Acta Scientiae Veterinariae, 2022. 50(Suppl 1): 748.

CASE REPORT
Pub. 748

ISSN 1679-9216

Granulomatous Lymphadenitis in a Dog Caused by *Mycobacterium intracellulare*

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ABSTRACT

***Background*:** Mycobacteriosis is caused by bacteria belonging to the genus *Mycobacterium*, with considerable zoonotic potential and risk to public health. Infection in dogs is rare and is usually associated with immunosuppression, resulting from eating meat or contact with contaminated soil or fomites. Dogs are also known as potential sources for the spread of atypical tuberculosis in humans and other animals. This paper aims to describe the clinical, cytological, histopathological, and molecular findings of a male canine seen at University Veterinary Hospital of Cuiabá, Mato Grosso, with generalized lymphadenomegaly associated *Mycobacterium intracellulare* infection.

***Case*:** A 2-year-old male Lhasa Apso dog was referred to the University Veterinary Hospital in Cuiabá city, located in the Midwest region of Brazil. The patient had a history of intermittent claudication of the left pelvic limb for approximately 6 months and lymphadenomegaly with progression for approximately 2 months. The dog had wheezing and generalized lymphadenopathy (submandibular, axillary, and popliteal lymph nodes); cryptorchidism was also observed. A complete blood count revealed nonspecific results, and in the serum biochemical profile, the values of urea, creatinine, albumin, and alanine aminotransferase were within the reference range. No changes were observed on the radiography of the femurotibiopatellar joints. Considering the generalised lymphadenopathy, fine needle aspiration cytology and histopathological examination through biopsy of the lymph nodes was performed. On the cytology and histopathology, numerous negative images of moderately refringent bacillary structures distending the cytoplasm from the macrophages was found. The samples were also subjected to special Ziehl-Neelsen staining, which confirmed an accentuated and diffuse granulomatous lymphadenitis associated with alcohol acid-resistant bacilli. The polymerase chain reaction (PCR) was used to amplify the DNA of the lymph node fragment for the *hsp65* gene, which was subjected to genetic sequencing and construction of a phylogenetic tree, with 99.77% genetic similarity for the species *M. intracellulare*. As treatment, doxycycline (10 mg/kg twice a day for 60 days) and enrofloxacin (5 mg/kg once a day for 10 days) were prescribed. However, the canine suffered car trauma leading to a fractured pelvis, which motivated the owner to opt for euthanasia at another veterinary establishment.

***Discussion*:** In the reported case, it was not possible to determine the source of infection, as the owners reported that the animal lived inside the house with only sporadic access to the street. The clinical signs manifested by this dog were non-specific, and only the signs of generalised lymphadenopathy could be correlated with the signs expected in the infection with this mycobacterium. The hematological and biochemical laboratory findings were nonspecific, and it did not demonstrate the involvement of other organs. Considering the findings in cytology and histology, mycobacterial infection can be suspected. The diagnosis was confirmed through pathological and molecular findings. In this case, the PCR technique was used with partial amplification of the *hsp65* gene and subsequent genetic sequencing, making it possible to identify a species like *M. intracellulare* (99.77% similarity). Due to euthanasia for another reason, it was impossible to monitor the dog's treatment and investigate other changes in the *post mortem* examination, especially the pulmonary lesions frequently described in *Mycobacterium intracellulare* infection in humans.

Keywords: *Mycobacterium avium* complex (MAC), canine, infection, non-tuberculous mycobacteriosis.

DOI: 10.22456/1679-9216.118107

Received: 3 October 2021 Accepted: 27 December 2021 Published: 6 February 2022

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INTRODUCTION

Mycobacterium genus contains several opportunistic and obligate pathogens with zoonotic potential, including *Mycobacterium avium* complex (MAC), which includes the species *Mycobacterium intracellulare* and four *Mycobacterium avium* subspecies: *M. avium* subspecies *avium*, *M. avium* subspecies *hominissuis*, *M. avium* subspecies *silvaticum*, and *M. avium* subspecies *paratuberculosis* [7]. Infections are acquired predominantly from environmental sources, such as soil, water, food, dust sources [7], plants and animals [9]. In dogs, infection results from eating meat or contact with contaminated soil or fomites [5] and may be associated with immunodeficiency [9].

The infection by the MAC complex can be characterized by primary cutaneous granulomatous lesions or progression of infection from the respiratory or gastrointestinal system [5] to multiple organs such as the lymph nodes, liver, spleen [9], intestine, lung, bone marrow, and even spinal cord [7] with poor prognosis [10].

This report aims to describe the clinical, laboratory, cytological, histopathological, and molecular findings in a dog with granulomatous lymphadenitis caused by *Mycobacterium intracellulare*.

CASE

An approximately 2-year-old male Lhasa Apso dog was referred to the University Veterinary Hospital in Cuiabá city located in the Midwest region of Brazil. The patient had a history of intermittent claudication of the left pelvic limb for approximately 6 months and lymphadenomegaly with progression for approximately 2 months.

On physical examination, the patient had a bodyweight of 4.8 kg, body temperature of 38.8°C, heart rate of 156 bpm, wheezing, and generalized lymphadenopathy (submandibular, axillary, and popliteal lymph nodes). In addition to a noticeable increase in volume upon palpation of the lymphatic chain, cryptorchidism was also observed, with the testicles in the inguinal region.

A complete blood count revealed a slight increase in haemoglobin (19.4 g/dL; reference range 12-18 g/dL), mild relative neutrophilia (78%; reference range 60-77%), and relative lymphopenia (9%; reference range 12-30%). For the serum biochemical profile, the values of urea, creatinine, albumin, and alanine aminotransferase were within the reference range. No changes were observed on the radiography of the femurotibiopatellar joints.

In the face of generalised lymphadenopathy, fine needle aspiration cytology of the lymph nodes was performed, and many macrophages with a distended cytoplasm were observed in the background of a slightly hyaline amorphous substance. There were numerous negative images of moderately refringent bacillary structures distending the cytoplasm from the macrophages, which were freely seen among them and immersed in the hyaline substance (Figure 1A). The cytological sample was also subjected to special Ziehl-Neelsen staining [11]. The morphological diagnosis was confirmed in accentuated and diffuse granulomatous lymphadenitis associated with alcohol acid-resistant bacilli.

Subsequently, an excisional lymph node biopsy was performed. Thus, the patient was anaesthetized for surgical removal of the left and right submandibular lymph nodes, which were subjected to histopathological examination and PCR. Macroscopically, the lymph nodes sent for histopathological examination measured approximately 2.0 × 1.5 × 1.0 cm with a yellowish colour and an oval and soft appearance. Histologically, there was marked diffuse infiltration of macrophages extending from the subcapsular sinus to the medullary region, replacing the lymph parenchyma and altering the lymph node tissue architecture with a loss of distinction between the cortical and medullary regions (Figure 1B). The macrophages exhibited large foamy cytoplasm (Figure 1C). Through Ziehl-Neelsen staining, an intense amount of acid-resistant structures in bacillary format was observed in the macrophages, which largely filled the cytoplasm (Figure 1D).

Genomic DNA was extracted from the lymph node samples using the phenol and chloroform method [12] with the addition of glass beads. PCR using the *hsp65* gene [13] resulted in the production of a 441 base pair fragment that was purified using the kit illustra™ ExoProStar[1], which was subsequently subjected to sequencing together with the BigDye™ Terminator[2] (BigDye™ Terminator Ready Reaction Cycle Sequencing) on an automatic sequencer[2] (ABI 3500 Genetic Analyser™).

The sequences were compared in the GenBank database using the Basic Local Alignment Search Tool program (BLAST) on the NCBI server (http:www.ncbi.nlm.nih.gov/BLAST) [1], processed by the CLC DNA Workbench 6.0 Program[3], and analysed using BLAST to

verify the identity with other corresponding strings available in GenBank. Phylogenetic analysis was performed on the Phylogeny.fr platform [2], and the sequence generated in the PCR was aligned using the MUSCLE program (v3.8.31) [3] with eight sequences from different *Mycobacterium* spp. isolates from various parts of the world available on GenBank. The phylogenetic tree was assembled using the maximum likelihood method using the PhyML software [6]. The product generated by PCR was like *M. intracellulare* 99.77% (AY536637.1), *M. yongonensi* 99.55% (KF224991.1), *M. kansasii* 99.07% (KF432774.1), and *M. marseillense* 98.18% (EU239787.1). The homologous sequence of the *hsp65* gene of *Nocardia cyriacigeorgica* (EF127509.1) was included as an outgroup in the phylogenetic tree (Figure 2). Finally, the sequence was deposited in GenBank under the accession number MW631935.1.

Given the diagnosis of infection by *M. intracellulare*, doxycycline[4] [Doxitec® - 10 mg/kg, orally, twice a day for 60 days] and enrofloxacin[5] [Zelotril® - 5 mg/kg, orally, once a day for 10 days] were prescribed as therapeutic approach. However, the canine suffered car trauma leading to a fractured pelvis, which motivated the owner to opt for euthanasia at another veterinary establishment.

DISCUSSION

Infection with MAC complex bacteria in dogs, although sporadic, can be a potential source of infection in humans and other animal species [10]. Despite the few cases diagnosed in dogs, there is a strong racial predisposition particularly for the Miniature Schnauzer and Basset Hound. For the Miniature Schnauzer, a recessively inherited defect in the CARD9 adapter protein has recently been documented to increase susceptibility to the MAC complex. For the Basset Hound breed, susceptibility presents itself in a similar way but seems to be less common than in Miniature Schnauzers. The underlying cause has yet to be elucidated [4].

Among the reported cases of MAC infection in dogs, many affected dogs were young, with a higher risk of developing signs within the first 3 years of age and rarely in dogs older than 5 years [4]. In the present case, the canine was approximately 2-year-old.

In the reported case, it was not possible to determine the source of infection, as the owners reported that the animal lived inside the house with only sporadic access to the street. Infection by direct contact is not common, occurring predominantly because of exposure to environmental contaminants, such as water, soil, food, and dust sources [9].

The clinical signs manifested by this dog were nonspecific, and only the signs of generalised lymphadenopathy could be correlated with the signs expected in the infection with this *Mycobacterium*. Respiratory and skin signs are the most reported in dogs, with some exhibiting signs of systemic granulomatous infection [5] often associated with immunocompromise [9]. However, these were not seen in the current case. In men, these manifestations are associated with concurrent HIV infection [7].

The hematological and biochemical laboratory findings were nonspecific, and it did not demonstrate the involvement of other organs. Histological analysis revealed that the presence of alcohol-acid resistant bacilli after the use of special Ziehl-Neelsen staining and presence of granulomatous inflammation, mycobacterial infection can be suspected [4], as occurred in this case report, in which the pathological and molecular findings confirmed the definitive diagnosis, as described in literature [5,7].

For identification of *Mycobacterium* species, PCR and sequencing with construction of the phylogenetic tree are the most recently adopted methods [5]. Conventional phenotypic tests cannot distinguish MAC complexes. Therefore, molecular methods must be performed. In this case, the PCR technique was used with partial amplification of the *hsp65* gene and subsequent genetic sequencing, making it possible to identify a species like *M. intracellulare* (99.77% similarity). Another important diagnostic method is isolation in culture, not performed in this report.

The treatment with doxycycline and enrofloxacin [9] cannot be followed since the guardian opted for euthanasia after the canine underwent automobile trauma. However, even with treatment, disseminated MAC infections are associated with poor prognosis [10]. In the literature, there is a report of a dog with granulomatous splenitis and lymphadenitis caused by *M. avium* subspecies *hominissuis*, with poor prognosis after worsening of clinical signs even with treatment [9].

Given the zoonotic potential of a MAC-infected dog, particularly for immunocompromised human patients, dogs affected by the disease represent a risk to public health. In this sense, the collaborative efforts of microbiologists, veterinarians, dog breeders, primary care physicians, and infectious disease specialists who

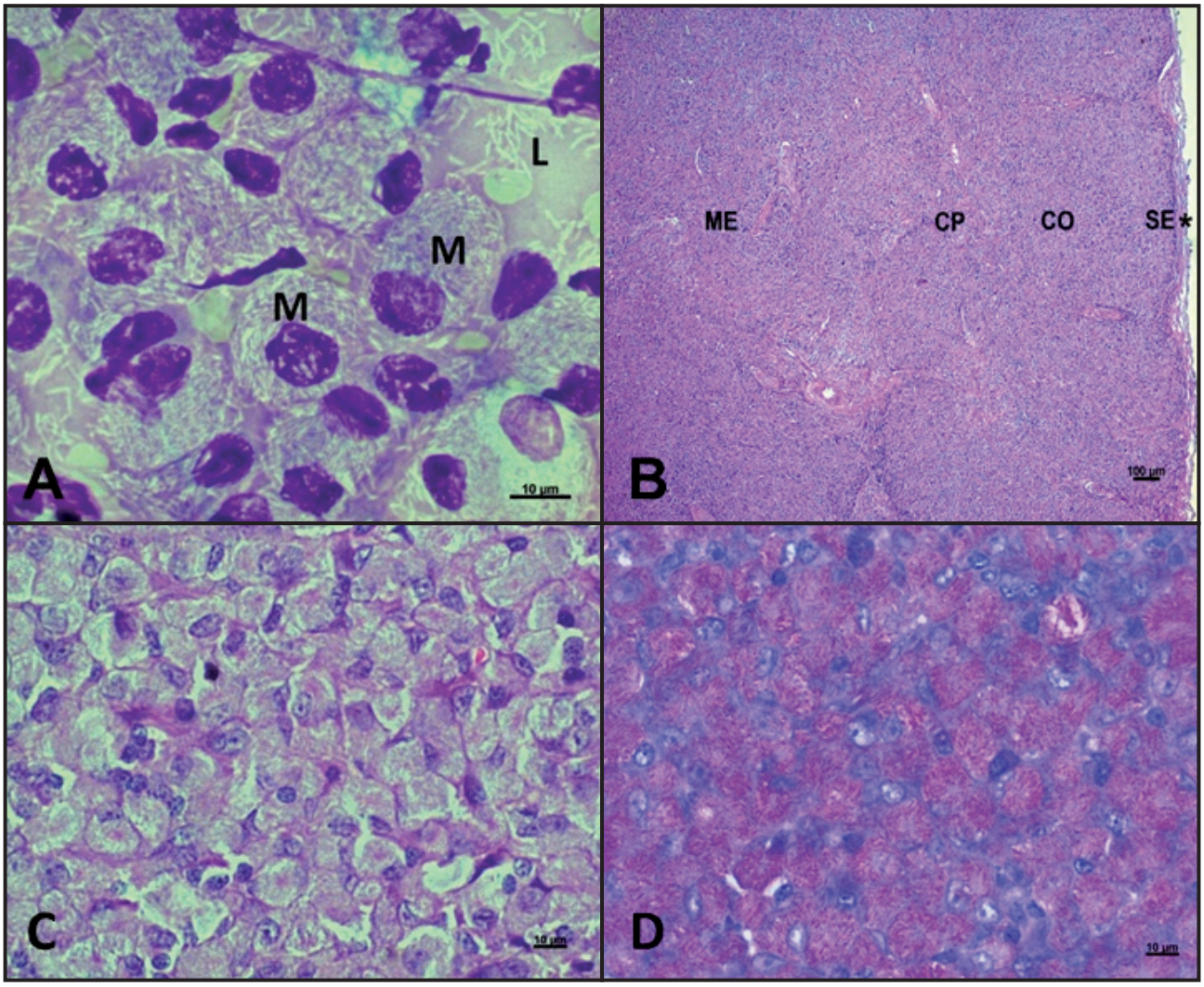

Figure 1. Dog with lymphadenitis caused by *Mycobacterium intracellulare*. A- Fine needle aspiration cytology of the submandibular lymph node. Cluster of macrophages (M) showing distended cytoplasm containing many negative images of bacillus-shaped structures. These structures can also be observed free between macrophages (L) [HE; 40x]. B- Histological section of the submandibular lymph node. There is a loss of lymph node histological architecture due to an intense infiltration of macrophages that starts below the capsule (*), in the subcapsular sinus (SE), occupying the region of the superficial cortex (CO) and the deep cortex (CP) with extension to the medullary region (ME) [HE; 5x]. C- Macrophages with broad cytoplasms and foamy appearances [HE; 20x]. D- Intense infiltrate of macrophages with cytoplasms distended by many acid-resistant bacillary structures strongly stained [Ziehl-Neelsen; 20x].

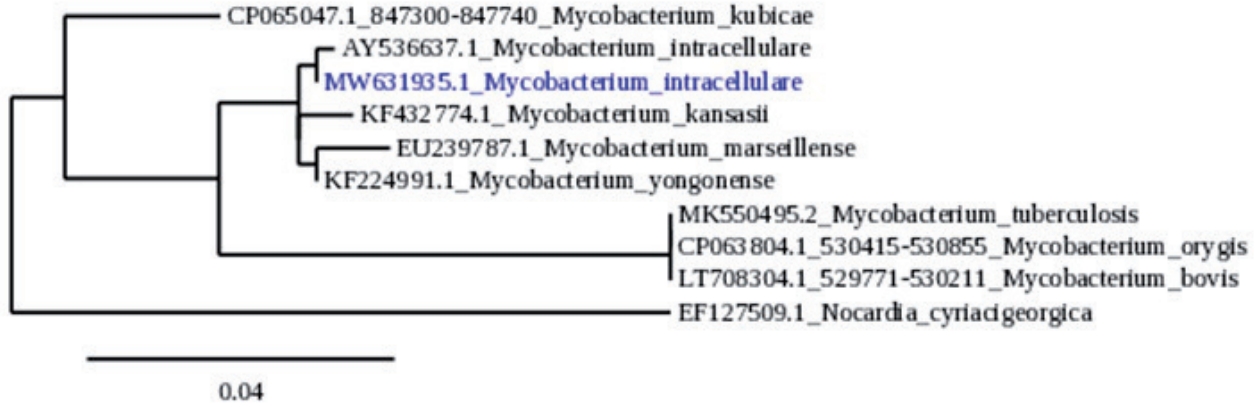

Figure 2. Phylogenetic tree built from the alignment of multiple sequences of the *hsp65* gene. It was assembled using the maximum likelihood method using the PhyML software. *Nocardia cyriacigeorgica* sequence was included as an outgroup.

apply the One Health concept are crucial for better management and prevention of MAC infections [4].

Despite the important findings, some limitations of this report included the impossibility of monitoring the treatment of the dog and investigating other alterations in the *post mortem* examination, especially if there were frequent pulmonary lesions in *M. intracellulare* infection in humans [8].

Chronic lymphadenomegaly, granulomatous inflammation and acid-resistant bacilli were clinical-histological findings that led to the diagnosis of non-tuberculous mycobacteriosis, and the genetic sequencing with the analysis of the phylogenetic tree concluded for infection by *Mycobacterium intracellulare*.

MANUFACTURERS

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Acknowledgments. The authors would like to thank the National Multiprofessional Residency in Health Commission by the scholarship granted. The authors would also like to thank Vitor Hugo Tadano da Conceição Padilha for his contribution to this work.

Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the contents and writing of the paper.

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