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Fish tank granuloma not associated to *Mycobacterium marinum* but to *Microbacterium paraoxydans*

ALBERTO FICA[1,2], ALEJANDRA KUTSCHER[1,2], CARLA LILLO[3], GABRIELA BUSTAMANTE[4], JAVIER OLMOS[5], JORGE HERNÁNDEZ[6], JUAN VARGAS[3,7]

[1]Servicio de Medicina, Hospital Base de Valdivia, Chile.
[2]Instituto de Medicina, Facultad de Medicina, Universidad Austral de Chile, Valdivia, Chile.
[3]Servicio de Anatomía Patológica, Hospital Base de Valdivia, Chile.
[4]Laboratorio Central, Hospital Base de Valdivia, Chile.
[5]Centro Salud Familiar Las Ánimas, Valdivia, Chile.
[6]Servicio de Traumatología y Ortopedia, Hospital Base de Valdivia, Chile.
[7]Instituto de Anatomía, Histología y Patología, Facultad de Medicina, Universidad Austral de Chile.

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Correspondencia a:
Dr. Alberto Fica.
Médico Infectólogo,
SubDepartamento de Medicina,
Hospital Base de Valdivia
Bueras 1003, Valdivia, Región De Los Ríos, Chile.
albertoficacubillos@gmail.com

Fish tank granuloma has traditionally been associated with Mycobacterium marinum. We report the case of a 54 old-year woman that worked in a freshwater fish pet store and developed a skin ulcer in a hand finger with a suppurative granuloma secondary to Microbacterium paraoxydans. This is a bacterial fish pathogen, rarely observed in human infections. Identification was performed by colony morphology and MALDI-TOF. She evolved satisfactorily after receiving a sequential treatment with beta-lactams and linezolid. To our knowledge, it is the first known description of this type.

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Keywords: *Granuloma; Microbacterium; Infections; Mass Spectrometry.*

Granuloma de pecera no asociado a *Mycobacterium marinum* sino a *Microbacterium paraoxydans*

El granuloma de pecera ha sido tradicionalmente asociado a infecciones por Mycobacterium marinum. Reportamos el caso de una paciente de 54 años que trabaja en una tienda de acuarios y que desarrolló una úlcera en uno dedo de la mano con un granuloma supurativo secundario a Microbacterium paraoxydans, un patógeno de peces que ha sido infrecuentemente observado en infecciones humanas. La identificación fue efectuada por el aspecto de la colonia y espectrometría de masas (MALDI-TOF). Evolucionó satisfactoriamente luego de recibir un tratamiento antibiótico secuencial con beta-lactámicos y luego linezolid. Según nuestro conocimiento, esta es la primera descripción de este tipo.

Palabras claves*: Granuloma; Microbacterium; Infecciones, Espectrometría de Masas.*

Fish tank granuloma has traditionally been associated with *Mycobacterium marinum* infection in individuals exposed to fresh or saltwater fish/seafood ponds[1]. *M. marinum* infection is usually confined to the skin and its diagnosis requires a high degree of suspicion, special cultures, histopathological analysis and/or microorganism detection by molecular methods. We report a case of cutaneous granuloma associated with a bacterial pathogen of freshwater fish. To our knowledge, it is the first known description of this type. The patient gave her informed consent to publish this report.

Case report

A 54-year-old woman presented to a primary care center for a 3-week painless progressive lesion on the distal end of the right index finger that progressed with ulceration and discharge (Figure 1A). She was otherwise healthy, and worked in a freshwater fish pet store, cleaning fish tanks and feeding animals by crushing snails with her hands, having frequent contact with the pond water. An empirical treatment with amoxicillin-clavulanate was given for 7 days. Because to a lack of response, a first biopsy was performed one month later reporting a granulomatous, partly necrotizing inflammatory process (Figure 2). Complementary stains did not show microorganisms; molecular tests for mycobacteria or *Bartonella* from the fixed tissue were negative. A satellite lesion appeared in the ipsilateral forearm (Figure 1B), with no response to empirical treatment with doxycycline for 10 days and cloxacillin for additional 7 days. The patient was referred to our hospital. She was in a good general condition, and no fever, lymphadenopathy,or visceromegaly were found. An x-ray study excluded osteomyelitis, and laboratory tests did not reveal anemia, leukocytosis, or platelet count abnormalities. The erythrocyte sedimentation rate and the C-reactive protein values were normal (8 mm/h and 0.23 mg/dL, respectively). A new biopsy was scheduled, with a microbiological study of a fresh tissue sample. Histopathological study showed a suppurative, granulomatous chronic inflammation, with regressive signs. No microorganisms were identified with special stains (Ziehl-Neelsen, KinJou and Gram) and PCR for *Mycobacterium tuberculosis* or atypical mycobacteria were negative, as was a direct Ziehl-Neelsen staining of fresh tissue. Fungal and mycobacterial culture in liquid medium were negative. The sample was also seeded on 5% sheep blood Columbia and PoilyViteX chocolate agar plates (BioMérieux) and incubated at 37 ° C under aerobic and microaerophilic conditions. After 72 hours, medium-sized, yellow, dry colonies with Gram-positive bacteria developed in both culture media, later identified as *Microbacterium paraoxydans* with 99.9% confidence by MALDI-TOF (Matrix Assisted Laser Desorption/Ionization Time-

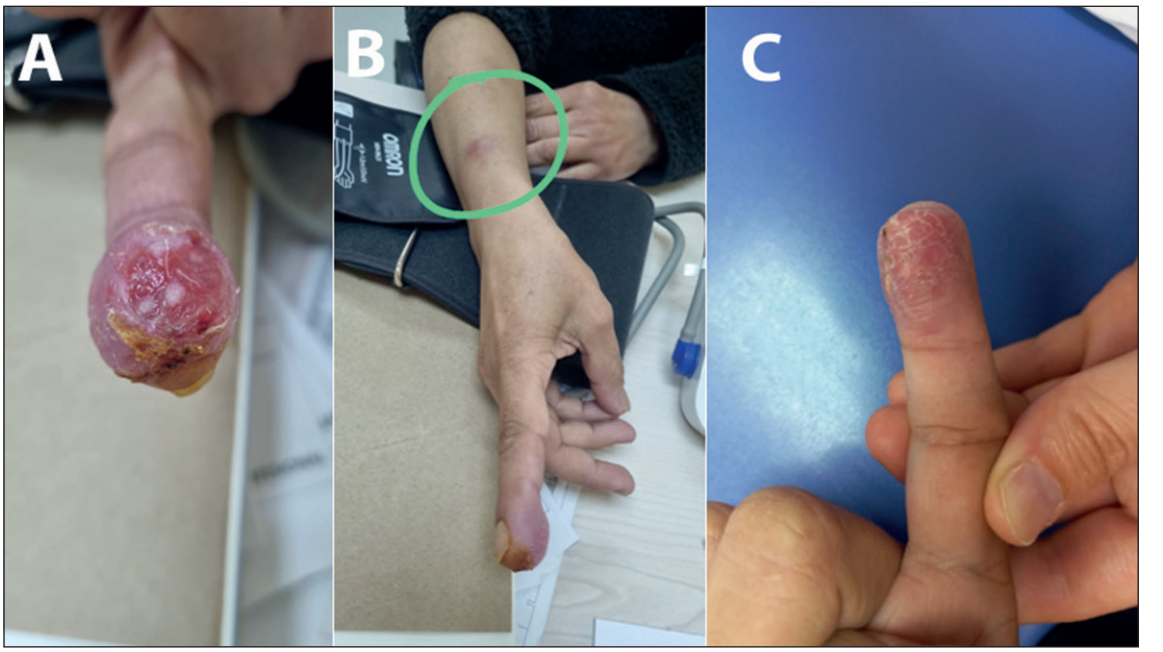

Figure 1. A. Initial picture taken approximately at 3 weeks of evolution. An ulcer is observed on the index finger of the right hand with edema and local erythema. **B.** Satellite lesion on the forearm. This lesion appeared after an initial amoxicillin-clavulanic treatment course. **C.** Control picture taken after 14 days of amoxycillin treatment followed by 10 days of oral linezolid. Improvement is evident as ulcer skin healed.

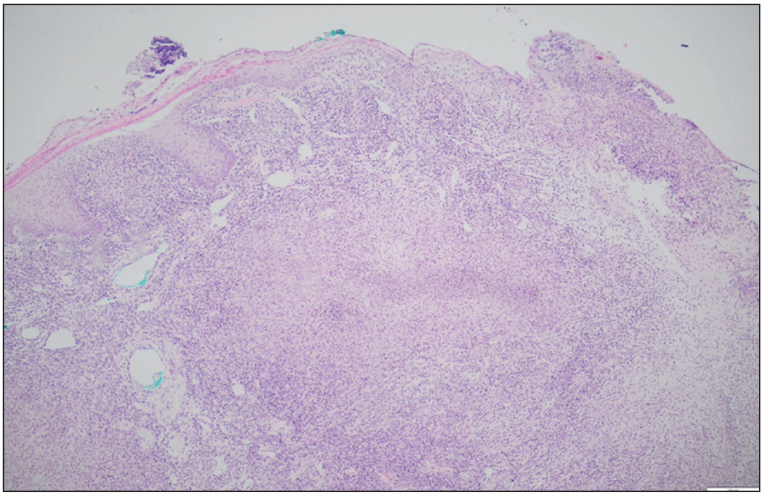

Figure 2. Microphotography of the histological study. Hematoxylin-eosin stain 4x. A granuloma with a suppurative center is observed.

of-Flight; VITEK MS; BioMérieux). Susceptibility test was carried out using the Kirby-Bauer method on a 5% Mueller Hinton sheep blood plate and inhibition halos reading were interpreted according to version 12.0 of the 2022 EUCAST Manual. The strain was susceptible to penicillin (44 mm halo), linezolid (42 mm halo), and vancomycin (30 mm halo). An antibiotic treatment was started orally with amoxicillin 1 g twice a day for 4 weeks but only taken during 14 days due to gastrointestinal intolerance. Treatment was replaced to oral linezolid 600 mg every 12 h for an additional 10 days. The lesion evolved favorably without relapse at 3 months of follow-up (Figure 1C).

Discussion

We believe the involvement of *M. marinum* in the granulomatous skin lesion was reasonably ruled out by a negative mycobacterial culture, two negative PCR molecular tests (one from a fresh tissue sample), and the absence of compatible bacillary forms in the staining of two histopathological samples obtained at different times. Unexpectedly, the culture revealed the presence of a bacterial fish pathogen, *M. paraoxydans*, which has occasionally been described in a wide range of infections in humans including bacteremia in immunosuppressed patients, peritoneal dialysis catheter-associated peritonitis, endophthalmitis, and urinary, articular, pleural or pulmonary infections[2-6]. Bacteremic cases have been associated to catheter-related infections, neutropenia or endocarditis, in most cases with a favorable outcome after antibiotic treatment and catheter removal[4]. More than 50 species are included in the genus *Microbacterium*, being *M. oxydans*, *M. paraoxydans* and *M. foliorum* the most frequent[3]. All they stain as Gram positive rods and grow on standard culture media as yellow-pigmented colonies[3]. Bacterial identification can be achieved by 16S rRNA gene sequencing and more recently by MALDI-TOF[3,5]. *Microbacterium* species are commonly distributed in the environment or sewage[4]. We are not aware that this pathogen had been previously described in association with skin infections associated with granulomas.

Granulomatous inflammation is composed of aggregates of epithelioid histiocytes with a peripheral border of lymphocytes and plasma cells. Its etiologies are several, including infections, autoimmune pathologies, toxins, allergens, drugs or neoplasms. Identification of the reaction pattern is important in the diagnosis. For example, well-defined granulomas include foreign body reactions, necrotizing, non-necrotizing, and suppurative granulomas. The prototypical example of a necrotizing granuloma is mycobacterial infection and for a non-necrotizing granuloma, sarcoidosis. Suppurative granulomas, as in our case, are seen more frequently in fungal infections, atypical

mycobacteria, deep mycoses, and some bacteria. The list includes *Actinomyces* spp., *Dirofilaria* spp., *Acanthamoeba* spp., *Balamuthia mandrillaris*, *Bartonella henselae*, *Blastomyces dermatitidis*, *Brucella* spp., *Chlamydia trachomatis* (serotypes L1, L2, L3 causing lymphogranuloma venereum), dematiaceous fungi, non -tuberculous mycobacteria, *Francisella tularensis*, *Prototheca* spp., *Sporothrix schenckii*, *Paracoccidioides brasiliensis*, *Yersinia* spp. and *Enterobius vermicularis*[7].

These granulomas are composed of epithelioid histiocytes surrounding a center of polymorphonuclear neutrophils[8]. In the context of a skin granuloma, it is routine to perform histochemical techniques in search of microorganisms. A basic panel includes Grocott's metamine stain and PAS for fungi, Ziehl-Neelsen for acid-fast bacilli, Gram for bacteria, and Fite or Fite-Faraco for other mycobacteria.

The different species of *Microbacterium*, including *M. paraoxydans*, are universally susceptible to linezolid, meropenem, doxycycline or vancomycin and into a lesser degree to penicillin (78%) and 3rd generation cephalosporins (72%)[3]. Susceptibility to quinolones is close to 50%. In our patient, various antibiotics active against *M. paraoxydans* were used during its evolution, including amoxicillin (initially with clavulanic acid), then doxycyclin, amoxicillin again, and finally linezolid. The accumulated treatment time reached 5 weeks and the agent was identified after at least 2 weeks of treatment, suggesting a difficult eradication.

This exceptional case was demonstrated by the finding of the agent in a tissue culture obtained under aseptic technique, a compatible working environment, the previous description of this species in a variety of human infections, the therapeutic response with appropriate treatment and exclusion of other causes. This report underlines the relevance of a histological and microbiological approach in persistent ulcerated skin lesions and expands the list of agents involved in suppurative granulomas.

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