

# Mycoplasma Bovis-pneumonia and Polyarthritits in Feedlot Calves in Argentina: First Local Isolation

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# Abstract

Bovine respiratory disease (BRD) is one the most frequently occurring clinical problem in weaned calves after their arrival at the feedlot. Infectious pneumonia usually has complex causes, involving several microorganisms. *Mycoplasma* is frequently detected in BRD, especially *Mycoplasma bovis*. This work reports the first local isolation of *M. bovis* from feedlot calves with pneumonia and polyarthritis in Argentina. Twenty four out of 545 calves showed progressive, subacute to chronic respiratory distress, coughing, and fever. Thirty percent of the affected calves also showed lameness and swelling of elbow or carpal, and knee or tarsal joints. Five necropsies were performed and severe multifocal to coalescent pulmonary nodules, containing white-yellowish caseous exudate encircled by fibrous tissue, and fibrinonecrotic arthritis and tenosynovitis were detected. *Mycoplasma* was isolated from lung and joint samples. The 16S-23S rRNA ITS consensus sequence obtained from these isolates showed 100 % similarity with the same region of *M. bovis* strains. This work should alert practitioners about the presence of mycoplasma infections as the cause of BRD in the region. Since there are no commercially available vaccines in the region for the prevention and control of *M. bovis* pneumonia and arthritis, surveillance is a priority to reduce the source of disease to naïve animals.

## Introduction

Bovine respiratory disease (BRD) is one the most frequently occurring clinical problem in weaned calves after their arrival at the feedlot (O'Connor et al. 2001), causing important economic losses in the beef industry (Schneider et al. 2009). Infectious pneumonia usually has complex causes, involving two or more microorganisms and is commonly predisposed by environmental factors (Taylor et al. 2010). Viruses most frequently associated with BRD are bovine herpesvirus type 1 (BoHV1), bovine parainfluenza virus type 3 (BPIV-3), bovine respiratory syncytial virus (BRSV), and bovine viral diarrhea virus (BVDV). Secondary bacterial infections are usually associated with *Mannheimia haemolytica*, *Pasteurella multocida* and *Histophilus somni*. Other bacteria frequently detected in BRD are mycoplasmas, especially *Mycoplasma bovis* (Nicholas and Ayling 2003; Gagea et al. 2006; Booker et al. 2008; Fulton et al. 2009).

*M. bovis* is most commonly recognized as a cause of pneumonia and arthritis in calves and mastitis in dairy cattle in North America and Europe (Gagea et al. 2006; Caswell et al. 2010; Murray et al. 2017). Margineda et al. (2017) reported for the first time the presence of *M. bovis* as a cause of pneumonia in feedlot cattle and dairy calves in Argentina. Nevertheless, the prevalence, morbidity, mortality, and economic relevance of *M. bovis* pneumonia in the BRD complex in Argentina is still unknown.

Although *M. bovis* has been previously isolated from other clinical presentations of dairy cattle (Cerdá et al. 2000) and *M. bovis*-pneumonia has been previously diagnosed in Argentina (Margineda et al. 2017), this work reports the first local isolation of *M. bovis* from feedlot calves with pneumonia and polyarthritis in Buenos Aires province, Argentina.

# Materials And Methods

## *Clinical history of the herd*

The outbreak occurred in a feedlot in Carlos Tejedor department (35°11'01"S 62°36'16"W), Buenos Aires province, Argentina. During December 2018 until January 2019, 545 early-weaned calves of 45-55 kg arrived at the feedlot, from three farms. Upon arrival, calves were twice tilmicosin-treated (metaphylaxis, days 0 and 21 post-arrival) and immunized using a commercial polyvalent vaccine against BoHV1, *P. multocida*, *Moraxella bovis*, *Clostridium chauvoei* and *Clostridium perfringens* (days 0, 21 and 42 post-arrival). The diet consisted of cracked corn, soybean expeller, wheat bran and a commercial vitamins-minerals premix. Calves were allocated in three different lots: 200 in lot A, 143 in lot B and 202 in lot C.

## *Post mortem examination and tissue sampling*

After clinical examination the five most clinically affected calves were euthanized according to the regulations of the Animal Ethics Committee of INTA and necropsied accordingly. Samples from central nervous system, heart, liver, spleen, kidney, muscle, lung, mediastinal lymph nodes and synovial capsules were collected and fixed in 10% neutral buffered formalin for histopathological and immunohistochemistry (IHC) examination. Also, lung, synovial fluid and capsules samples were collected for microbiological examination.

## *Histopathology and Mycoplasma bovis immunohistochemistry*

Formalin fixed tissues were paraffin embedded, sectioned at 4-5 µm and stained with hematoxylin and eosin (HE) for histologic examination. Formalin fixed and paraffin embedded lung and synovial capsules were examined using IHC for the detection of *M. bovis* as described previously (Haines et al. 2004) using mouse anti-*M. bovis* monoclonal antibody (Millipore MAB970) at 1:100 dilution. Positive and negative controls were included (Margineda et al. 2017). No other *Mycoplasma* spp. were tested by IHC in this study.

## *Microbiology*

Lung and synovial fluid samples were inoculated onto Mycoplasma Base Medium with Selective Mycoplasma supplement -MM- (Oxoid Ltd., Wad Road, Basingstoke, UK) and 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. Genera were classified according to the Bergey's Manual of Determinative Bacteriology (Bergey and Holt 1994). Lung smears were heat-fixed and stained using Ziehl – Neelsen (ZN) methods to detect acid-fast bacteria (AFB).

## *PCR and sequencing*

Molecular detection of *Mycoplasma* was performed for both, clinical samples and to confirm the presence of the agent after culture. Briefly, DNA was extracted using the commercial kit Puri-Prep S (Inbio Highway, Argentina) according to the manufacturer's instructions. For mycoplasma detection, a nested PCR targeting 16S-23S rRNA intergenic spacer region (ITS) was performed under the conditions reported by Tang et al. (2000) using primers previously reported (Nakagawa et al. 1992; Harasawa et al. 1993). To identify the mycoplasma species, the obtained PCR products were purified (Puriprep-GP Kit, Inbio Highway), quantified and sequenced (ABI 3130xl; Applied Biosystems) using the inner primers described by Harasawa et al. (1993). The sequences were curated using the BioEdit software and aligned using Clustal Omega software. Since all the sequences were identical, a unique consensus sequence was obtained and then aligned against the database using nucleotide BLAST (<http://www.ncbi.nlm.nih.gov/blast>) excluding uncultured and environmental sample sequences.

### *Virology*

Lung samples were homogenized in Eagle's minimum essential medium (MEM) (Gibco, 4150034; Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum, and inoculated on Madin-Darby Bovine Kidney (MDBK) cells. Cell cultures were incubated at 37°C and 5% CO<sub>2</sub> for 5 days and examined daily for cytopathic effect. After four consecutive passages, cultures were tested for BoHV-1, BPIV-3, BRSV and BVDV by direct fluorescent antibody tests.

## **Results**

Clinical cases were initially reported in lots A (9 cases) and B (13 cases). The first two cases occurred during December 2018, 1 on January, 9 during February and 10 during March 2019. All the affected calves (24 out of 545; morbidity = 4.4%) showed progressive, subacute to chronic respiratory distress, coughing, hyperpnea, poor body condition, lethargy, dehydration and fever (39.5 to 41.3°C). Thirty percent of the affected calves also showed lameness, grinding noise when they walk, and pain in one or more joints associated with visible swelling of the affected front (Fig. 1a) and hind leg joints (elbow or carpal, and knee or tarsal joints, respectively).

*Post mortem* examinations were performed in five affected calves. Fibrinonecrotic arthritis and tenosynovitis were detected in the affected joints (Fig. 1b). The lungs of the five calves showed cranioventral consolidation affecting 20 to 50% of the pulmonary parenchyma. All the examined lungs showed multifocal to coalescent white nodules, some of them protruding above the pleural surface (Fig. 1c). On section, white nodules ranged 0.3 to 2.5 cm and contained white-yellowish caseous exudate, encircled by fibrous tissue (Fig. 1d). Pleural fibrosis was observed in one of the examined animals (Fig. 1e) and chronic pleural adhesions in two calves. No other gross lesions were observed in the affected necropsied calves.

Histologically, multifocal necrotic areas in the pulmonary parenchyma were observed. These foci contained many necrotic inflammatory cells that retained their cellular outlines but had intensely

eosinophilic cytoplasm and lysis of the nuclei (Fig. 2a and 2b). These foci were delineated by a band of neutrophils and macrophages, encircled by a layer of fibroblasts macrophages, lymphocytes, and plasma cells. The smaller bronchioles contained an accumulation of necrotic leukocytes in the bronchiolar lumen and the epithelium was discontinuous. The bronchiolar walls were thickened by edema and infiltrate of lymphocytes with fewer neutrophils and macrophages. Similar foci of caseous necrosis that contained recognizable necrotic leukocytes were occasionally present in the alveoli. Macrophages and scarce plasmocytes were infiltrating the interlobular septa. Follicular hyperplasia was observed in mediastinal lymph nodes. In joint capsule, fibrin admixed with many neutrophils was adherent to the synovium, with areas of hyperplasia alternating with areas of necrosis or denudation of synoviocytes. The subsynovial stroma had severe infiltration of many neutrophils, macrophages, lymphocytes, and plasma cells; and prominent fibroblast hyperplasia (Fig. 2c). No other microscopical lesions were detected in the affected calves.

Immunohistochemistry showed abundant *M. bovis* antigen in the lungs and joint capsule of calves. In lungs, the positive staining was observed mainly at the margin of the necrotic lesions, and to a lesser extent in the center of the necrotic foci. In bronchioles containing caseous debris, antigen was present within the debris and adjacent to bronchiolar epithelial cells (Fig. 2d). Antigen of *M. bovis* was identified in synovium and stroma subsynovial within the debris and adjacent neutrophils and macrophages. Staining for *M. bovis* antigen was not visible in the sections of negative controls.

Typical *Mycoplasma* fried-egg-shaped colonies were observed in all the lung samples from the five calves (Fig. 2e). In none of the synovial fluid samples compatible-*Mycoplasma* colonies were observed. Lung and synovial fluid sampled during the necropsies of calves #1, #2, #3 and #4 resulted negatives for the isolation of aerobic and microaerophilic bacteria using the routine diagnostic procedures. *Trueperella pyogenes* and *H. somni* were isolated from calf #5 lung sample. No AFB were observed in the ZN staining of lung smears.

The lung samples from all calves and synovial samples from calves #1, #3, and #4 rendered PCR positive results. The presence of the agent was also confirmed in the lung cultures. The 16S-23S rRNA ITS consensus sequence obtained showed 100 % similarity with the same region of *M. bovis* strains NADC59 (CP042939.1), MJ1 (CP042938.1), KG4397 (AP019558.1), NADC61 (CP022599.1), NADC67 (CP022596.1), NADC62 (CP022595.1), NADC58 (CP022594.1), NADC57 (CP022593.1), NADC56 (CP022592.1), NADC55 (CP022591.1), NADC54 (CP022590.1), NADC18 (CP022589.1), MJ4 (CP022588.1), MJ3 (CP022587.1), MJ2 (CP022586.1), JF4278 (LT578453.1), Ningxia-1 (CP023663.1), 08M (CP019639.1), 72242 (KX687011.1), 393B08 (KX687010.1), 268B07 (KX687009.1), HB0801-P115 (CP007589.1), NM2012 (CP011348.1), 1982-M6152 (CP058969.1), 2019-043682 (CP058968.1), PG45 (CP002188.1), 70-213 (AY779747.1) and ATCC 25523 (AY729934.1).

BoHV-1, BPIV-3, BRSV and BVDV isolation resulted negative in the lung samples tested.

## Discussion

The bovine respiratory disease causes important economic losses in the beef industry (O'Connor et al. 2001) and is described as multifactorial with different etiological agents involved (Gagea et al. 2006; Fulton et al. 2009). Although *M. bovis* is frequently detected in association with BRD worldwide (Gagea et al. 2006; Fulton et al. 2009; Murray et al. 2017), only one description of the disease is available in Argentina (Margineda et al. 2017).

This report describes an outbreak and the first isolation of *M. bovis* in feedlot calves with chronic pneumonia and polyarthritis in Argentina. Clinical signs observed in the animals are similar to the previous reports: subacute to chronic respiratory distress with fever and severe lameness resulting from polyarthritis (affecting carpal and tarsal joints, mainly), also known as “pneumonia-arthritis syndrome” (Adegboye et al. 1996; Gagea et al. 2006). Failure of antibiotic treatment (Ayling et al. 2000) and retarded growth are other characteristics of the disease (Shahriar et al. 2002), as it was observed in this outbreak. According to the information recorded during the occurrence of the outbreak, 4.4% of the exposed calves were affected. However, the incidence of mycoplasma pneumonia can be as high as 100% (Pfützner and Sachse 1996). Nevertheless, before the disease was confirmed in this feedlot, some of the calves in the affected lots (A and B) were moved into different lots with other animals. Then, *Mycoplasma*-like disease was observed in these animals (veterinary practitioner personal communication), showing how easy transmission occurred (Pfützner 1990). Therefore, the exact epidemiological rates of this outbreak are actually unknown. Certain animals may act as reservoirs of *Mycoplasma* in the respiratory tract without developing the clinical disease (Thomas et al. 2002) and probably, reservoir calves may have been introduced in December or January, providing the source for infection to in-contact calves, as it was previously reported (Allen et al. 1992). No previous history of these calves was available to explain this issue. Tilmicosin-treatment of these calves was probably not efficient in order to reduce their reservoir status, since this is not recommended as effective for *M. bovis* therapy. On the other hand, enrofloxacin, florfenicol and spectinomycin would be better options as metaphylactic antibiotic treatment (Caswell et al. 2010).

*Post mortem* diagnostic during BRD should be carried out in untreated animals in the initial stages of the clinical disease. Therefore, diagnosis of BRD due to *M. bovis* sometimes have some difficulties, since chronically affected calves probably have been already treated with a variety of antimicrobials (Cooper and Brodersen 2010). Nevertheless, pathological changes associated with *Mycoplasma* pneumonia are characteristic and can provide useful information. *Mycoplasma* pneumonia is characterized as subacute or chronic suppurative bronchopneumonia with multiple foci of caseous necrosis (Adegboye et al. 1995; Pfützner and Sachse 1996; Gagea et al. 2006; Caswell et al. 2010), as they were observed during the five necropsied calves in this outbreak. Histopathologically, foci of acute coagulative necrosis surrounded by a densely basophilic border of necrotic leukocytes (“oat cells”) are also morphologically distinctive from other bacterial etiologies of BRD (Pfützner and Sachse 1996; Gagea et al. 2006).

Bovine respiratory disease is usually caused by multiple microorganisms and their identification in tissue samples from an affected calf should be carefully interpreted (Hodgins et al. 2002; Nicholas and Ayling 2003). In one of the sampled lungs, *T. pyogenes* and *H. somni* were isolated. These bacteria could be

responsible for BRD. However, the clinical history and the pathological findings resemble “pneumonia-arthritis syndrome” previously associated with *M. bovis* (Adegboye et al. 1996; Gagea et al. 2006).

Molecular diagnostics have substituted classic diagnostic procedures such as culture for *Mycoplasma* spp and other fastidious microorganisms, providing very specific, sensible and rapid tests (Cooper and Brodersen 2010). During this work, PCR was applied as a screening test, and then, the tissue samples were cultured and *Mycoplasma* was isolated. Although previous reports mentioned similar isolation success on lung and synovial samples (Adegboye et al. 1996), in this work, only lung samples resulted positive for *Mycoplasma* isolation. Previous reports DNA amplicons were then sequenced and 100% nucleotide identity was observed with *M. bovis* reference strains, confirming the etiological agent involved during this outbreak. Nevertheless, bacteriological results should be interpreted in conjunction with the presence of pathological changes associated with this infection, since *M. bovis* can be part of the microbiota of healthy bovine upper respiratory tract.

In accordance with Margineda et al. (2017), this work should alert practitioners about the presence of *Mycoplasma* infections as the cause of BRD in Argentina, moreover considering that other species of Mollicutes causing arthritis and pneumonia as *U. diversum*, *M. bovigenitalum*, *M. bovirhinis*, *M. alkalescens* and *M. leachii* has been reported in Argentina (Seitz et al. 2018; Sosa et al. 2018; Neder et al. 2019).

Since there are no commercially available vaccines in the region for the prevention and control of *M. bovis* pneumonia and arthritis, and the disease caused by *M. bovis* is refractory to delayed antimicrobial therapy, surveillance is a priority to reduce the source of disease to naïve animals. Removal of clinically affected animals and quarantine of the affected lots is useful to reduce the dissemination of mycoplasmosis to unaffected lots.

## Conclusions

This work reports the first local isolation of *M. bovis* from feedlot calves with pneumonia and polyarthritis in Argentina. Further work should be done in order to broaden the regional information about the clinical prevalence of this pathogen.

## Declarations

### Funding

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### Competing Interests

None of the co-authors have any financial or personal relationships that could inappropriately influence or bias the content of the paper.

### **Availability of data and materials**

All data and materials are available for publication.

### **Ethical approval**

The manuscript in part or in full has not been submitted or published anywhere. The manuscript will not be submitted elsewhere until the editorial process is completed.

### **Consent to participate and for publication**

All the co-authors have agreed for authorship, read and approved the manuscript, and given consent for submission and subsequent publication of the manuscript.

### **Authors' contributions**

Germán Cantón conceived of the presented manuscript. Germán Cantón, Ignacio Llada, Facundo Urtizbiría and Sofía Fanti performed the post mortem examination and sampling of the animals. Germán Cantón, Ignacio Llada, Carlos Margineda, Valeria Scioli and Eleonora Morrell carried out the histopathological analysis. Carlos Margineda performed the immunohistochemical analysis of the tissue samples. María Andrea Fiorentino, Enrique Louge Uriarte, Erika Sticotti and Pablo Tamiozzo carried out the microbiological examination of the specimens. All authors discussed the results and contributed to the final manuscript.

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## **References**

1. Adegboye DS, Halbur PG, Cavanaugh DL, Werdin RE, Chase CCL, Miskimins DW, Rosenbusch RF (1995) Immunohistochemical and pathological study of *Mycoplasma bovis*-associated lung abscesses in calves. JVDI 7: 333-337. <https://doi.org/10.1177/104063879500700306>
2. Adegboye DS, Halbur PG, Nutsch RG, Kadlec RG, Rosenbusch RF (1996) *Mycoplasma bovis*-associated pneumonia and arthritis complicated with pyogranulomatous tenosynovitis in calves. JAVMA 209: 647-649.
3. Allen JW, Viel L, Bateman KG, Rosendal S (1992) Changes in the bacterial flora of the upper and lower respiratory tracts and bronchoalveolar lavage differential cell counts in feedlot calves treated for respiratory disease. Can J Vet Res 56: 177-183.

4. Ayling RD, Baker SE, Peek ML, Simon AJ, Nicholas RA (2000) Comparison of *in vitro* activity of danofloxacin, florfenicol, oxytetracycline, spectinomycin and tilmicosin against recent field isolates of *Mycoplasma bovis*. *Vet Rec* 146: 745-747. <http://dx.doi.org/10.1136/vr.146.26.745>
5. Bergey DH, Holt JG (1994). *Bergey's manual of determinative bacteriology*. Ninth Edition. Williams & Wilkins, Baltimore, USA.
6. Booker CW, Abutarbush SM, Morley PS, Kee Jim G, Pittman TJ, Schunicht OC, Perrett T, Wildman BK, Kent Fenton R, Guichon T, Janzen ED (2008). Microbiological and histopathological findings in cases of fatal bovine respiratory disease of feedlot cattle in Western Canada. *Can Vet J* 49: 473-481.
7. Caswell JL, Bateman KG, Cai HY, Castillo-Alcala F (2010) *Mycoplasma bovis* in respiratory disease of feedlot cattle. *Vet Clin North Am Food Anim Pract* 26: 365-379. <http://dx.doi.org/10.1016/j.cvfa.2010.03.003>
8. Cerdá R, Xavier J, Sansalone P, de la Sota R, Rosenbush R (2000) Isolation of *Mycoplasma bovis* during an outbreak of bovine mastitis at a dairy farm in the province of Buenos Aires. First report in the Republic of Argentina. *Rev Latinoam Microb* 42: 7-11.
9. Cooper VL, Brodersen BW (2010) Respiratory disease diagnostics of cattle. *Vet Clin North Am Food Anim Pract* 26: 409-416. <http://dx.doi.org/10.1016/j.cvfa.2010.04.009>
10. Fulton RW, Blood KS, Panciera RJ, Payton ME, Ridpath JF, Confer AW, Saliki JT, Burge LT, Welsh RD, Johnson BJ, Reck A (2009) Lung pathology and infectious agents in fatal feedlot pneumonias and relationship with mortality, disease onset, and treatments. *JVDI* 21: 464-477. <https://dx.doi.org/10.1177/104063870902100407>
11. Gagea MI, Bateman KG, Shanahan RA, van Dreumel T, McEwen BJ, Carman S, Archambault M, Caswell JL (2006) Naturally occurring *Mycoplasma bovis*-associated pneumonia and polyarthritis in feedlot beef calves. *JVDI* 18: 29-40. <https://dx.doi.org/10.1177/104063870601800105>
12. Haines DM, Moline KM, Sargent RA, Campbell JR, Myers DJ, Doig PA (2004) Immunohistochemical study of *Haemophilus somnus*, *Mycoplasma bovis*, *Mannheimia hemolytica* and bovine viral diarrhea virus in death losses due to myocarditis in feedlot cattle. *Can Vet J* 45: 231-234.
13. Harasawa R, Mizusawa H, Nozawa K, Nakagawa T, Asada K, Kato I (1993) Detection and tentative identification of dominant mycoplasma species in cell cultures by restriction analysis of the 16S-23S rRNA intergenic spacer regions. *Res Microbiol* 144: 489-493. [https://dx.doi.org/10.1016/0923-2508\(93\)90057-9](https://dx.doi.org/10.1016/0923-2508(93)90057-9)
14. Hodgins DC, Conlon JA, Shewen PE (2002) Chapter 12. Respiratory viruses and bacteria in cattle. In: Brogden KA, Guthmiller JM (eds) *Polymicrobial diseases*. ASM Press, Washington, USA.
15. Margineda CA, Zielinski GO, Jurado S, Alejandra F, Mozgovej M, Alcaraz AC, López A (2017) *Mycoplasma bovis* pneumonia in feedlot cattle and dairy calves in Argentina. *Braz J Vet Path* 10: 79-86. <https://dx.doi.org/10.24070/bjvp/1983-0246.v10i2p79-86>
16. Murray GM, More SJ, Sammin D, Casey MJ, McElroy MC, O'Neill RG, Byrne WJ, Earley B, Clegg TA, Ball H, Bell CJ, Cassidy JP (2017) Pathogens, patterns of pneumonia, and epidemiologic risk factors

- associated with respiratory disease in recently weaned cattle in Ireland. JVDI 29: 20-34.  
<https://dx.doi.org/10.1177/1040638716674757>
17. Nakagawa T, Uemori T, Asada K, Kato I, Harasawa R (1992) *Acholeplasma laidlawii* has tRNA genes in the 16S–23S spacer of the rRNA operon. J Bacteriol 174: 8163-8165.  
<https://dx.doi.org/10.1128/jb.174.24.8163-8165.1992>
  18. Neder VE, Allassia M, Amadio A, Calvino LF (2019) First report of *Mycoplasma leachii* isolation associated with disease in dairy calves in Argentina. Rev Arg Microbiol 51: 18-21.  
<https://doi.org/10.1016/j.ram.2018.01.004>
  19. Nicholas RA, Ayling RD (2003) *Mycoplasma bovis*: disease, diagnosis, and control. Res Vet Sci 74: 105-112. [https://dx.doi.org/10.1016/s0034-5288\(02\)00155-8](https://dx.doi.org/10.1016/s0034-5288(02)00155-8)
  20. O'Connor A., Martin SW, Nagy E, Menzies P, Harland R (2001) The relationship between the occurrence of undifferentiated bovine respiratory disease and titer changes to bovine coronavirus and bovine viral diarrhea virus in 3 Ontario feedlots. Can J Vet Res 1: 143-150.
  21. Pfützner H (1990) Epizootiology of the *Mycoplasma bovis* infection of cattle. Zentralblatt für Bakteriologie Supplement 20: 394-399.
  22. Pfützner H, Sachse K (1996) *Mycoplasma bovis* as an agent of mastitis, pneumonia, arthritis and genital disorders in cattle. Rev Sci Tech Off Int Epiz 15: 1477-1494.
  23. Schneider MJ, Tait RG, Busby WD, Reecy JM (2009) An evaluation of bovine respiratory disease complex in feedlot cattle: Impact on performance and carcass traits using treatment records and lung lesion scores. J Anim Sci 87: 1821-1827. <https://dx.doi.org/10.2527/jas.2008-1283>
  24. Seitz J, Sticotti E, Giraudo J, Tamiozzo P (2018) Detección de *Ureaplasma diversum* en vacas con y sin vulvovaginitis granular en Argentina. Ab Intus 1: 89-92.
  25. Shahriar F, Clark EG, Janzen E, West K, Wobeser G (2002) Coinfection with bovine viral diarrhea virus and *Mycoplasma bovis* in feedlot cattle with chronic pneumonia. Can Vet J 43: 863-868.
  26. Sosa C, Tirante L, Chaves J, Pol M, Ambrogia A, Giraudo JA, Tamiozzo P (2018) Identificación de especies de *Mycoplasma* y de *Ureaplasma diversum* en rodeos lecheros de Argentina. Rev Arg Microbiol 50: 31-35. <https://doi.org/10.1016/j.ram.2017.02.010>
  27. Tang J, Hu M, Lee C, Roblin R (2000) A polymerase chain reaction based method for detecting *Mycoplasma/Acholeplasma* contaminants in cell culture. J Microbiol Methods 39: 121-126.  
[https://dx.doi.org/10.1016/s0167-7012\(99\)00107-4](https://dx.doi.org/10.1016/s0167-7012(99)00107-4)
  28. Taylor JD, Fulton RW, Lehenbauer TW, Step DL, Confer AW (2010) The epidemiology of bovine respiratory disease: What is the evidence for predisposing factors? Can Vet J 51: 1095-1102.
  29. Thomas A, Dizier I, Trolin A, Mainil J, Linden A, Ball H, Bell C (2002) Isolation of mycoplasma species from the lower respiratory tract of healthy cattle and cattle with respiratory disease in Belgium. Vet Rec 151: 472-476. <http://dx.doi.org/10.1136/vr.151.16.472>

## Figures

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### Figure 1

Post mortem findings in necropsied calves. (a) Bilateral swollen carpal joints of a calf with mycoplasma arthritis. (b) Necropsy #3. Fibrinonecrotic carpal arthritis and tenosynovitis in calf with Mycoplasmosis. (c) Necropsy #4. Focally extensive subacute to chronic pneumonia affecting approximately 90% of the right lung (apical, cranial and caudal lobes) of a calf with mycoplasma pneumonia. Multifocal caseonecrotic nodules are present in the cranial lung lobe (blue arrows). (d) Necropsy #1. Multifocal caseonecrotic nodules in the lung parenchyma. Formalin fixed tissue. (e) Necropsy #1. Focally extensive subacute to chronic pneumonia mainly affecting the whole right apical and cranial lobes and the cranial region (20% approximately of parenchyma) of the right caudal lobe of a calf with mycoplasma pneumonia (\*).

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### Figure 2

Laboratory findings. (a) Necropsy #4. Lung. Necrotic focus delineated by a band of neutrophils and macrophages, encircled by a layer of fibroblasts, macrophages, lymphocytes, and plasma cells. Hematoxylin and eosin, 100×. (b) Necropsy #3. Lung. Large area of caseous necrosis with mineralization surrounded by inflammatory infiltrate characterized by fibroblast, macrophages and lymphocytes, mainly, typical of Mycoplasma pneumonia. Macrophages and scarce plasmocytes were infiltrating the interlobular septa. Hematoxylin and eosin, 40×. (c) Necropsy #4. Carpal joint. Necrotic foci or denudation

of synoviocytes with severe infiltration of neutrophils, macrophages, lymphocytes, and plasma cells; and prominent fibroblast hyperplasia. Hematoxylin and eosin, 40×. (d) Necropsy #3. Lung. Immunohistochemistry labelling of *M. bovis* in the lung observed mainly in bronchioles containing caseous debris. (e) Typical *Mycoplasma* fried-egg-shaped colonies were observed in the lung culture collected during necropsy #1.