

## ***Mycoplasma bovis*-polyarthritis and pneumonia in grazing beef steers: outbreak in Buenos Aires province**

Poliartritis y neumonía por *Mycoplasma bovis* en novillos en pastoreo: brote en la provincia de Buenos Aires

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**ABSTRACT.** *Mycoplasma spp.* infection causes different clinical diseases in livestock. In cattle, infection with *Mycoplasma bovis* usually is clinically manifested with arthritis, mastitis, pneumonia and/or otitis. This paper describes an outbreak of polyarthritis and pneumonia caused by *M. bovis* in grazing steers in Buenos Aires province, scarcely reported in the region. Clinical and pathological findings were recorded. Three 1-year-old steers were euthanized and *post mortem* examined. Tissue samples were collected for histopathology and bacteriological analysis. Different degrees of lameness were observed in 4.6% of the herd of 1000 steers. Mild respiratory signs were observed in half of the lame animals. Macroscopically, polyarthritis was present in different joints of the hind and front limbs, characterized by the presence of severe caseous tenosynovitis. In addition, multiple foci of different extension with caseous content were observed in the pulmonary parenchyma. *Mycoplasma spp.* was isolated and *M. bovis* DNA was amplified from articular content and lungs of all three animals. This clinical presentation has not been frequently reported in the region, particularly in grazing beef cattle.

**KEY WORDS:** mycoplasmosis; *Mycoplasma bovis*; grazing; pastoral; cattle; Argentina.

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**RESUMEN.** Las infecciones por *Mycoplasma* spp. causan diferentes cuadros clínicos en el ganado. En bovinos, la infección con *Mycoplasma bovis* usualmente provoca artritis, mastitis, neumonía y/u otitis. Existe escasa información sobre cuadros clínicos asociados a infecciones con *Mycoplasma* spp. en novillos en pastoreo en Argentina. Este trabajo describe un brote de poliartritis y neumonía causada por *M. bovis* en novillos en pastoreo en la provincia de Buenos Aires, escasamente reportada en la región. Se registraron los signos clínicos y hallazgos patológicos. Se realizaron necropsias a tres novillos de un año de edad. Se recolectaron tejidos para realizar estudios histopatológicos y bacteriológicos. Se registraron diferentes grados de claudicación en el 4,6% de los animales de un rodeo de 1000 novillos. Se observaron signos respiratorios leves en la mitad de los novillos que manifestaron claudicación. Macroscópicamente, se observó poliartritis en las articulaciones de los miembros delanteros y/o traseros, caracterizada por la presencia de tenosinovitis caseosa severa. Además se observaron múltiples focos de contenido caseoso en el parénquima pulmonar. Se aisló *Mycoplasma* spp. y se amplificó ADN de *Mycoplasma bovis* de todas las muestras de contenido articular y pulmón de los tres animales. Esta presentación clínica no ha sido frecuentemente reportada en la región, particularmente en bovinos de carne en pastoreo.

**PALABRAS CLAVE:** mycoplasmosis; *Mycoplasma bovis*; pastoreo; engorde; bovinos; Argentina.

## INTRODUCTION

*Mycoplasma* spp. infection causes different clinical manifestations in livestock. In cattle, the main clinical signs associated with *Mycoplasma bovis* are arthritis, mastitis, pneumonia, and otitis (Pfützner *et al.*, 1996). *Mycoplasma* spp. can be transmitted directly through milk from cows with mastitis to their calves or through respiratory secretions (aerosols). Moreover, it could be less frequently indirectly transmitted, through the consumption of water and feed contaminated with secretions from infected animals (Nicholas *et al.*, 2003; Fox *et al.*, 2008). The incubation period ranges from 3 weeks to 6 months (Pfützner *et al.*, 1996). Once the infection is established in the respiratory system, the bacteria can spread by the hematogenous route and reach the joints causing, arthritis, in one or more joints (Maunsell, 2009).

Although *M. bovis* has been previously isolated from other clinical presentations of dairy (Cerdá *et al.*, 2000) and feedlot cattle (Cantón *et al.*, 2021), this work describes an outbreak of polyarthritis and mild respiratory signs in beef grazing steers of different origins associated with *M. bovis*.

## CASE DESCRIPTION

### Epidemiology

On February of 2021 Aberdeen Angus steers of approximately 100 kg of live weight, from different origin arrived to a farm located in Olavarría (36°38'14.1"S 60°24'43.2"W), Buenos Aires

province, Argentina. At arrival they were vaccinated with two doses against clostridial and respiratory diseases (Bioclostrigen® and Biopoligen®, Biogénesis-Bagó, Argentina). They also received copper-zinc supplementation (Suplenut®, Biogénesis-Bagó, Argentina), and were de-wormed with levamisole (Fosfamisol®, Biogénesis-Bagó, Argentina) and ivermectin (Bagomectina®, Biogénesis-Bagó, Argentina). These animals were grazing on polyphitic pastures without any supplementation.

The outbreak of mycoplasmosis affected 4.6% of 1,000 steers. Approximately half of the affected steers died four days to a week after the initiation of clinical presentation, with negative response to subcutaneous administration of tilmicosin (10 mg/kg) (Maxitil®, Biogénesis-Bagó, Argentina).

### Clinical signs

Forty-six steers showed poor body condition and different degrees of lameness (1 to 5, in the lameness score described by Sprecher *et al.*, 1997) with enlarged hind and/or front limb joints. In a few steers, mild coughing was also present. Clinical examination was carried out in three affected steers (Table 1). Euthanasia was performed in these three severely affected steers according to the methodology approved by the Animal Ethics Committee of INTA Balcarce.

	Rectal temperature (°C)	Heart rate (beats per minute)	Respiratory rate (movements per minute)
Steer #1	39.5	92	28
Steer #2	38.6	80	20
Steer #3	38.5	128	60
Reference values (Constable <i>et al.</i> , 2016)	< 39.2	< 90	<30-40

**Table 1.** Rectal temperature, heart and respiratory rates of the three affected steers and references values.

## Necropsy

During *post mortem* examination, enlargement of different joints was the most prominent pathological change in the three steers, and to a lesser extent, in the lungs. In some cases, only one joint was affected, but in most of the cases several joints were enlarged. Steer #1 showed tenosynovitis in the right tarsus with proliferation of caseous material (figure 1), and excessive accumulation of synovial fluid in the left femoro-tibio-patellar joint. Multifocal white-yellow round lesions of different sizes that contained caseous material (interpreted as sequestrations) (figure 2) were observed affecting 10% of the right lung parenchyma, in the cranio-ventral lobe. In steer #2, abundant translucent opaque synovial fluid with the presence of fibrin was observed in the right coxo-femoral joint. Seventy percent of the left lung was consolidated and multifocal to coalescent caseous areas surrounded by fibrous material (“sequestration”) were observed. Mediastinal lymphadenomegalia was also present. Tenosynovitis was observed in the right femoro-tibio-rotulian and humero-radio-ulnar joints of steer #3, with caseous content and presence of abundant opaque translucent synovial fluid. Twenty percent of the left ventral lung lobe was also affected, presenting consolidation and multiple caseous foci.

## Laboratory results

Tissue samples (synovial capsule, lung, mediastinal lymphnodes, heart, kidney, liver, spleen, abomasum, rumen, duodenum and colon) were collected from steers #1, #2 and #3, fixed in 10% neutral buffered formalin, paraffin-embedded, sectioned at 4-5 µm and stained with hematoxylin and eosin (HE) for histologic examination. Microscopically, in different joints of the three steers, the synovial stroma had multifocal necrotic areas similar to those observed

in the pulmonary parenchyma. These areas were encircled by moderate amount of granulation tissue. In addition, severe infiltration of many neutrophils, macrophages, lymphocytes, and plasma cells; and prominent fibroblast hyperplasia were observed in the synovial stroma. Furthermore, multifocal necrotic areas were observed in the lung (figure 3). These foci were characterized by the presence of necrotic inflammatory cells that had intense eosinophilic cytoplasm and lysis of the nuclei (karyorrhexis and karyolysis). In addition, these foci were delineated by a band of neutrophils and macrophages, encircled by a layer of fibroblasts, macrophages, lymphocytes, and plasma cells. The lumen of the bronchi and bronchioles contained moderate infiltration of neutrophils and macrophages, and the subepithelium was infiltrated by lymphocytes and macrophages. The interalveolar and interlobular septa were infiltrated by macrophages and scarce neutrophils and plasmocytes. No other relevant microscopic lesions were present in the affected animals.

Synovial capsule, synovial fluid, and lung samples were inoculated onto *Mycoplasma* Base Medium with Selective *Mycoplasma* supplement (MM) (Oxoid Ltd., Wad Road, Basingstoke, UK), Columbia Blood Agar (CBA) (Oxoid Ltd., Wad Road, Basingstoke, UK) with 7% bovine blood and MacConkey agar (MC) (Oxoid Ltd., Wad Road, Basingstoke, UK). All plates were incubated at 37°C, MM under 5% CO<sub>2</sub>, CBA under 10% CO<sub>2</sub> and MC under aerobiosis, and examined at 96, 48 and 24 h, respectively. *Mycoplasma spp* was isolated from articular content and the lung of steers #1, #2 and #3. *Mycoplasma* species confirmation was conducted through a specie-specific PCR, previous DNA extraction using a commercial kit (Puri-Prep S, Inbio Highway, Argentina), according to the authors conditions (Thomas *et al.*, 2004) using as positive control DNA from a previous study (Sosa

*et al.*, 2018). *Mycoplasma bovis* was identified in all the isolates. No other bacterial pathogens were identified in CBA and MC media. Lung smears were heat-fixed and stained using Ziehl – Neelsen (ZN) methods and no acid-fast bacteria were detected.

## DISCUSSION AND CONCLUSIONS

Mycoplasmosis is usually characterized by polyarthritis and pneumonia in cattle (Nicholas *et al.*, 2003; Caswell *et al.*, 2016; Cantón *et al.*, 2021). Nevertheless, outbreaks of these clinical signs are scarcely reported in grazing beef cattle of Argentina (Margineda *et al.*, 2017; Cantón *et al.*, 2021). To the best of our knowledge, this is the first description of this clinical presentation associated with *M. bovis* in this production system in Argentina, because of the presence of the agent has been reported in feedlot (Cantón *et al.*, 2021) and in dairy farms (Cerdá *et al.*, 2000, Sosa *et al.*, 2018, Neder *et al.*, 2021).

The affected steers were introduced to the farm several months before the first clinically affected animal was detected. They were bought from different locations so it is difficult to establish if some carrier animals were the point-source of the disease. *Mycoplasma spp.* can survive in clinically healthy infected carrier animals (Thomas *et al.*, 2002).

Upon arrival, the steers were kept in a semi-intensive grazing system and possibly the close contact between animals allowed a high infection rate. After confirming the diagnosis of mycoplasmosis, affected animals were isolated from the herd. All the affected animals and the rest of the herd were subcutaneously treated with enrofloxacin (2.5 mg/kg) (Baytril®, Bayer, Argentina). Nevertheless, the most severely affected steers did not show any clinical recovery. Early treatment is likely to be more successful before extensive tissue necrosis develops (Adegbove *et al.*, 1996). Furthermore, the efficacy of antimicrobial treatment is limited and, although animals occasionally recover, infections can be persistent (Shahriar *et al.*, 2002).

Since there are no commercially available vaccines in the region for the prevention of *M. bovis* infection, after the suspicion of mycoplasmosis, it is important to carry out active surveillance to identify and remove clinically affected animals and possible sources of infection to the other animals in the herd

(Nicholas *et al.*, 2003). Metaphylactic treatment has shown variable results (Gourlay *et al.*, 1989; Daniels *et al.*, 2000). Although it could delay the clinical appearance of the disease, it is difficult to eliminate the infection (Godinho *et al.*, 2005).

These clinical presentations have not been widely described in the region, mainly in grazing systems. They should be included in the differential diagnosis of polyarthritis and pneumonia in grazing young cattle.

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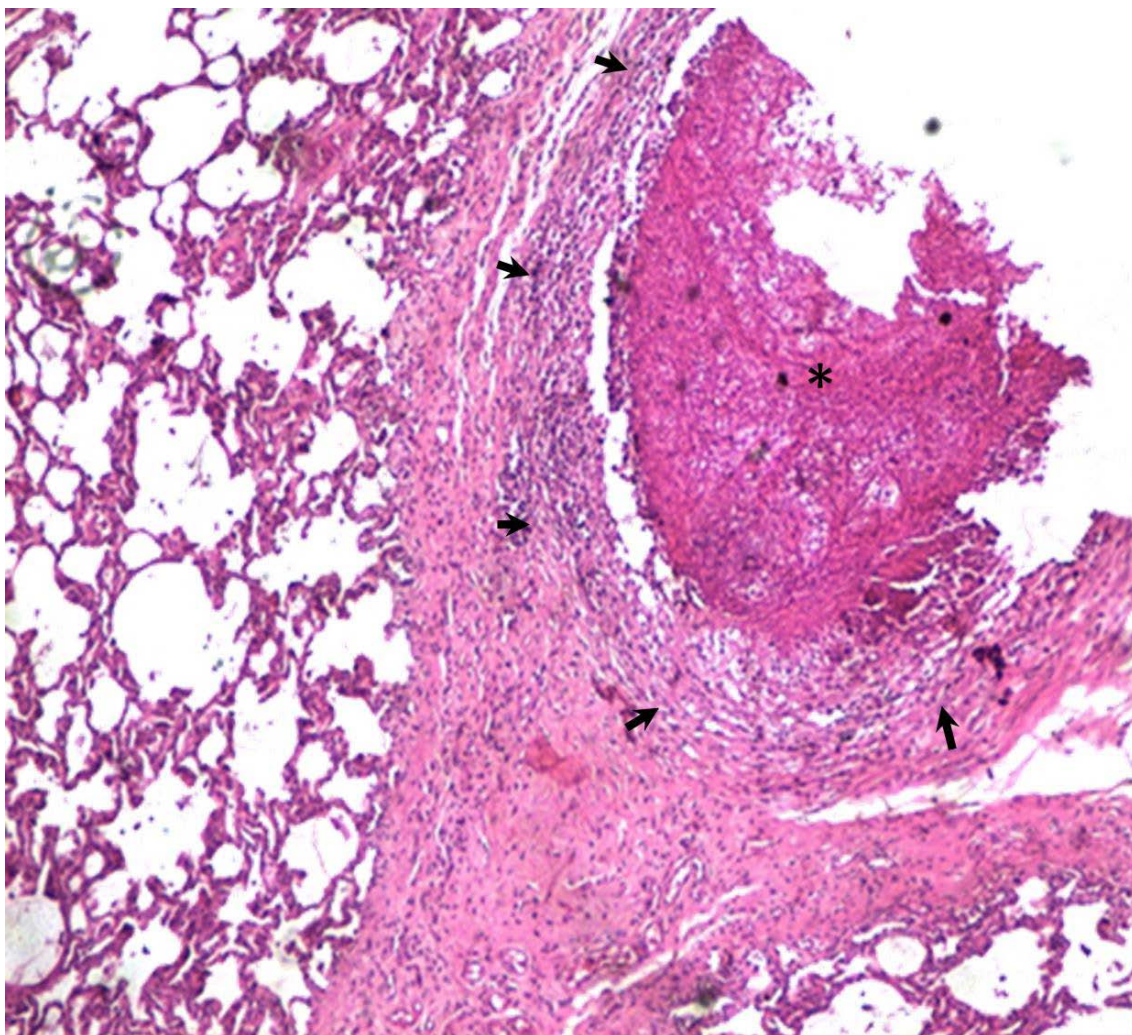
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Figure 1. Caseous tenosynovitis in the right tarsus of steer #1.



**Figure 2.** Numerous foci of caseous necrosis with sequestration in the lung parenchyma of steer #1.



**Figure 3.** Encapsulated necrotic foci (asterisk) in the lung of steer #3, encircled by moderate amount of granulation tissue (arrow-heads).