fish were sampled and histopathological, microbiological, and biomolecular exams were carried out. Histopathology showed systemic granulomatosis. Microbiological and biomolecular exams allowed us to identify the *M. fortuitum* as a main species. Finally, some considerations on the legal aspects of such disease are discussed.

Introduction

Fish farming is a fast-growing industry, where innovation and new outlets are being explored. In order to adapt production to market conditions, aquaculture has benefited economically from the introduction of alien species (e.g. rainbow trout, Pacific oyster) and from the farming of new species, which do not occur in an area owing to biogeographical barriers.

It should be noted that there is a significant trade in alien organisms, mainly fish, as ornamental species, but the keeping of these organisms in pet shops, garden centers and commercial and private aquaria is not covered by the Common Fisheries Policy.

Considering the potential role of these animal's movements in transmitting diseases/zoonoses from one region to another, it is necessary to limit the introduction of new species and to secure animal health, promoting the application of Code of Practice on the introduction and transfer of alien/exotic aquatic organism.

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Currently, many live ornamental fish are imported per week e.g. via airfreight from different countries. Imported animals carrying disease agents can come into European countries and be sold to the final consumer/owner, well before any disease would become apparent. To compound this problem, typical tropical fish wholesalers and retailers have no bio-security procedures.

The threat of disease introduction and establishment in native species as a result of the international trade in ornamental fish is well recognized, and there are disease agents in farmed ornamental fish, which are known to have a carrier state. But hazard identification should not be limited to known agents or diseases but should be inclusive of data where epidemiological evidence suggests the presence of a significant, but unidentified or unconfirmed, pathogen that can cause human infection, e.g. by contact with affected fish or contaminated water, i.e. Mycobacteriosis.

Mycobacterium fortuitum, as well as *Mycobacterium marinum* and *Mycobacterium chelonae*, are the mycobacterial species commonly associated with fish tuberculosis[1] and are considered a potential risk for human beings. Generally, the genus *Mycobacterium* causes diverse disease in humans (Table 1).

M. marinum and *M. fortuitum*, slowly growing bacteria that occur in bodies of fresh or saltwater in various parts of the world, cause Tuberculosis in fish and can cause infection in humans and other species after contact with contaminated water (e.g. from fish tanks)[2] or marine animals.

Nontuberculous mycobacterial infections of the skin

Nontuberculous mycobacteria (NTM) are slender, nonmotile, acid-fast bacilli that are present in a variety of environments worldwide. Fast- and slow-growing groups are distinguished; the latter subdivided according to pigment-forming properties in the culture. With the advent of the AIDS epidemic and the introduction of immunosuppressive therapies, the incidence of NTM-associated diseases has increased dramatically and NTM have been acknowledged as important pathogens.[3]

Six major clinical syndromes caused by NTM can be differentiated, including pulmonary infection, local non-tender lymphadenitis, skin and soft-tissue infections, disseminated infection, catheter-related infections, and chronic granulomatous infections of bursae, joints, tendon sheaths, and bones.

Almost all NTM species have been incriminated in cutaneous disease. The most common species in the United States and Europe are *M. marinum* and the rapidly growing mycobacteria *Mycobacterium abscessus*, *M. fortuitum*, and *M. chelonae*. *Mycobacterium ulcerans* is endemic in at least 32 countries in Africa, western Pacific, Asia, and South America.[3]

Disease in fish and human

M. marinum was first isolated in 1926 by Aronson[4] from saltwater fish carcasses in the Philadelphia aquarium. Baker and Hagan[5] discovered that the mycobacterium caused tuberculosis in freshwater platyfish and called it *Mycobacterium platypoecilus*. It was recognized as a human pathogen by Linell and Norden[6] who isolated it from skin lesions of swimmers from a swimming pool in Sweden. They called the bacterium *Mycobacterium balnei*; *M. platypoecilus* and *M. balnei* were subsequently found to be the same and are now called *M. marinum*.[7] The disease due to *M. marinum*, initially described as swimming pool granuloma,[8] is also called fish tank granuloma.[9] It can cause disease in a variety of species of fish[10] and zoonotic infections in humans exposed to fish and contaminated water[2] responsible for outbreaks of disease.

M. marinum causes the most common chronic bacterial disease in ornamental fish and it can affect both the temperate and tropical species in freshwater and marine environments.[10]

Fish tuberculosis is a systemic, chronic disease characterized by the presence of granulomatous reaction in visceral organs accompanied by continuing mortalities in the infected stock.[11–13]

In human, *M. marinum* is a well-known cause of cutaneous infection manifested by skin ulcers and nodular lymphangitis, that can progress to tenosynovitis, arthritis, and osteomyelitis.[14,15] These latter types of deep infection result from direct extension of the cutaneous infection and can be very resistant to treatment. Surgical debridement is usually required.[16]

Swimming pool granulomas most commonly appear as a solitary papulonodular lesion on an extremity over a bony prominence, which is prone to trauma. The papule gradually enlarges to form a nodule or plaque. Occasionally, the lesion may be pustular or even ulcerate.

Although infection may be caused by direct injury from the fish fins or bites, most are acquired during the handling of the aquariums such as cleaning or changing the water.[17,18] Indirect

Table 1 Main species of *Mycobacterium* related to the way of transmission, host and disease (by Stamm and Brown, 2004 modified).

Species	Source or mode of transmission	Host	Disease
M. marinum	Water	Fish	Fish tuberculosis
		Human	Fish tank/swimming pool granuloma
M. tuberculosis	Aerosol droplets	Humans	Tuberculosis
M. bovis	Aerosol droplets	Cattle	Cattle tuberculosis
	Milk from infected animals	Humans	Tuberculosis
M. kansasii	Soil and water	Humans	Tuberculosis
M. avium	Soil and water	Humans, swine	Tuberculosis in immunodepressed

infection has also been described due to a child's bath that was used to clean out a fish tank.[19] The incubation period is on the average 3 weeks, but may take up to 9 months.[18]

People who have breaks in the skin such as cuts and scrapes may be at risk when (1) in contact with water from an aquarium or fish tank, (2) handling, cleaning, or processing fish, and (3) while swimming or working in freshwater or saltwater bodies.

M. marinum infection that occurrs worldwide, may be an occupational hazard for certain professionals (for example, pet shop workers), but many infections occur in fish fanciers who keep an aquarium at home; hence, the name ''fish fanciers' finger syndrome''.[20]

For people with immune system deficit (i.e. HIV, cancer patients undergoing chemotherapy, etc.), *M. marinum* infection can become severe.

The correct diagnosis in human beings can be difficult for the clinician,[8] because the presentation is often insidious and nonspecific, key historical information may not be obtained and the diagnosis is therefore commonly delayed.[21,18] Clues in the clinical history include skin injuries associated with fish, aquariums, or more seldomly swimming pools.

Tissue biopsy for histology and culture is important to establish the diagnosis. Histological appearances vary and depend on the age of the lesion. Granulomas lend support to the diagnosis, but are not pathognomonic. Organisms are seldom seen in the histological sections.[17]

Differential diagnoses include other mycobacterial infections (both tuberculous and nontuberculous), sporotrichosis, deep fungal infections, leishmaniasis, tularemia, sarcoidosis, tumors, and foreign-body reactions.[16]

Aim of the study

On the basis of the above considerations and of some cases of Mycobacteriosis in imported ornamental fish observed during routinary diagnostic activity in aquarium fish in Sicily, we carried out some legal reflections.

Material and methods

Seven fish belonging to the species *Danio rerio* were infected by cohabitation with 27 fish, which was the object of our previous study (Fig. 1).[22] Spontaneously died fish were necropsized. Samples were sent for histopathology. Tissue samples were fixed in 10% buffered formalin solution, routinely processed and stained with E.E., PAS and Ziehl Neelsen. Two teleosts were sent to the IZS of Palermo for bacteriology. On bacterial isolates, biochemical tests were performed to identify them. DNA was extracted from tissue and water using the *Gene Elute kit* (Sigma Chemical). Two different PCR methods were carried out: the first was aimed at finding sequences referable to the genus *Mycobacterium*, while the second one was used to define specific sequences. In the first method, primers Int1 and Ext2 (5′-CCCCATCGACCTACTACG-3′; 5′-CCCGGACAGGCCGAGTTT-3′) were used. In the second method, generic primers for the ITS sequences common for all the eubacteria and, then, to mycobacteria were used. After confirmation by electrophoresis of PCR products, and their purification, we carried out sequencing by the genetic analyzer Applied Biosystems 3130.

Fig. 1 *Carassius auratus* Several whitish tubercles were spread in all coelomic organs.

The sequences obtained were analyzed using WU BLAST 2 to detect the most possible ones.

Results

All the examined subjects show externally a severe emaciation and ascitis. No changes were reported in tissues and organs of fish, probably due to the small size of the latter. Sections obtained allowed us to observe the classical features of a systemic granulomatosis involving all tissues. Ziehl Neelsen elective staining showed small reddish bacteria within necrotic foci in the inner part of granulomas (Figs. 2–5). Microbiological exam confirmed the presence of mycobacteria in the examined tissues; the different colonies isolated, already distinguishable morphologically in culture medium, when stained with Ziehl Neelsen, showed a sharp difference in microscopical morphology. Biomolecular exam confirmed the pathogen and indicated the presence of different species of the same bacterium in the tank. The main bacterial species was *M. fortuitum*. A first result of this study is the proposal of a new investigation method that, starting from the suspect of mycobacterial infection, leads to the identification of the species by ribotypization. One of the sequences found is the one showed in Table 2.

The high pathogenicity of the bacterial isolates was confirmed by the death of the fish, 3 weeks after the infection.

Considerations and conclusions

Particularly, in the absence of specific recommendations concerning the diseases in ornamental fish, the authors propose the following points:

(i) A list of third world countries or parts thereof, from which Member States are authorized to import live fish in the Community, should be established.
(ii) It is necessary to lay down specific animal health conditions and model certificates for those third countries, taking into account the animal health situation of the third country concerned and of the fish to be imported, in order to prevent the introduction of disease agents that could cause significant impact to

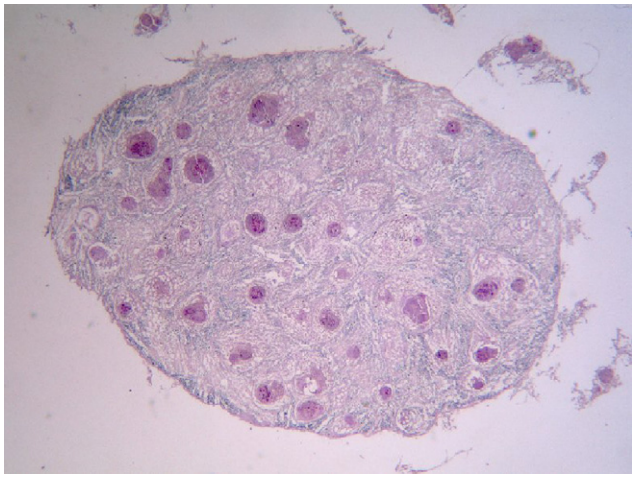

Fig. 2 *Danio rerio* Histological section of the spleen showing differently sized granulomas (H&E 2,5x).

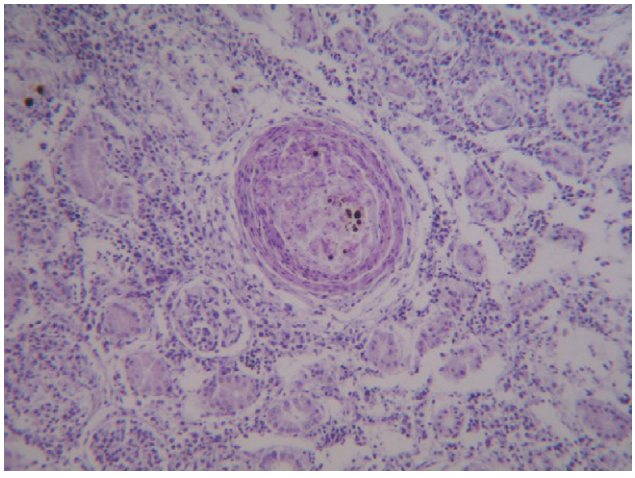

Fig. 3 *Danio rerio* Kidney section with a well differentiate granuloma (H&E 10x).

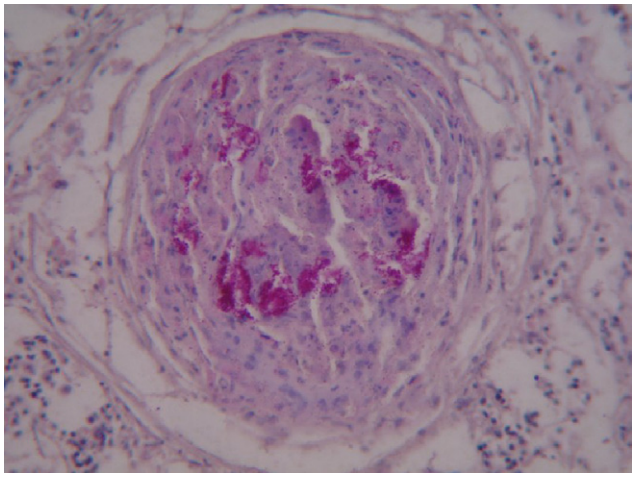

Fig. 4 *Danio rerio* Section obtained from liver tissue showing reddish bacteria within the necrotic centres of the granulomas (Z.N. 40x).

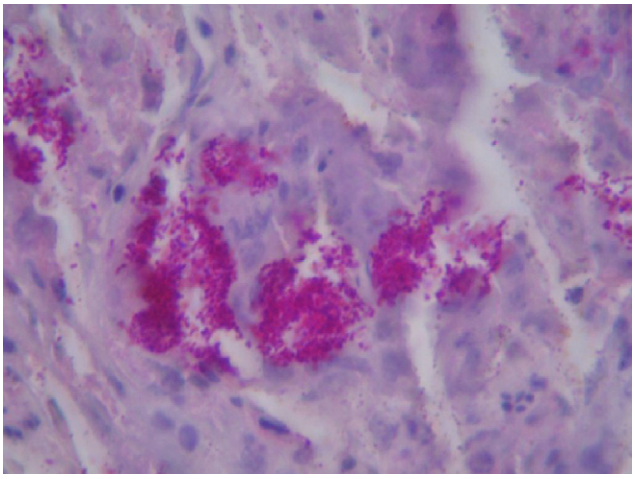

Fig. 5 *Danio rerio* Higher magnification of the previous image showing the morphology of such small acid-alcohol resistant bacteria (Z.N. 100x).

the fish stock in the Community. Attention should be paid to emerging diseases and diseases that are exotic to the Community.

Within Europe (Belgium, Denmark, France, Germany, Holland, Italy, Spain, and United Kingdom) the Commission Decision 2003/858/EC and the Council Directive 2006/88/EC do not apply to tropical ornamental fish kept permanently in aquaria, pet shops, garden ponds and specially without any direct contact with natural waters in the Community or to ornamental fish that are equipped with an effluent treatment system, reducing the risk of transmitting diseases to the natural waters to an acceptable level. However, individual EU countries do require health certification for imported ornamental fish under the Council Directive 91/67/EEC.

However, there is a great variation in policy for importation of ornamental fish between countries within regions. For example, France requires that a EU Directive 2003/858/EC health certificate-derived template be used for all imported live fish (including tropical ornamentals).

The Regulation EC no. 998/2003 on the animal health requirements applicable to the noncommercial movement of pet animals (including ornamental tropical fish) establishes that these animals must be accompanied by a certificate issued by an official veterinarian attesting the health of fish in consignment and that their source was free of specified disease agents.

(iii) It is necessary that countries or parts thereof from which Member States are authorized to import live fish must apply conditions for disease control, and monitoring at least equivalent to Community standards as laid down in Directives introducing minimum Community measures for the control of certain farming fish diseases.

(iv) The sampling and testing methods used for the detection and confirmation of certain fish diseases may be in accordance with those laid down in the International Office of Epizootics (OIE) Manual of Diagnostic Tests for Aquatic Animals.

Table 2 The sequence of *Mycobacterium* found by biomolecular exam.

TGGCGCCGGCTTGTGCACAACAAATTGAAAGCTGCCAGACACACTATTGGGCTTTGAGACAACAGGCCCGCATCCTGTCCCGTTGGGGG-CAGGGGGTGTGTTGTTGCCTCACTTTGGTGGTGGGGTGTGGTGTTTGATTTGTGGATAGTGGTTGCGAGCATCTAGCACGCA-TAGGGTGTGGCTGGGGCCTTCGGGTTTCGGTCGCGTTTGTGTGTGTTGATGTGCAATTTCTTTTGAAACTCATTTTTTGGTTTTTGTGTTG-TAAGTGTTTAAGGGCGCATGGTGGATGCCTTGGCA

(v) Finally, a public education program on the risks related to imported ornamental fish, with emphasis on responsible pet ownership, aquarium management and disease investigation, should be implemented.

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