

Mastitis in cattle due to *Mycobacteroides abscessus* – case report

[*Mastite bovina por Mycobacteroides abscessus* – relato de caso]

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ABSTRACT

Mastitis is considered the main disease that affects dairy cattle worldwide, and it is caused mainly by *Staphylococcus aureus* and environmental *Streptococcus* spp. Eventually, nonconventional pathogens, as rapidly growing mycobacteria (RGM), may also cause chronic mastitis, which will not be responsive to antibiotic treatments. Diagnosis of mastitis caused by RGM is a difficult task, and most of time this agent may be misdiagnosed. Here we describe a case of clinical mastitis caused by the RGM *Mycobacteroides abscessus* in a cow from Southern Brazil, confirmed by microbiological and molecular characterization. Our results reinforce the necessity of a detailed laboratorial identification of the agent and to include this agent in differential diagnosis of chronic clinical mastitis nonresponsive to treatment.

Keywords: RGM, rapidly growing mycobacteria, milk, bovine

RESUMO

A mastite é considerada uma das principais doenças que afetam rebanhos leiteiros ao redor do mundo e é causada principalmente por *Staphylococcus aureus* e *Streptococcus* spp. ambientais. Eventualmente, patógenos não convencionais, como *Mycobacterium de crescimento rápido* (RGM), podem também causar mastite crônica, a qual não responderá ao tratamento com antimicrobianos. O diagnóstico de mastite causada por RGM é uma tarefa difícil, e a maioria das vezes esse agente pode ser subdiagnosticado. Neste relato, descreveu-se um caso de mastite clínica em uma vaca, no sul do Brasil, causada por RGM *Mycobacteroides abscessus*, confirmado por caracterização microbiológica e molecular. Os resultados reforçam a necessidade de um diagnóstico laboratorial detalhado para a identificação do agente e a inclusão deste como diagnóstico diferencial no reconhecimento de mastite crônica não responsiva ao tratamento.

Palavras-chave: RGM, *Mycobacterium de crescimento rápido*, leite, bovino

INTRODUCTION

Mastitis is an inflammation of the mammary gland, generally associated with intramammary infection. It is the main disease which affects dairy cattle worldwide, causing expressive economic losses to the dairy producer (Bradley, 2002). Organisms such as bacteria, yeasts and algae have been described as causes of bovine mastitis, which is normally divided in clinical or subclinical manifestation, which have a negative

impact in milk production and quality (Bradley, 2002).

The main pathogens causing mastitis worldwide are *Staphylococcus aureus* and environmental streptococci (Riekerink *et al.*, 2008). However, nonconventional pathogens may also cause chronic mastitis, which will not be responsive to antibiotic treatments. Some of those nonconventional pathogens include *Prototheca* spp. and environmental *Mycobacterium* sp. (or *Mycobacteroides* sp.) (Marques *et al.*, 2008; Machado *et al.*, 2015).

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Diagnosis of mastitis-causing rapidly growing mycobacteria (RGM) is a difficult task. Most of time, RGM species may be misdiagnosed due to their similarity with other conventional pathogens, such as *Corynebacterium* sp. At this point, the laboratory diagnosis of mastitis is of extreme importance, since it will be able to make the correct diagnosis and to perform the molecular identification/differentiation. Then, we describe a case of clinical mastitis in a cow due to RGM *Mycobacteroides abscessus* (formerly *Mycobacterium abscessus*) and its phenotypical and molecular characterization.

CASUISTRY

A Jersey cow, 4 years-old, from a small farm located in Southern Brazil, presented recurrent clinical mastitis nonresponsive to antibiotic treatment. The animal belonged to a small herd composed of four lactating cows which were manually milked. The affected cow had mastitis in previous lactation and was treated with antibiotic (systemic and intramammary therapy). As soon as the next lactation started, it was observed that the animal had pain in one quarter and the milk had a pinkish color, suggestive of blood. Immediately, the animal was treated with corticosteroid anti-inflammatory and parenteral antibiotic therapy composed by gentamicin and amoxicillin, followed by intramammary therapy with gentamicin. After those treatments, the owner started a homeopathic treatment using 12 drops of each, twice a day. The clinical signs disappeared, but the milk production remained reduced. To perform the identification of mastitis-causing agents, milk samples were aseptically collected from affected quarters and sent to laboratory to perform culture and antibiogram (SB 02/19 protocol). The milk sample for microbiological diagnosis was collected 25 days after the onset of clinical signs, which coincided with the period in which the antimicrobial and homeopathic treatment was performed. The milk sample was plated onto 5% sheep blood (BA) and MacConkey agar plates and incubated for 48h. Antimicrobial susceptibility assay was performed according to the routine protocol (CLSI, 2013).

RESULTS AND DISCUSSION

After 48h of sample inoculation, small, dry, and greyish colonies were observed only in BA

plates. On Gram stain, the colonies looked like Gram-positive bacilli shaped, and didn't color well. The isolate was non-motile, had fermentative metabolism of glucose, was positive in catalase, aesculin hydrolysis and indole tests, and negative to urease and oxidase enzymes. It was negative to hydrogen sulphide production and did not use citrate as a sole carbon source.

On antimicrobial susceptibility testing using disc-diffusion method, the isolate showed no inhibition halo to enrofloxacin (5µg), amoxicillin (10µg), penicillin (10µg), tetracycline (30µg), ampicillin (10µg), ceftiofur (30µg), sulfazotrim (25µg), cephalexin (30µg), doxycycline (30µg), ciprofloxacin (5µg) and cefepime (30µg), and showed a halo of inhibition for neomycin (30µg), amikacin (30µg) and gentamycin (10µg).

We suspected *Mycobacterium* sp. or *Corynebacterium* sp. according to the microbiological results, clinical signs, and laboratory findings. However, considering that *Corynebacterium* sp. normally is not a common cause of clinical mastitis and does not present a marked resistant profile in antimicrobial assays, identified colonies were submitted to PCR and nucleotide sequencing for 16s rDNA region (Lane, 1991) and a duplex PCR for hsp65 gene of *Mycobacterium* species, which could differentiate between *tuberculosis* or non-*tuberculosis* (Kim et al., 2006). The isolate was positive in both PCR, and the amplicons were submitted to DNA sequencing and molecular analysis.

The average nucleotide identity of the amplicons was analyzed using the Basic Local Alignment Search Tool (BLAST). SB 02/19 presented higher nucleotide identity with *Mycobacteroides abscessus* in the 16S rDNA region (100% with CP065287.1) and hsp65 gene (99.27 to 100% with CP060408.1 and AP018436.1). Phylogenetic tree of 16S rDNA region was built in MEGA X software using the maximum likelihood method with 1,000 bootstrap replicates, using alignment of nucleotide sequences obtained from GenBank (Fig. 1). SB 02/19 clustered with *M. abscessus* and apart from another *Mycobacterium* spp. and *Mycobacteroides* spp.

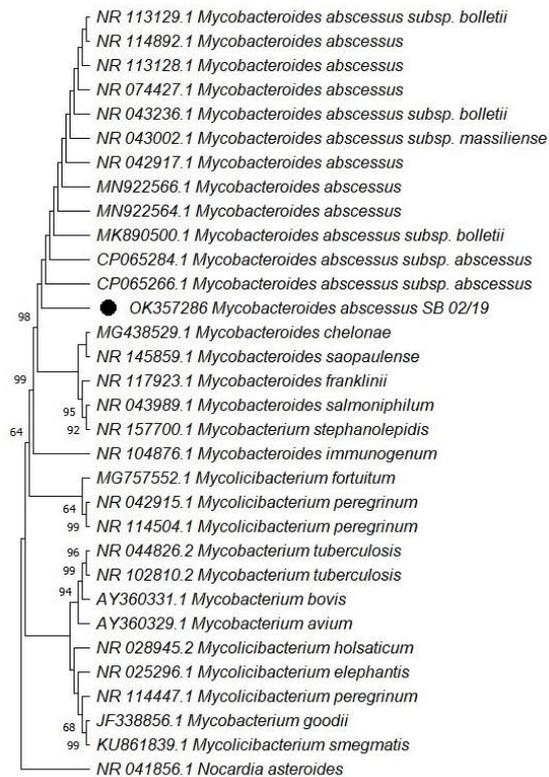


Figure 1. Phylogenetic tree based on sequence of 16S rDNA region. The evolutionary history was inferred using maximum likelihood method with 1000 bootstrap replicates. The evolutionary distances were computed using the Tamura-3 parameter model and implemented by MEGA X. Values > 60% are shown. The SB 02/19 is identified with a black circle.

M. abscessus is a ubiquitous environmental organism and an opportunistic pathogen that can cause various diseases in humans and animals (Lunn *et al.*, 2005). The occurrence of infection caused by *M. abscessus* is extensively described in humans, especially in cases of infection of the surgical site and lung infection, being a major problem for people with cystic fibrosis (Jönsson *et al.*, 2007; Leão *et al.*, 2010). In animals, a range of different infections have been described in many species (Bercovier and Vincent, 2001; Lunn *et al.*, 2005).

M. abscessus causing mastitis in cattle is not a common pathogen described, but other RGM associated with mastitis cases have been described more frequently, as *M. goodii* (Machado *et al.*, 2015) and *M. elephantis* (Ji *et al.*, 2017). Once it is an environmental organism,

and according to the mastitis case related by Ji *et al.*, 2017 caused by another *Mycobacterium non-tuberculosis*, we believe that in this case the mastitis started after a contamination from the environment, in an ascending route. *M. abscessus* probably entered the udder by contaminated materials, such as antibiotic cannula, reinforcing the need for using clean and sterile materials during mastitis treatment.

The presence of *M. abscessus* as well other non-tuberculous *Mycobacterium* in samples, such as milk, may be a big deal for disease control, once people and animals infected may have the agent but only develop the disease on an immune system failure (Jönsson *et al.*, 2007). In this case, the farmer reported that the cow had recurrent mastitis, which was treated before and regressed, but when a new lactation started the disease was manifested again.

CONCLUSIONS

Our findings showed that RGM species, as *M. abscessus*, should be also considered in the investigated of recurrent clinical mastitis, especially in cases that are not responsive to antibiotic treatment.

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