MYCOBACTERIUM INTRACELLULARE INFECTION IN A CAPYBARA (*HYDROCHOERUS HYDROCHAERIS*)

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MYCOBACTERIUM INTRACELLULARE INFECTION IN A CAPYBARA (*HYDROCHOERUS HYDROCHAERIS*)

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Abstract: This report describes the first case of *Mycobacterium intracellulare* infection with typical granulomatous lesions of mycobacteriosis in a capybara (*Hydrochoerus hydrochaeris*). The individual was a captive-bred young female, part of the control group of an experimental study on stress. Multiple granulomatous lesions were detected in a mesenteric lymph node of this young female. Mycobacterial infection was confirmed by bacteriologic culture and molecular identification methods. Clinical lesions were characterized by histopathology.

Key words: Granuloma, *Mycobacterium avium-intracellulare* complex, wildlife.

BRIEF COMMUNICATION

The *Mycobacterium avium* complex, collectively known as nontuberculous mycobacteria (NTM), consists of opportunistic pathogens, such as *M. avium* subsp. *avium* and *M. avium* subsp. *paratuberculosis*, as well as environmental species such as *M. intracellulare*, each capable of causing diseases in wildlife, livestock, and humans.[1,5] *M. intracellulare* is ubiquitous in the environment, and it causes opportunistic disease, known generically as mycobacteriosis, especially found in immunocompromised individuals and those with preexisting or concurrent disease processes.

This study describes the first case of mesenteric mycobacterial infection caused by *M. intracellulare* in a capybara (*Hydrochoerus hydrochaeris*). The individual was a captive-bred subadult female, part of an experimental study aimed at evaluating the effect of induced stress on parasitism and physiologic parameters. The experiment was conducted with 26 young females acquired from a capybara breeding farm. They were transported to Estación Zoológica Experimental Granja La Esmeralda, Santa Fe, Argentina, on 28 August 2009.[2] The treatments consisted of stress by food restriction or manipulation, and control groups were not subject to these stressors. After 3 mo, animals were euthanized, organs of the digestive, respiratory, reproductive, and immune systems and accessory glands were examined and multiple samples were collected.

Multiple granulomatous nodules, measuring 0.5–1.5 cm in diameter, some of them having a whitish fibrous capsule, were detected in a mesenteric lymph node of a female of the control group (Fig. 1). The lesion was consistent with a tuberculosis granuloma, because it had central caseous necrosis and mineralization. No further lesions of this nature were found in this or other animals. The entire lymph node was taken and cut into two pieces, with half the granuloma present in each of them. One piece was placed into 10% buffered formalin for histopathologic examination, and after 24 hr fixation, the sample was embedded in paraffin, cut in 4-μm sections, and stained with hematoxylin andeosin (Biopur SRL, Rosario-2000, Argentina) and Ziehl-Neelsen's acid-fast stains (Ziehl kits, Biopur SRL, Rosario-2000, Argentina). The other half of the lymph node was kept frozen for microbiologic investigations. After the tissue sample was decontaminated following Petroff's method[4] using 4% NaOH, it was incubated at 37°C for 30 min. After centrifuged at 3,000 r.p.m. for 15 min, the supernatant was discarded and the deposit was neutralized

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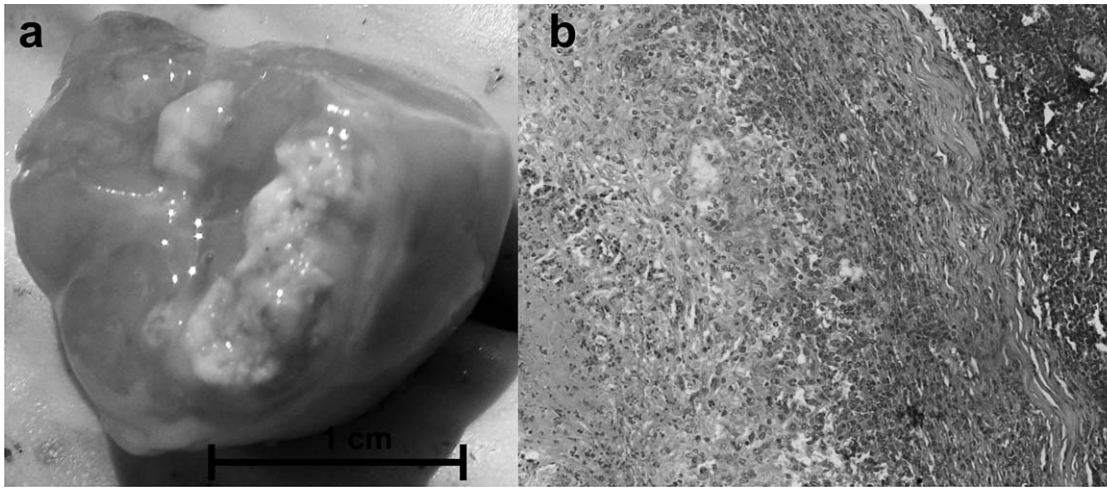

Figure 1. Mesenteric lymph node with multiple granulomatous nodules (**a**). The histo-architecture of the granuloma (**b**) showed central caseous necrosis, epithelioid tissue proliferation with Langhans giant cells, and thin fibrous capsule. On the right, there is lymphoid tissue characteristic of a lymph node. H&E, ×10.

with phenol red and sulfuric acid. Sediment material was cultured onto selective Löwenstein-Jensen and Stonebrink media (Laboratorios Britania, CABA-1283, Argentina) by duplicate. Aerobic incubation at 30°C was conducted for several weeks. A Ziehl-Neelsen stain was also carried out with sediments, and mycobacteria were isolated on Stonebrink agar as loopfuls.

For DNA extraction, a loopful of bacteria was resuspended in 250 µl of distilled water in a 1.5 ml tube and was incubated in a 95°C dry bath over a span of 45 min. The identification of the isolate was performed on the basis of three polymerase chain reaction (PCR) assays: amplification of the IS*6110* (specific of *M. tuberculosis complex*), amplification of IS*1245* (specific of *M. avium* subsp. *avium* and *M. avium* subsp. *hominissuis*), and amplification of the *hsp65* gene (present in the *Mycobacterium* genus) to perform PCR restriction analysis (PRA). Amplification of the fragments of 245 bp, 247 bp, and 440 bp belonging to IS*6110*, IS*1245*, and *hsp65*, respectively, was attempted following previous work.[3,4,9–11] PCR products were column purified. For the sequencing reaction, the primer 5′CTTAACACATGCAAGTCGAAC 3′ (Invitrogen, CABA-1427, Argentina) was used.

All PCR products were separated by electrophoresis on a 2% agarose gel (LE-Agarose 1200, Genbiotech, CABA-1427, Argentina) stained by ethidium bromide (Sigma-Aldrich, St. Louis, Missouri 63178, USA) solution (1 mg/ml) and after that visualized with an ultraviolet transilluminator.[8] The identification of the *Mycobacterium* group was made by comparison of the PRA pattern detected with those included in the PRAsite database (http://app.chuv.ch/prasite/index.html).

Further specific identification of the *Mycobacterium* isolate was performed by sequencing the16S rDNA gene with a 16-capillary ABI3130xl sequencer (Applied Biosystems, Carlsbad, California 92008, USA) using Big Dye Terminator v3.1 (Cycle Sequencing Kit, Applied Biosystems) in the Genomic Unit facility of the Biotechnology Institute of INTA. The resulting DNA sequence was compared with those available in the Ribosomal Differentiation of Medical Microorganisms (RIDOM) database (http://rdna.ridom.de/).

Histologically, there was an architectural disorganization of the lymph node due to multiple coalescing small granulomas, characterized by an eosinophilic necrotic central area surrounded by a few multinucleated giant cells, macrophages, lymphocytes, some eosinophils (Fig. 1). A fine fibrous capsule was present, and mineralization occurred in the central necrotic area. These microscopic findings suggested a *Mycobacterium* infection.

Ziehl-Neelsen stain revealed scarce acid-resistant bacteria in some macrophages' cytoplasm, but no acid-fast bacteria were seen. Culture was successful after a 58-day period.

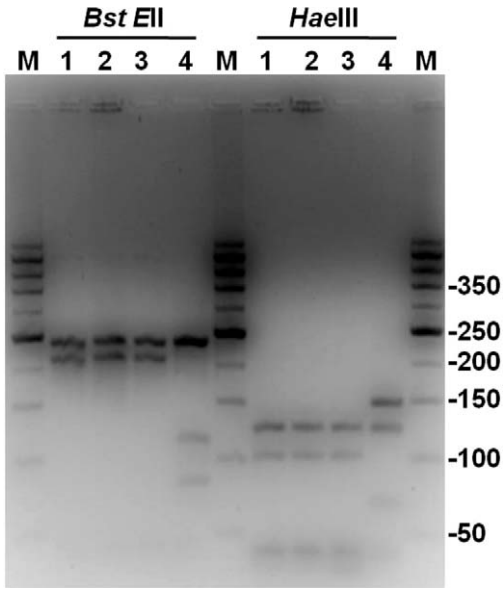

Figure 2. Restriction analysis of the polymerase chain reaction product with Bst*EII* and Hae*III*. M: cincuenta marker (Biodynamics, 1370, Buenos Aires, Argentina). Molecular weights are detailed on the right side, 1: sample; 2: duplicated sample; 3: *Mycobacterium avium* subsp. *avium* control; 4: *Mycobacterium bovis* control.

The PCR results of IS*6110* and IS*1245* were negative, but *hsp65* was positive. These results suggested that the isolate was a NTM. The restriction pattern of *hsp65* amplicon obtained from the PRA technique (*Bst*EII 235/210 bp and *Hae*III 130/105 bp) was compatible with *M. avium* subsp. *avium* type I, *M. avium* s. *paratuberculosis* type 1, and *M. avium* s. *silvaticum* type 1 pattern according to the PRAsite database (Fig. 2).

The 16S rDNA sequence alignment had an identity of 99.7% with *M. intracellulare* and 97.6% with *M. avium* subsp. *avium* in the RIDOM database. The novel sequence was deposited in GenBank (KF135658). Therefore, the isolate was characterized as *M. intracellulare*.

Although the experimental study included the examination and sampling of several organs by histopathology and molecular biology methods, lesions were only found in a single mesenteric lymph node.

Environmental reservoirs of NTM are believed to include soil and water sources. NTM are resistant in the environment because of their relatively slow growth and impermeable cell wall. They are capable of surviving and multiplying at extremes of pH and temperature, in low-oxygen and low-nutrient environments, and after treatment with chlorine or ozone.[7] In this case, transmission most likely occurred through ingestion of contaminated soil particles or water drops.

Given that wildlife surveillance initiatives are practically nonexistent in the developing world, infections with mycobacteria or other pathogens in wildlife are likely to be largely underestimated, posing a risk to public health, livestock, and biologic conservation.

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