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First Report of Ulcerative Dermatitis Due to a Simultaneous Infection by Mycobacteria and Dermatophytes in a Dog

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ABSTRACT

A five-month-old male pitbull dog was referred to the Small Animal Clinic, College of Veterinary Medicine, Universidad Autonoma de Nuevo Leon, presenting with numerous skin ulcerative lesions in the sternal and axillary regions as well as the hindquarters, with an evolution of two weeks. The objective of this study was to describe the morphological features observed in the skin of a dog infected with acid-fast bacilli and dermatophytes. Microscopically, the dermis showed numerous acid-fast bacilli, both within phagocytic cells and free in the tissue. Grocott's dye revealed numerous bifurcated and septate hyphae with chlamydo spores in the dermis along with evidence of the inflammatory reaction. Based on the observed morphological characteristics of the microorganisms, the diagnosis was ulcerative dermatitis caused by a simultaneous infection with *Mycobacterium* sp. and *Trichophyton* sp. In conclusion, this is believed to be the first report of its kind in a dog in Mexico.

Key words: *Mycobacterium* sp., *Trichophyton* sp., dog, ulcerative dermatitis

INTRODUCTION

Mycobacterium is a genus of Gram-positive bacteria with a worldwide distribution. Mycobacteria are non-motile, rod-shaped, aerobic microorganisms that cause lung and systemic diseases in mammals, although approximately one-third of the known species are primarily saprophytic. They have a lipid cell wall that resists dehydration and enables the bacteria to survive and multiply within phagocytic host cells, generating an inflammatory response in the host that can be granulomatous, pyogranulomatous or ulcerative (Bercovier and Vincent, 2001). Diagnosis is difficult as the microorganisms are fastidious and difficult to culture (Malik *et al.*, 2013). Prognosis is also problematic as spontaneous regression is unpredictable. In dogs, mycobacteria cause nodular or ulcerative skin infections. Localized lesions occur frequently in immune-competent dogs while widespread lesions are more common in immune-compromised animals.

Dermatophytes are fungi that infect keratinaceous tissues, such as skin, hair and nails. They are classified into three genera: *Epidermophyton*, *Microsporum* and *Trichophyton*. The latter is one of the most common causes of skin problems in domestic animals (Chermette *et al.*, 2008).

Superficial mycoses associated with *Trichophyton* are known as ringworm and rarely invade subcutaneous or deeper tissues. When deep infections occur, they are usually accompanied by wounds or irritation of the skin and are particularly likely if contaminated hair follicles rupture which can spread the infection to the dermis (Copetti *et al.*, 2006). Both *Mycobacterium* sp. and *Trichophyton* sp. can be difficult to diagnose definitively if one is not familiar with them. Furthermore, symptoms are similar to infection by other pathogens, such as *Nocardia* sp. or *Rhodococcus equi*.

Herein, we document what we believe is the first reported case of simultaneous infection by acid-fast organisms and ringworm in a dog in Mexico.

MATERIALS AND METHODS

Case description

Animal clinical condition: A five-month-old male pitbull dog weighing about 10 kg was referred to the Small Animal Clinic, Faculty of Veterinary Medicine, Universidad Autonoma de Nuevo Leon. Numerous well-defined, rapidly growing skin ulcers were found upon physical examination, mainly distributed in the ventral part of the body, especially in the sternum, axillary and hind-limbs areas. The condition had developed over about two weeks. There was no history of trauma. The dog was the only pet in the house and was confined to the back yard. The animal was underweight and depressed but there was no fever or dehydration. On palpation, there were no visible subcutaneous nodules in areas adjacent to ulcers and regional lymph nodes seemed normal. Ulcers were circular, elevated and with thickened edges, about 0.5 to 3 cm in diameter. An odorless, serosanguineous or purulent fluid flowed from the ulcers. Granulation tissue was present at the edges and center of the larger ulcers. The dog's vaccinations and dewormings were up to date. Prior to being admitted to the clinic, the wounds had been treated by the veterinarian with saline physiological solution and nitrofurazone for a couple of days without improvement. The presumptive clinical diagnosis was based on the multiple lesions which were consistent with nocardiosis, cutaneous canine leprosy or infection by *Mycobacterium*.

Biopsy study: Ulcer biopsies were taken and referred to the Diagnostic Pathology Department at the UANL for histopathological diagnosis. Samples were rapidly fixed in 10% buffered formaldehyde (pH 7.4) and subsequently embedded in paraffin. Sections (5 μm -thickness) were cut and then stained with hematoxylin and eosin, Ziehl-Neelsen and Grocott. Finally, the samples were examined at 40 \times and 100 \times with a light microscope.

Mantoux test: No blood samples were taken for laboratory analysis but intradermal tests in the thigh were performed with purified protein derivative, [PPD; 250 UI/0.1 mL (kindly provided by Arce-Mendoza, Immunology Department, Faculty of Medicine, UANL)] and chest X-rays were performed to observe the involvement of mycobacteria.

Treatment: The animal was treated with rifampicin (10 mg kg⁻¹ day⁻¹) and isoniazid (15 mg kg⁻¹ day⁻¹). The patient died apparently of pneumonia 10 days after initializing the treatment and no more tests were conducted on the animal due to the dog owner refusal.

RESULTS

Skin tissue was observed under the microscope after staining. When stained with hematoxylin and eosin, hyperplasia of the spinous layer and hyperkeratosis were observed as well as loss of epidermal tissue in some areas which exposed the dermal connective tissue. The dermis had

disrupted collagen fibers, coagulative necrosis, fibroblast proliferation, fibrosis and numerous hemorrhagic areas. There was evidence of a severe inflammatory response with the presence of neutrophil polymorphonuclears, macrophages, epithelioid cells, lymphocytes and occasional giant cells surrounded by discrete calcification areas. Many translucent, circular structures of approximately 20 μm in diameter, poorly stained with eosin were present in the middle of the area where the inflammatory response was evident. Upon staining with Ziehl-Neelsen dye, numerous rod-shaped, intensely stained, red microorganisms were visible, that were either single or in groups; these were present inside macrophages and giant cells or were free in the tissue (Fig. 1). Staining with Grocott dye revealed numerous septate and branched hyphae, brown or brownish ocher in color, distributed along the surface and deep in the dermis. These were accompanied by numerous spherical 20 μm diameter dilated chlamydo spores arranged in chains at the center of the area of the inflammatory reaction (Fig. 2). The chlamydo spores were surrounded by numerous

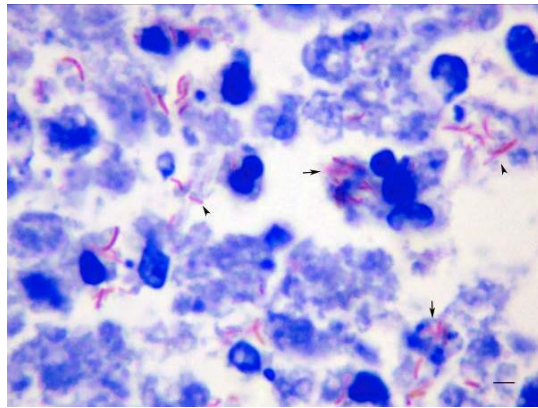


Fig. 1: Dermis. Numerous acid-fast bacilli inside macrophages (arrows) and free in the alveoli spaces (arrowheads). Ziehl-Neelsen stain. Bar = 5 μm

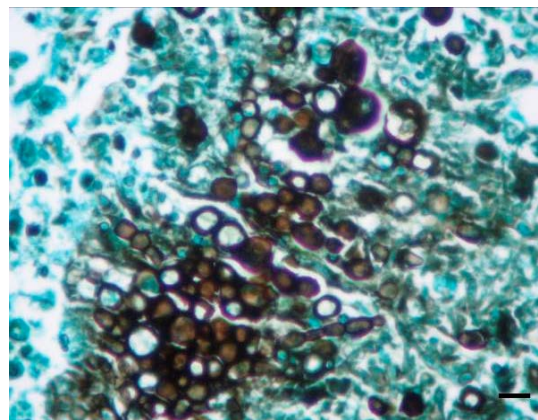


Fig. 2: Dermis. Numerous chlamydo spores organized in chains surrounded by numerous inflammatory cells. Grocott stain. Bar = 25 μm

macrophages, lymphocytes and giant cells. The surrounding tissue had numerous fibroses and necrotic areas. Similarly, an inflammatory response involving neutrophils and macrophages was also observed, associated with birefringent structures similar to hair fragments in the deep dermis. The pathologic diagnosis was a severe acute ulcerative dermatitis pyogranulomatous due to *Mycobacterium* sp. and *Trichophyton* sp.

DISCUSSION

This is considered the first report in Mexico of pyogranulomatous ulcerative dermatitis in a dog caused by simultaneous infection by acid-fast bacilli and a dermatophyte. A number of considerations leads us to suggest the combined participation of *Mycobacterium* sp. and *Trichophyton* sp., in this case: These consideration include the morphological characteristics of the microorganisms as observed with microscopy combined with the use of several different staining techniques as well as the histopathology of the lesions. The diagnosis of mycobacterial infections in histological samples requires demonstrating the presence of acid-fast bacilli which can be detected with Ziehl-Neelsen dye, within the lesion. While microorganisms were apparent upon staining with this stain, *Mycobacterium* sp. are not the only bacteria in veterinary medicine that will react positively to Ziehl-Neelsen stain. *Nocardia* sp. and *Rhodococcus equi* also react, although weakly (Greene and Moore, 2013). However, *Nocardia* sp. develop mycetomas and microscopic filamentous structures and microcolonies with the accompanying inflammatory response, called "sulfur granules" (Salinas-Carmona, 2000) which were not observed in this case. *Rhodococcus* sp. have branched or septate vegetative filaments or coccobacillary forms, neither of which were observed in these samples (Santoro *et al.*, 2008). Besides Ziehl-Neelsen staining, bacteriological culture or molecular tests, such as PCR are recommended to isolate and identify mycobacteria (Bercovier and Vincent, 2001). Samples were taken to the Department of Bacteriology (Veterinary College-UANL) for egg-enrichment media, Lowenstein-Jensen (with and without glycerol, for *M. tuberculosis* and *M. bovis*, respectively). However, we obtained negative results. The intradermal injection of PPD, a test for tuberculosis, resulted in hemorrhagic indurated swelling at the site of injection 72 h after inoculation. Delayed hypersensitivity to this antigen is considered a positive reaction in both humans and other mammals and both tuberculous and non-tuberculous mycobacteria cross react to a considerable extent in this test. Thus, this test is a reliable indicator of mycobacterial infection. Furthermore, the dog's rectal temperature increased by 1.1°C 12 h after PPD injection, considered to be a positive test result (Greene and Moore, 2013). The type of inflammatory reaction in this study is also consistent with mycobacterial infection. Chest radiography showed multiple whitish concretions uniformly distributed in the lung parenchyma, consistent with spread of the disease. Identification of the species of mycobacteria infecting the dog is difficult. There are no tests that would differentiate between the different species that infect the skin. Four different types of mycobacteria groups have been described in dogs, based on the characteristics of their growth and type of injury with which they are usually associated (Greene and Moore, 2013; Malik *et al.*, 2013). The microorganisms observed in this study were numerous within phagocytic cells, consistent with infections by *Mycobacterium* sp. microorganisms (O'Brien *et al.*, 2011) which caused pyogranulomatous and ulcerative skin lesions. This case also has some similarities to infection by *M. tuberculosis* and *M. bovis*. In such infections, microorganisms within inflammatory cells are rather scarce and were only occasionally observed extracellularly which was similar to what we observed in this case. Subsequent studies with molecular biology, like PCR and direct fluorescent antibodies techniques, will allow us to fully

identify the microorganisms involved. Mycobacterial skin infections are rare in dogs, although apparently the incidence is increasing due to a growing population of immune-compromised individuals, either because of treatment or constant stress (Foley *et al.*, 2002). There is no evidence for particular susceptibility to infection by any breed or either sex, although infections are more common in short-haired breeds, such as the pitbull in this study (Santoro *et al.*, 2008). The origin of the *Mycobacterium* infection in this case is unknown but it has been reported that most of the canine cutaneous ulcerative lesions occur by saprophytic organisms that normally inhabit the environment and that are introduced to the skin via percutaneous inoculation through wounds or arthropods (Malik *et al.*, 2013). The latter may have occurred in this case as ectoparasites in the dog were apparent during his physical examination.

The other microorganism observed in the ulcerative lesions in this case was identified as a dermatophyte of the genus *Trichophyton* (Van Rooij *et al.*, 2012). This identification was based on its morphological characteristics. Members of this genus have been associated with ringworm in dogs in various parts of the world (Copetti *et al.*, 2006). The fungus in this case had the typical dilated, septate hyphae and chlamydospore structures organized in chains, suggesting a diagnosis of *T. verrucosum*. This fungus, like the dermatophytes *Microsporum* and *Epidermophyton*, cause superficial skin infections of the stratum corneum, follicular sheath and nails or claws (Chermette *et al.*, 2008). However, the fungal infection in this case was in the superficial and deep dermis, a rare condition in dogs. Deep dermatophyte infections usually develop from superficial dermatophytosis (Ates *et al.*, 2008) but no gross lesions or histopathologic lesions suggestive of ringworm were present in the dog. Absence of the fungus in the follicular sheath and epidermis in dogs with evidence of gross ringworm infection has been noted previously. It has been proposed that dermatophytosis in deep skin layers may be due to follicular rupture that spreads the fungus directly into the dermis (Nweze, 2001).

Trichophyton infection is common in domestic dogs and the usual mode of transmission is through direct contact with chlamydospores or segmented or fragmented hyphae from infected individuals. Fleas, houseflies and rodents can also transmit this disease (O'Brien *et al.*, 2011) and it can be mechanically transmitted via contaminated brushes and collars or digging through infested sites or surfaces. The dog's habitat-including confinement, heat and moisture may have predisposed him to infection. Warm, humid conditions can lead to softening and maceration of the skin which increases the ability of dermatophytes to penetrate it and enhances germination. It was not possible to determine neither the origin of the co-infection nor the order in which microorganisms appeared on the skin. Both organisms have a worldwide distribution and are prevalent in domestic animals of the northeastern region of Mexico. It is possible that the dog had a period of partial immune-suppression or maybe both microorganisms gained access to the lower layers of the skin due to loss of a physical barrier that confers protection, in this case, the skin.

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