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ORIGINAL ARTICLE

Mycoplasma bovis-pneumonia and polyarthritis in feedlot calves in Argentina: First local isolation

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KEYWORDS
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Abstract Bovine respiratory disease (BRD) is one of the most frequent clinical concerns in weaned calves after their arrival at the feedlot. This work reports the first local isolation of *Mycoplasma 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. 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.

PALABRAS CLAVE
Mycoplasma bovis;
Neumonía;
Poliartritis;
Feedlot;
Bovinos

Neumonía y poliartritis por *Mycoplasma bovis* en terneros de feedlot: primer aislamiento local

Resumen La enfermedad respiratoria bovina es uno de los problemas sanitarios más frecuentes en terneros recién destetados luego de su arribo a los corrales de encierre *(feedlots)*. Este trabajo describe el primer aislamiento local de *Mycoplasma bovis* de terneros de *feedlot* con neumonía y poliartritis en Argentina. Se vieron afectados 24 de 545 terneros; mostraron

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enfermedad respiratoria progresiva, subaguda a crónica, tos y fiebre. De los terneros afectados, el 30% presentó, además, problemas en articulaciones carpales o tarsales. Se realizaron 5 necropsias de terneros afectados y se observaron nódulos pulmonares multifocales a coalescentes, que contenían un exudado caseoso blanco-amarillento, rodeados de tejido fibroso, artritis y tenosinovitis fibrinonecrótica. Se aisló *Mycoplasma* de muestras de pulmón y articulación. La secuencia consenso del gen codificante del ARNr 16S-23S rRNA obtenido de estos aislamientos mostró un 100% de similitud con la misma región de cepas de *M. bovis*. Teniendo en cuenta que no hay vacunas disponibles comercialmente en la región para la prevención y el control de neumonías y poliartritis por *M. bovis*, es importante realizar una vigilancia epidemiológica a fin de reducir las fuentes de infección para animales susceptibles.

Introduction

Bovine respiratory disease (BRD) is one the most frequently occurring clinical concerns in weaned calves after their arrival at the feedlot[19], causing important economic losses in the beef industry[22]. Infectious pneumonia usually has complex causes, involving two or more microorganisms and is commonly predisposed by environmental factors[27]. 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*[5,9,10,18].

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[6,10,15]. Margineda et al.[14] 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 are still unknown.

Although *M. bovis* has been previously isolated from other clinical presentations of dairy cattle[7] and *M. bovis*-pneumonia has been previously diagnosed in Argentina[14], this study 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 weighing 45–55 kg arrived at the feedlot, from three farms. Upon arrival, calves were twice treated with tilmicosin (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, respiratory signs and polyarthritis were observed in affected calves. The five most severely affected calves were euthanized in accordance with the regulations of INTA's Animal Ethics Committee and necropsied accordingly. Samples from the 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. Furthermore, lung, synovial fluid and capsule 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 previously described[11], using mouse anti-*M. bovis* monoclonal antibody (Millipore MAB970) at 1:100 dilution. Positive and negative controls were included[14]. 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_2, CBA under 10% CO_2 and MC under aerobiosis, and examined at 96, 48 and 24 h, respectively. Genera were classified in accordance with the Bergey's Manual of Systematic Bacteriology[4]. 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 Puri-Prep S commercial kit (Inbio Highway, Argentina) according to the manufacturer's instructions. For *Mycoplasma* detection, a nested PCR targeting the 16S-23S rRNA intergenic spacer region (ITS) was performed under the conditions reported by Tang et al.[26], using primers previously reported[12,16]. 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[12]. 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_2 for 5 days and examined daily for evidence of 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 in 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 (39.5–41.3 °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

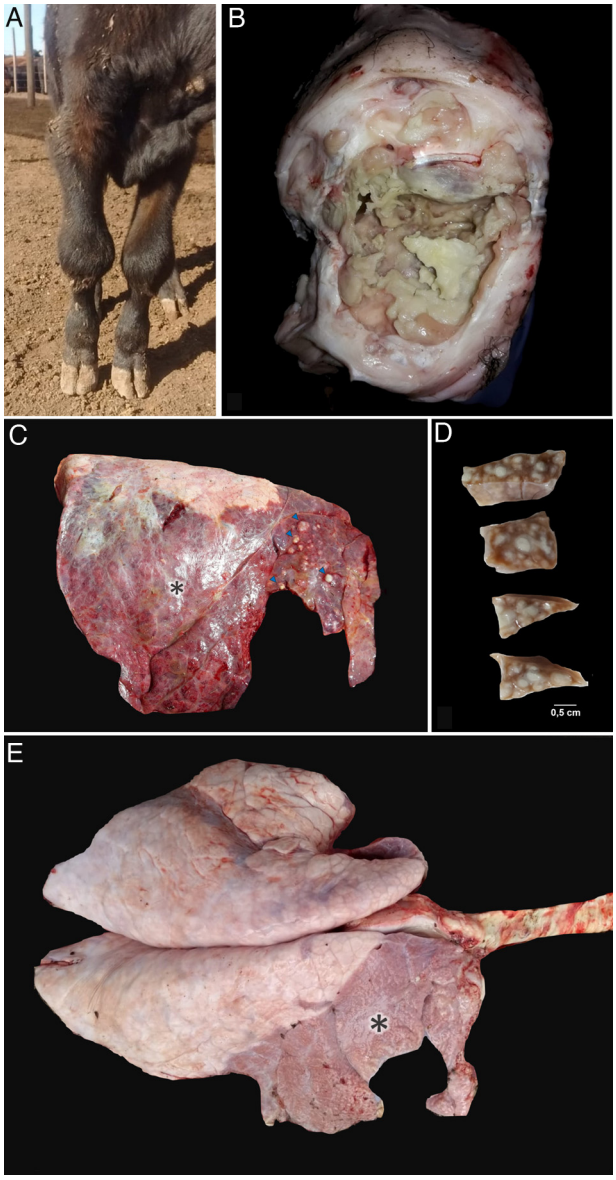

Figure 1 Post mortem findings in necropsied calves. (a) Bilateral swollen carpal joints of a calf with mycoplasma arthritis. (b) Necropsy #3. Fibrinonecrotic carpal arthritis and tenosynovitis in calf with Mycoplasmosis. (c) Necropsy #4. Focally extensive subacute to chronic pneumonia affecting approximately 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 (*).

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 0.3–2.5 cm and contained white-yellowish caseous exudate, encircled by fibrous tissue

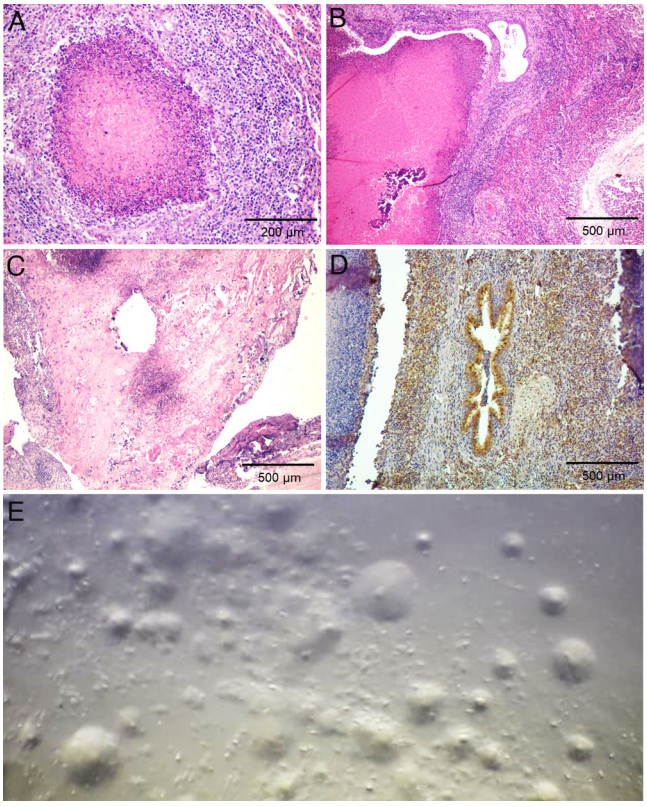

Figure 2 Laboratory findings. (a) Necropsy #4. Lung. Necrotic focus delineated by a band of neutrophils and macrophages, encircled by a layer of fibroblasts, macrophages, lymphocytes, and plasma cells. Hematoxylin and eosin, 100×. (b) Necropsy #3. Lung. Large area of caseous necrosis with mineralization 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 labeling of *M. bovis* in the lung was observed mainly in bronchioles containing caseous debris. (e) Typical *Mycoplasma* fried-egg-shaped colonies were observed in the lung culture collected during necropsy #1.

(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 nuclei lysis (Fig. 2a and b). 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 infiltrated 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 at the center of the necrotic foci. In bronchioles containing caseous debris, the antigen was present within the debris and adjacent to bronchiolar epithelial cells (Fig. 2d). *M. bovis* antigen was identified in synovial and subsynovial stroma 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 were negative for the isolation of aerobic and microaerophilic bacteria using 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).

The lung samples tested were negative for BoHV-1, BPIV-3, BRSV and BVDV.

Discussion

Bovine respiratory disease causes important economic losses in the beef industry[19] and is described as multifactorial with different etiological agents involved[9,10]. Although *M. bovis* is frequently detected in association with BRD worldwide[9,10,15], only one description of the disease is available in Argentina[14].

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 previous reports: subacute to chronic respiratory distress with fever and severe lameness resulting from polyarthritis (mainly affecting carpal and tarsal joints), also known as ''pneumonia-arthritis syndrome''[1,10]. Failure of antibiotic treatment and retarded growth are other characteristics of the disease[24], as was observed in this outbreak. Based on 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%[21]. Nevertheless, before the disease was confirmed in this feedlot, some of the calves in the affected lots (A and B) were moved to different lots with other animals. Then, *Mycoplasma*-like disease was observed in these animals (as reported by the veterinary practitioner), suggesting rapid transmission of the agent to susceptible animals[20]. 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[28] and probably, reservoir calves may have been introduced in December or January, providing the source for infection to in-contact calves, as was previously reported[3]. No previous history of these calves was available to explain this issue. Tilmicosin treatment of these calves was probably not efficient to reduce their reservoir status, since this is not recommended as effective for *M. bovis* therapy. Moreover, enrofloxacin, florfenicol and spectinomycin would be better options as metaphylactic antibiotic treatment[6].

Post mortem diagnosis during BRD should be carried out in untreated animals in the initial stages of the clinical disease. Therefore, the diagnosis of BRD due to *M. bovis* sometimes have some difficulties, since chronically affected calves have probably been already treated with a variety of antimicrobials[8]. 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[1,6,10,21], as observed during the five necropsied calves in this outbreak. With regard to histopathology, 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[10,21].

Bovine respiratory disease is usually caused by multiple microorganisms and their identification in tissue samples from an affected calf should be carefully interpreted[13,18]. *T. pyogenes* and *H. somni* were isolated from one of the sampled lungs. These bacteria could be responsible for BRD. However, the clinical history and the pathological findings resemble ''pneumonia-arthritis syndrome'' previously associated with *M. bovis*[1,10].

Molecular diagnostics have substituted classic diagnostic procedures such as culture for *Mycoplasma* spp and other fastidious microorganisms, providing very specific, sensitive and rapid tests[8]. 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 in lung and synovial samples[2], only the lung samples were positive for *Mycoplasma* isolation in this study. Previously reported 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, the 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 the healthy bovine upper respiratory tract.

In accordance with Margineda et al.[14], this work should alert practitioners about the presence of *Mycoplasma* infections as the cause of BRD in Argentina, specifically considering that other species of Mollicutes causing arthritis and pneumonia such as *U. diversum*, *M. bovigenitalum*, *M. bovirhinis*, *M. alkalescens* and *M. leachii* have been reported in Argentina[17,23,25].

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.

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.

Ethical disclosures

Affected calves were euthanized according to the regulations of the Animal Ethics Committee of INTA.

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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 inmmunohistochemical 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.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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References

1. Adegboye DS, Halbur PG, Cavanaugh DL, Werdin RE, Chase CCL, Miskimins DW, Rosenbusch RF. Immunohistochemical and pathological study of *Mycoplasma bovis*-associated lung abscesses in calves. J Vet Diagn Invest. 1995;7:333–7, http://dx.doi.org/10.1177/104063879500700306.
2. Adegboye DS, Halbur PG, Nutsch RG, Kadlec RG, Rosenbusch RF. *Mycoplasma bovis*-associated pneumonia and arthritis complicated with pyogranulomatous tenosynovitis in calves. J Am Vet Med A. 1996;209:647–9.
3. Allen JW, Viel L, Bateman KG, Rosendal S. 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. 1992;56:177–83.
4. Vos P, Garrity G, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer K-H, Whitman WB, editors. Bergey's manual of systematic bacteriology. Vol. 3: the firmicutes. 2nd ed. New York, USA: Springer-Verlag; 2009.
5. Booker CW, Abutarbush SM, Morley PS, Kee Jim G, Pittman TJ, Schunicht OC, Perrett T, Wildman BK, Kent Fenton R, Guichon T, Janzen ED. Microbiological and histopathological findings in cases of fatal bovine respiratory disease of feedlot cattle in Western Canada. Can Vet J. 2008;49:473–81.
6. Caswell JL, Bateman KG, Cai HY, Castillo-Alcala F. *Mycoplasma bovis* in respiratory disease of feedlot cattle. Vet Clin North Am Food Anim Pract. 2010;26:365–79, http://dx.doi.org/10.1016/j.cvfa.2010.03.003.
7. Cerdá R, Xavier J, Sansalone P, de la Sota R, Rosenbush R. 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. 2000;42:7–11.
8. Cooper VL, Brodersen BW. Respiratory disease diagnostics of cattle. Vet Clin North Am Food Anim Pract. 2010;26:409–16, http://dx.doi.org/10.1016/j.cvfa.2010.04.009.
9. Fulton RW, Blood KS, Panciera RJ, Payton ME, Ridpath JF, Confer AW, Saliki JT, Burge LT, Welsh RD, Johnson BJ, Reck A. Lung pathology and infectious agents in fatal feedlot pneumonias and relationship with mortality, disease onset, and treatments. J Vet Diagn Invest. 2009;21:464–77, http://dx.doi.org/10.1177/104063870902100407.
10. Gagea MI, Bateman KG, Shanahan RA, van Dreumel T, McEwen BJ, Carman S, Archambault M, Caswell JL. Naturally occurring *Mycoplasma bovis*-associated pneumonia and polyarthritis in feedlot beef calves. J Vet Diagn Invest. 2006;18:29–40, http://dx.doi.org/10.1177/104063870601800105.
11. Haines DM, Moline KM, Sargent RA, Campbell JR, Myers DJ, Doig PA. 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. 2004;45:231–4.
12. Harasawa R, Mizusawa H, Nozawa K, Nakagawa T, Asada K, Kato I. Detection and tentative identification of dominant mycoplasma species in cell cultures by restriction analysis of the 16S-23S rRNA intergenic spacer regions. Res Microbiol. 1993;144:489–93, http://dx.doi.org/10.1016/0923-2508(93)90057-9.
13. Hodgins DC, Conlon JA, Shewen PE. Respiratory viruses and bacteria in cattle. In: Brogden KA, Guthmiller JM, editors. Polymicrobial diseases. Washington, USA: ASM Press; 2002 [chapter 12].
14. Margineda CA, Zielinski GO, Jurado S, Alejandra F, Mozgovoj M, Alcaraz AC, López A. *Mycoplasma bovis* pneumonia in feedlot cattle and dairy calves in Argentina. Braz J Vet Path. 2017;10:79–86, http://dx.doi.org/10.24070/bjvp/1983-0246.v10i2p79-86.
15. 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. Pathogens, patterns of pneumonia, and epidemiologic risk factors associated with respiratory disease in recently weaned cattle in Ireland. J Vet Diagn Invest. 2017;29:20–34, http://dx.doi.org/10.1177/1040638716674757.
16. Nakagawa T, Uemori T, Asada K, Kato I, Harasawa R. Acholeplasma laidlawii has tRNA genes in the 16S–23S spacer of the rRNA operon. J Bacteriol. 1992;174:8163–5, http://dx.doi.org/10.1128/jb.174.24.8163-8165.1992.
17. Neder VE, Allassia M, Amadio A, Calvinho LF. First report of *Mycoplasma leachii* isolation associated with disease in dairy calves in Argentina. Rev Arg Microbiol. 2019;51:18–21, http://dx.doi.org/10.1016/j.ram.2018.01.004.
18. Nicholas RA, Ayling RD. *Mycoplasma bovis*: disease, diagnosis, and control. Res Vet Sci. 2003;74:105–12, http://dx.doi.org/10.1016/s0034-5288(02)00155-8.
19. O'Connor A, Martin SW, Nagy E, Menzies P, Harland R. 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. 2001;1:143–50.
20. Pfützner H. Epizootiology of the *Mycoplasma bovis* infection of cattle. Zentralb Bakteriol Suppl. 1990;20:394–9.
21. Pfützner H, Sachse K. *Mycoplasma bovis* as an agent of mastitis, pneumonia, arthritis and genital disorders in cattle. Rev Sci Tech Off Int Epiz. 1996;15:1477–94.
22. Schneider MJ, Tait RG, Busby WD, Reecy JM. 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. 2009;87:1821–7, http://dx.doi.org/10.2527/jas.2008-1283.
23. Seitz J, Sticotti E, Giraudo J, Tamiozzo P. Detección de *Ureaplasma diversum* en vacas con y sin vulvovaginitis granular en Argentina. Ab Intus. 2018;1:89–92.
24. Shahriar F, Clark EG, Janzen E, West K, Wobeser G. Coinfection with bovine viral diarrhea virus and *Mycoplasma bovis* in feedlot cattle with chronic pneumonia. Can Vet J. 2002;43:863–8.
25. Sosa C, Tirante L, Chaves J, Pol M, Ambrogia A, Giraudo JA, Tamiozzo P. Identificación de especies de *Mycoplasma* y de *Ureaplasma diversum* en rodeos lecheros de Argentina. Rev Arg Microbiol. 2018;50:31–5, http://dx.doi.org/10.1016/j.ram.2017.02.010.
26. Tang J, Hu M, Lee C, Roblin R. A polymerase chain reaction based method for detecting *Mycoplasma*/*Acholeplasma* contaminants in cell culture. J Microbiol Methods. 2000;39:121–6, http://dx.doi.org/10.1016/s0167-7012(99)00107-4.
27. Taylor JD, Fulton RW, Lehenbauer TW, Step DL, Confer AW. The epidemiology of bovine respiratory disease: What is the evidence for predisposing factors? Can Vet J. 2010;51:1095–102.
28. Thomas A, Dizier I, Trolin A, Mainil J, Linden A, Ball H, Bell C. Isolation of mycoplasma species from the lower respiratory tract of healthy cattle and cattle with respiratory disease in Belgium. Vet Rec. 2002;151:472–6, http://dx.doi.org/10.1136/vr.151.16.472.