




Case Report

First report of *Mycobacteroides abscessus* subsp. *massiliense* and *Erysipelothrix rhusiopathiae* as causative agents of pneumonia and hepatitis in a boa (*Boa constrictor*)

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Abstract

An adult boa with acute epistaxis, without other clinical signs, was found dead in its terrarium. Macroscopic examination of the specimen revealed multiple yellow foci in the lung and liver. Microscopic findings included severe and multifocal granulomas as well as vasculitis, hemorrhages and thrombosis in liver and lungs, with the presence of intralesional Gram positive and acid-fast bacilli. PCR end point analysis and sequencing using total DNA extracted from formalin-fixed paraffin-embedded samples of liver and lungs identified *Mycobacteroides abscessus* subsp. *massiliense* and *Erysipelothrix rhusiopathiae*. Mycobacteria are organisms that can affect a wide range of animals, including reptiles. In snakes, reports are rare and mycobacterial species are highly variable. *Erysipelothrix rhusiopathiae* is a pathogenic bacillus related to multiple organ disorders, which until now has not been reported in boas. This report describes the first case report of *Mycobacteroides abscessus* subsp. *massiliense* and *Erysipelothrix rhusiopathiae* detection in a boa.

Keywords: *Boa constrictor*, *Mycobacteroides abscessus*, *Erysipelothrix rhusiopathiae*, PCR, pneumonia, hepatitis, granulomas.

Introduction

Mycobacteriaceae comprises more than 100 species of bacteria that share morphological features, such as being Gram-positive, aerobic and acid-fast bacilli (8). These microorganisms can affect a wide range of animals, including reptiles of different families (21). Most species are known as environmental mycobacteria or referred as non-tuberculosis mycobacteria (NTM) and are found as saprophytes, commensals and symbionts in different ecosystems (1). NTM are ubiquitous organisms that can be found in water sources and soil, thanks to the lipid-rich cell wall that facilitates their survival in harsh environments due to the biofilm that provides natural protection against desiccation and antimicrobial agents, but also promotes slow growth and

adherence to surfaces, which guarantees an outstanding advantage compared to other microorganisms (17). Unlike the species causing tuberculosis, NTM can infect an organism through the environment. Among this NTM group, *Mycobacterium abscessus* complex (MABC), recently designated *Mycobacteroides abscessus* complex based on the analysis of specific markers (7, 23), has recently come to attention as responsible for opportunistic pulmonary, skin and soft tissue infections. Some bacteria that are part of this group include *M. abscessus* subsp. *massiliense*, *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *bolletii* (13, 27). The main virulence trait of these organisms remains in the intracellular life adaptation thanks to their cell wall that allows them to survive as intracellular pathogens into macrophages, dendritic and epithelial cells (14).

In addition, it has been proved the ability of the bacteria to cross the blood-brain barrier causing severe nervous damage due to meningitis or meningoencephalitis (22).

Cases of snakes infected with mycobacteria are relatively rare, and the species reported are highly variable (9, 16, 21, 24). The pathogenic species of mycobacteria can affect different organs and systems of the same animal; in reptiles, the distribution of the lesions may be more common in some locations, being the respiratory and skin systems the most affected. Particularly in snakes, when infection occurs naturally, mycobacteriosis are mainly reported as systemic and lung disease (16).

Erysipelothrix rhusiopathiae is a non-sporulating Gram positive bacillus with worldwide distribution. It is mainly associated with endocarditis (10), pneumonia (15), and involvement of various organs, including multisystemic forms of the disease (28, 30). The disease presentations caused by *E. rhusiopathiae* have similar characteristics in humans and animals (26).

This ubiquitous organism is capable of surviving for long periods of time in different environments, including marine locations (4). *E. rhusiopathiae* can act as a commensal or as pathogen (29); animals can become infected by contact with organic products or waste released by infected animals such as saliva, urine, feces or sputum. Identified virulence factors include capsule, hyalurodinase, neuraminidase, which contribute to the pathogenicity of this microorganism (2, 25).

Pigs and birds are considered the most important reservoirs for this bacterium (29). Currently, its prevalence and frequency as a possible pathogen of reptiles, including snakes, is unknown.

Case description

We present the case of an adult boa, a companion animal, with clinical history of acute epistaxis that was found dead in its terrarium. The animal was sent for *post mortem* study to the Department of Pathology of the Faculty of Veterinary Medicine and Zootechnics of the National Autonomous University of Mexico (UNAM).

On the macroscopic evaluation, multiple white-yellowish granular foci were found in the lungs and liver (Fig. 1A); on histological sections stained with hematoxylin and eosin they corresponded to intraparenchymal granulomas (Fig. 1B, 1C, 1D) containing abundant intrahistiocytic Gram positive (Fig. 1E) acid-fast (Fig. 1F) bacilli, with occasional multinucleated giant cells in the periphery. Additionally, aleatory foci of mild hemorrhages, lymphocytic vasculitis and thrombosis were observed in both lungs and liver, as well as edema on lung parenchyma sections. No lesions were observed in any other tissue, including septicemia changes.

The presence of granulomas and the positive Ziehl-Neelsen staining suggested the presence of mycobacteria.

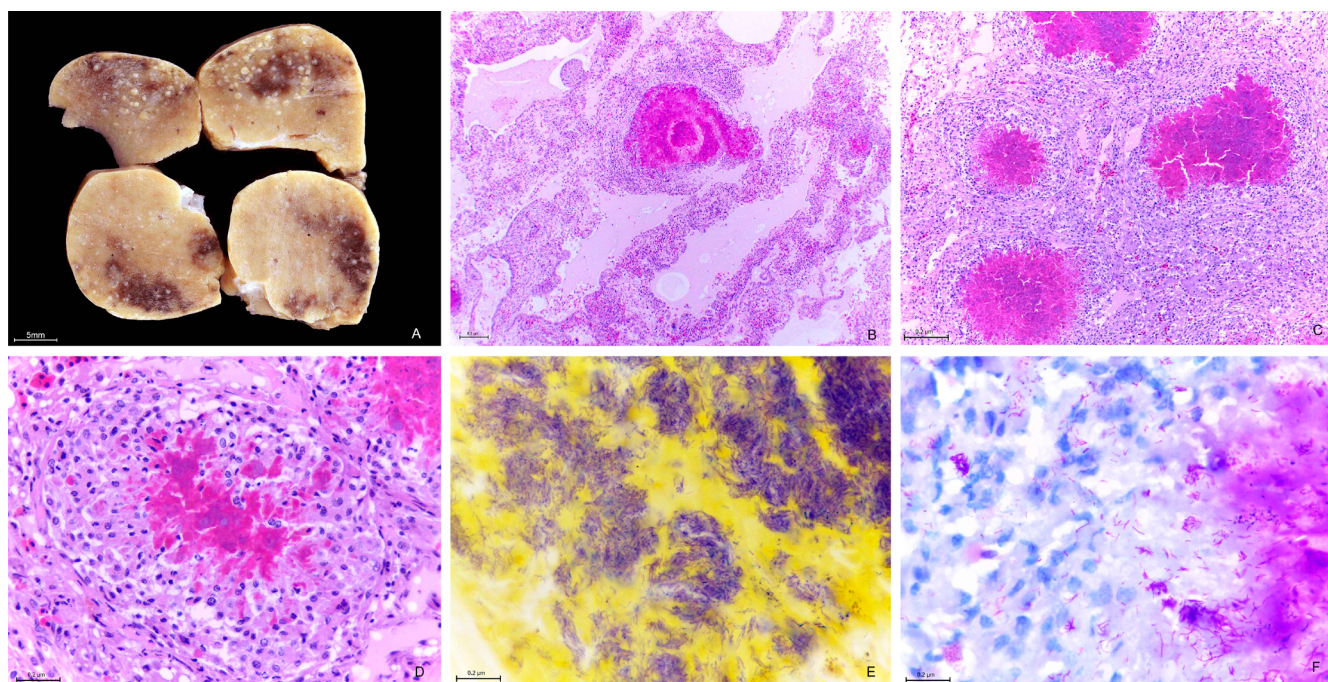


Figure 1. Pathological findings. A- The cut surface of the liver was yellow-tan and had multiple white foci and areas of hemorrhage. B- Lung. Faveoli are infiltrated by numerous histiocytes and show moderate hemorrhage and edema. Obj. 4x, H&E. C- and D- Liver. Most of the parenchyma is replaced by multiple granulomas with some histiocytes with blueish cytoplasm. Obj. 4x and 40x, H&E. E- Lung. Abundant Gram-positive bacteria were identified by Gram stain, Obj. 100x. F- Acid-fast bacilli were also seen by Ziehl-Neelsen stain, Obj. 100x.

We then extracted total DNA from paraffin-embedded sections of lungs and liver, using the DNeasy blood & tissue kit (QIAGEN, Ventura CA, USA), according to the manufacturer's instructions. In order to identify the involved species of mycobacteria, we followed two different strategies at the same time.

On one hand, we used a multiplex PCR reported by Chae *et al.*, to identify all mycobacterial species and discriminate between *Mycobacteroides abscessus* subsp. *abscessus*, *Mycobacteroides abscessus* subsp. *massiliense* and *Mycobacterium avium* complex (3). Primers 5'-GAGATACTCGAGTGGCGAAC-3' and 5'-CAACGCGACAAACCACCTAC-3' identify all mycobacterial species by amplifying a 506 bp fragment of 16S rRNA gene; primers 5'-GCTTGTTCCCGGTGCCACAC-3' and 5'-GGAGCGCGATGCGTCAGGAC-3' of *mass_3210* gene identify *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *massiliense*, as they amplify a 310 bp for *M. abscessus* subsp. *abscessus* or a 1145 bp for *M. abscessus* subsp. *massiliense*. We amplified the 506 bp fragment of 16S rRNA gene belonging to all mycobacterial species and a 1145-bp fragment which corresponds to *Mycobacteroides abscessus* subsp. *massiliense* (Fig. 2A).

On the other hand, we performed another PCR using two universal primer pairs (27F 5'-AGAGTTTGATC-MTGGCTCAG-3' and 1492R 5'-TACGGYTACCTTGT-TACGACTT-3') targeting the bacterial 16S rRNA gene. We amplified a ~1500-bp fragment that was purified using a QIAquick Gel Extraction Kit (Qiagen, Ventura CA, USA) according to the manufacturer's instructions and sequenced in both directions. The sequence was submitted to a BLAST search of all available databases at the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov>) and showed a 97.7% homology of *Erysipelothrix rhusiopathiae*. We submitted this sequence to the GenBank (accession

number OQ931241). The sequence analysis result made us to perform another PCR in order to confirm it. We used specific primers for *Erysipelothrix rhusiopathiae* (Ery-WT1 5'-CGATTATATTCTTAGCACGCAACG-3' and Ery-WT2 5'-TGCTTGTGTTGTGATTTCTTGACG-3') that amplified a 937-bp fragment of the DNA polymerase IV gene of *E. rhusiopathiae* (Fig. 2B) (19, 31).

These results confirmed the presence of both agents, *Mycobacteroides abscessus* subsp. *massiliense* and *Erysipelothrix rhusiopathiae*, affecting the lungs and liver of this boia.

Discussion

The PCR technique let us confirm the diagnosis of *Mycobacterium* spp. in the tissues examined by histology but also, it allowed us to identify the species (*Mycobacteroides abscessus* subsp. *massiliense*) as well as the presence of *Erysipelothrix rhusiopathiae*.

The described lesions in addition to the presence of intralésional bacteria (and its identification through PCR) in the liver and lungs, were associated with different mechanisms of damage by those two different microorganisms. Histiocytic granulomas on both organs were mainly related with the mycobacteria infection due to a direct immune response against the phagocytosis evasion mechanism. On the other hand, we consider that the vasculitis, thrombosis and hemorrhages described on both organs and the presence of lung edema were mostly related to *E. rhusiopathiae*, probably due to vascular damage, like previously reported in other species (6, 10, 11, 12). *E. rhusiopathiae* has several virulence factors such as neuraminidase, rhusiopathiae surface proteins A (RspA) and B (RspB), haemolysin, hyaluronidase, and some others (20). Neuraminidase mediates the vascular damage,

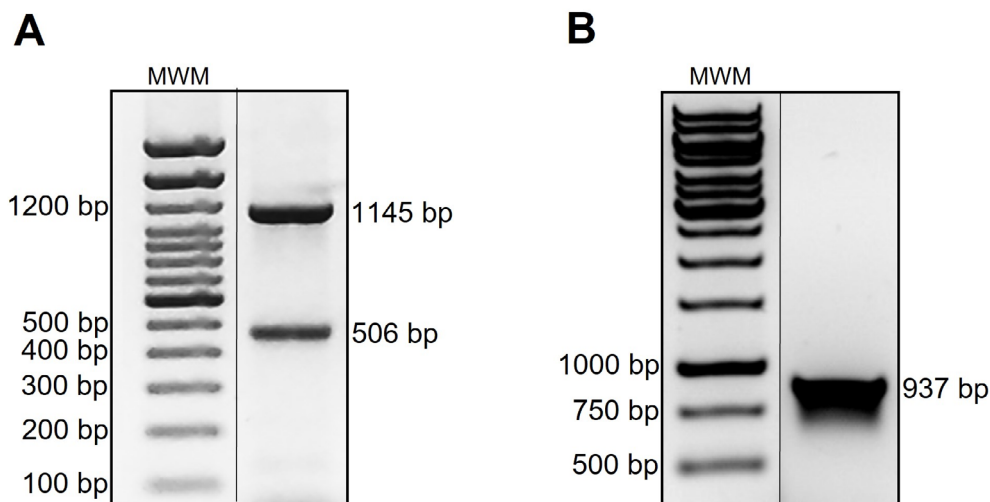


Figure 2. PCR identification. A- Amplification of the 506- and 1145-bp fragments corresponding to *M. abscessus* subsp. *massiliense*; MWM: 100 bp. B- Amplification of the 937-bp fragment corresponding to *E. rhusiopathiae*; MWM: 1 KB plus. DNA was extracted from paraffin embedded liver and lung tissues. 1% agarose gels stained with Sybr safe.

which also leads to thrombosis and interference with microcirculation in capillaries and venules at many sites (20, 25).

Based on the authors research, to the date there are not case reports or prevalence studies of *Erysipelothrix rhusiopathiae* or *Mycobacteroides abscessus* subsp. *massiliense* in reptiles or snakes. Although the pathogenesis of these bacteria in reptiles remains unknown, their identification can contribute to the understanding and possibly prevention of emergent diseases with potential zoonotic risk. Other mycobacterium species isolated from snakes are *Mycobacterium leprae*, *Mycobacterium haemophilum*, and *Mycobacterium marinum* (16).

The demonstration of the outstanding capability of MABC to survive successfully on several environments as well as within a host as a pathogen, has placed these bacteria into the first places of the list of the most threatening and dangerous pathogens, especially on immunosuppressed patients (14, 18). Its natural resistance in addition to an extremely low sensitivity to antibiotics provides a true challenge to deal with, on infected animals (18), thereby, it is necessary to consider them as a real threat on affected patients.

E. rhusiopathiae is an important opportunistic pathogen which can affect a wide range of domestic and wild animals, including birds, mammals, rodents, reptiles, fishes and arthropods (5), but no reports on snakes have been described yet. Described clinical forms of the disease are not characterized in reptiles, maybe due to insufficient clinical or pathological reports. Furthermore, it has been recovered from soil, decomposing plant material, fresh water and marine environment, being viable in the environment for long periods (12). These habitat features suggest that in this case, water and food sources, as well as the enclosure, could have been the source of infection for the boa. Suboptimal or inadequate environmental conditions that favored an immune decline could also contribute, as those same conditions are described with some others pathogens in reptiles (21).

Conflict of Interest

The authors declare no competing interests.

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