



Case Report

Facial paralysis in feedlot cattle associated with otitis caused by *Mycoplasma bovis*: first report in Argentina

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Abstract

The paper describes an outbreak of *Mycoplasma bovis*-associated bronchopneumonia and otitis clinically associated with facial paralysis in feedlot steers in Argentina. Three of sixty-one feedlot animals from the same pen developed unilateral facial paralysis primarily with ear drooping, droopy eyelids (eyelid ptosis), and head tilt. Postmortem examination revealed cranioventral consolidation of the lungs (bronchopneumonia) and the presence of abundant purulent exudate in the tympanic bulla (otitis). The affected lung had a nodular appearance. Histopathological examination of the middle and inner ear showed necrotizing, suppurative otitis, and bronchopneumonia with bronchiectasis compatible with *M. bovis* infection. Immunohistochemistry of the lung, and middle and inner ear revealed abundant *M. bovis* antigen. The ELISA tests revealed that the two animals showing facial paralysis had seroconverted for *M. bovis*. To our knowledge, these are the first reported cases of *M. bovis* otitis in Argentina. These findings should alert veterinarians and diagnosticians of the relevance of *M. bovis* as a cause of otitis and facial paralysis in cattle.

Keywords: *Mycoplasma bovis*, pathology, otitis.

Introduction

Otitis is a common disorder in cattle worldwide (21). Although dairy and beef calves are at continuous risk of infection, the true prevalence of this disease has yet to be established with precision and is probably underestimated (15). According to the site of inflammation, otitis is classified as external, middle, and internal (21). It is most commonly caused by bacteria such as *Trueperella pyogenes* (1, 9, 15), *Corynebacterium pseudotuberculosis* (14), *Histophilus somni* (19, 22), *Pasteurella multocida* (2, 10, 14, 27), *Pseudomonas* spp. (14), *Streptococcus* spp. (14), *Staphylococcus* spp. (28), and several types of mycoplasmas, particularly *M.*

bovis (9, 10, 15, 16). According to some reports, *M. bovis* is the most frequently isolated agent in bovine cases of otitis media/interna (2).

Mycoplasma bovis belongs to the class Mollicutes, a group of bacteria so named because they lack cell walls and are instead enveloped by a complex plasma membrane (5). Typically, these organisms inhabit the mucosal surfaces in contact with the external environment, such as the respiratory, urogenital, eyes, and mammary glands (5). *M. bovis* is a frequent participant in the bovine respiratory disease complex (5, 12, 18) and causes arthritis (4, 11), mastitis (5, 7), otitis (15, 16), and meningitis (26) in cattle. This study documents an outbreak of otitis media and internal caused

by *M. bovis* infection in feedlot steers in Argentina and summarizes the etiology and epidemiology of otitis in other scientific publications.

Case description

The outbreak of respiratory disease and otitis associated clinically with facial paralysis occurred in (Carcarañá, Santa Fe, Argentina) a feedlot housing 14000 steers in February 2017. After five weeks in a fattening pen housing 61 steers (8-9 months old), signs of facial paralysis were observed in 3 animals. The affected steers had unilateral drops of the ear auricle (ear-ptosis), droopy eyelids (eyelid ptosis), and head tilt. In addition, all three animals had hyperthermia (above 40°C) and mild dyspnea with mucopurulent nasal discharge. All three steers were initially treated with two doses of tilmicosin and then given penicillin-streptomycin. There was no clinical response to this antibiotic therapy. When the owner notified the outbreak and one month after the onset, blood samples were taken from two steers with facial paralysis and three others without any clinical signs. Blood sera were tested for *M. bovis* using a commercial indirect ELISA Kit (Bio-X Diagnostics, Rochefort, Belgique) following the manufacturer's instructions. When the owner notified about the outbreak, two animals with facial paralysis were treated with long-acting enrofloxacin (treatment time: 23 days); after 31 days, the owner observed the animals and expressed that they had a complete recovery.

The ELISA tests revealed that the two animals showing facial paralysis and two of the three asymptomatic steers had seroconverted for *M. bovis*. One of the three animals exhibiting facial paralysis (Fig. 1A) was euthanized according to the regulations of the Animal Ethics Committee of INTA and necropsied. Exudate samples from the middle and internal ear, brain, and lung were submitted for bacteriological culture. Then, samples were inoculated onto blood agar and MacConkey agar plates and incubated aerobically in duplicates under microaerophilic conditions (5% CO₂) at 37 °C for 48 h. Culture for *Mycoplasma* spp. was not performed. Lung samples were homogenized in Eagle's minimal essential medium supplemented with 10% fetal bovine serum and inoculated on Madin-Darby Bovine Kidney cells. Cell cultures were incubated at 37°C and 5% CO₂ and examined daily for five days. After the fourth passage, cultures were tested for BVDV by PCR.

Tissue samples collected at the necropsy were fixed in 10% phosphate-buffered formalin for routine histopathology and immunohistochemistry (IHC). Temporal bones, including the tympanic bullae and auditory tubes, were decalcified with 5% nitric acid for five days, rinsed, and processed for histopathological examination. Six different tissue sections from the middle and inner ears were examined microscopically. The examination of the brain included the frontal and occipital neocortex, thalamus, corpora

quadrigemina, pons cerebri, cerebellum, and medulla oblongata. Blocks of selected paraffin-embedded tissues were subsequently submitted for *M. bovis* IHC. Epitope retrieval was done by autoclaving in citrate buffer at pH 6.0 for 15 min. The IHC staining for *M. bovis* tissues was performed using two antibodies: a) mouse anti-*M. bovis* monoclonal antibody (MAB970, Millipore, Burlington, MA, USA) applied for 12 hr. a dilution of 1:100; and b) rabbit anti-*M. bovis* polyclonal antibody (supplied by the California Animal Health and Food Safety Lab System-Bacteriology section) applied for 32 min. at a dilution of 1:5000. Negative controls were prepared by replacing the primary antibody with non-immune rabbit serum.

The postmortem examination revealed abundant purulent exudate in the right ear (right unilateral otitis) extending from the middle ear into the inner ear and surrounding bone (Figs. 1B). The tympanic bulla had hyperemic mucosa and was conspicuously filled with caseous exudate. The bone surrounding the exudate was soft and irregularly shaped (Fig. 1C). There was also cranioventral consolidation of the lungs involving 20% of pulmonary parenchyma, and the affected lung had a nodular appearance and texture. These nodules ranged from 0.5-1 cm in diameter and consisted of focal to confluent areas of necrosis, which on a cut surface appeared as white, thick caseonecrotic exudate. Bacterial agents were not isolated from the middle ear and lung.

Microscopically, the affected ear showed extensive inflammatory infiltrates filling and obliterating auditory tubes and ducts (Fig. 1D). These infiltrates comprised neutrophils admixed with abundant hypereosinophilic granules and necrotic debris. The epithelium lining the middle and inner ear was focally hyperemic, effaced, or had undergone squamous metaplasia (Fig. 1E). The underlying bone tissue was extensively infiltrated with fibroblasts, neutrophils, plasma cells, and macrophages (Fig. 1F). Affected trabecular bone exhibited pronounced osteoclastic resorption and formation of new lamellar bone interpreted as reactive bone remodeling. The cranial nerve fibers (VII) adjacent to the tympanic section showed moderate degeneration, poorly stained with H&E, and contained lymphocytes between the fibers (Fig. 1G). The most severely affected fiber groups were lost altogether, leaving remnants of endoneural and perineural tissues.

Microscopically, the lungs showed well-demarcated areas of necrosis surrounded by a thick layer of fibroblasts infiltrated with macrophages, lymphocytes, and a few plasma cells (Fig. 1H). The center of the necrotic foci contained abundant hypereosinophilic finely granular material, while the periphery comprised bronchial walls, some of which were dilated or effaced by the inflammatory reaction (Fig 1H). There was also thickening of the alveolar septa and suppurative bronchitis and bronchiolitis, as well as hyperplasia of the bronchus-associated lymphoid tissue (BALT). Immunohistochemistry revealed that *M. bovis* antigen was abundant in the ear and lungs (Figs. 1D and 1I). The margin of the necrotic lesions showed a marked

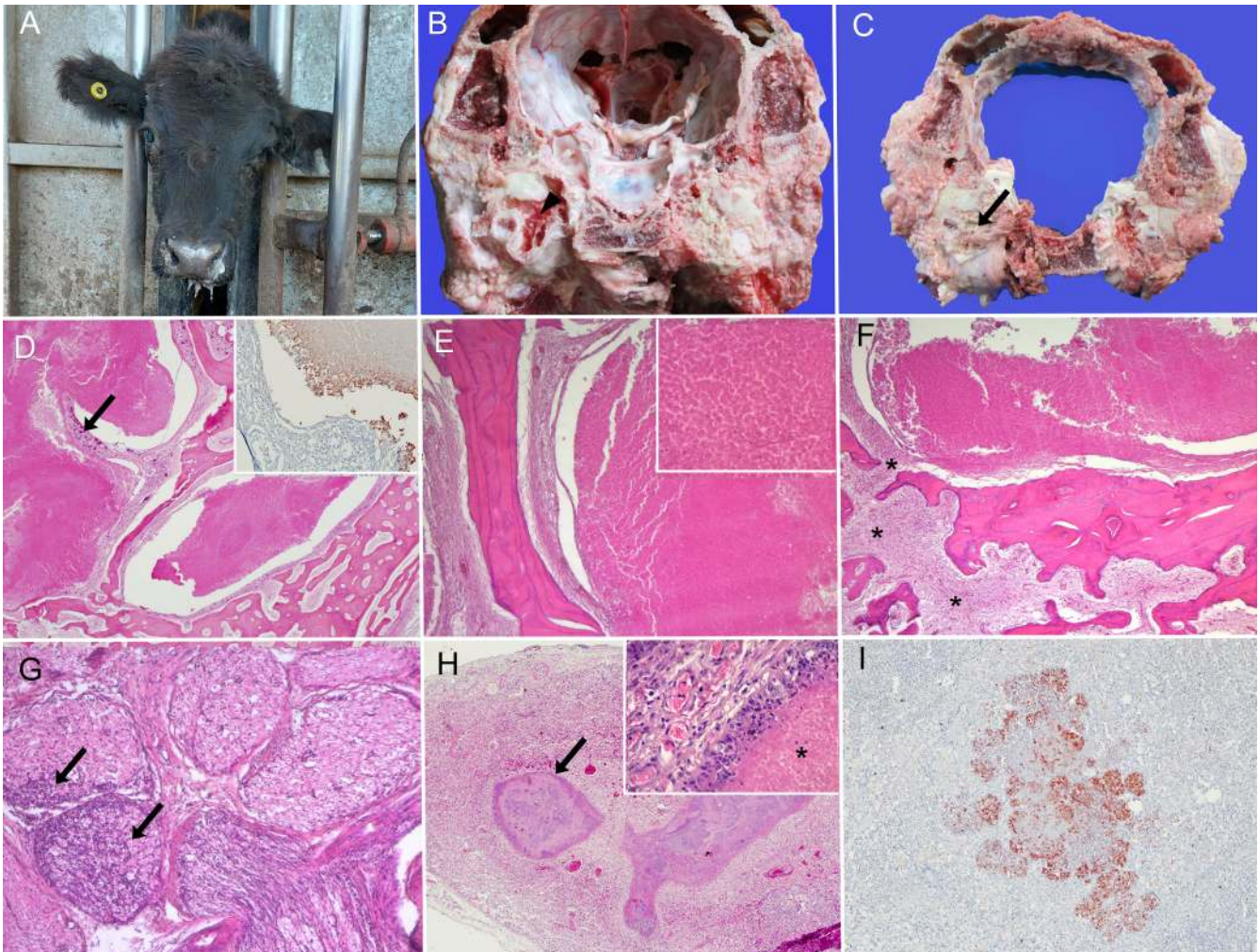


Figure 1. Clinical and pathologic findings in a case of mycoplasmosis in steers. **A-** Steer showing unilateral facial paralysis characterized by eyelid and ear droop and head tilt. **B-** Skull, transversal section. The left tympanic bulla appears normal with no evidence of exudate (arrowhead); the right side bulla is filled with purulent exudate. **C-** The tympanic bulla is filled with purulent exudate extending into the surrounding trabecular bone (arrow). **D-** Middle ear and cochlea, histopathology. Cellular and necrotic debris fill the auditory tube and efface the epithelium (arrow). HE, obj. 5x. Inset, immunohistochemistry. Note abundant positive antigen for *M. bovis* in the exudate filling the middle ear. *M. bovis*-IHC, obj. 10x. **E-** Middle ear, histopathology. Cellular and necrotic debris fill the lumen of the auditory tube. HE, obj. 5x. Inset: exudate with a granular eosinophilic appearance. HE, obj. 40x. **F-** Middle ear and adjacent bone, histopathology. The Eustachian tube is filled with neutrophils and cell debris. The inflammatory process extends into the adjacent bone, and the trabecular bone is replaced by fibrous connective tissue (asterisks). HE, obj. 40x. **G-** Transverse sections of the facial nerve, histopathology. Lymphocytic neuritis. Mononuclear cell infiltrate in the endoneurium and epineurium of two adjacent nerve fibers. HE, obj. 40x. **H-** Lung, histopathology. Necrotic airways filled with exudate. Inset: Close-up of necrotic tissue filled with finely granular cell debris (asterisk) surrounded by fibroblasts, macrophages, lymphocytes, and plasma cells. HE, obj. 40x. **I-** Lung, immunohistochemistry. Abundant positive *M. bovis* antigen within the cellular and necrotic debris. *M. bovis*-IHC, obj. 10x.

positive staining, and the centre of the necrotic foci presented a lesser extent positive staining.

Table 1 includes papers published in the last 40 years on otitis media occurring in cattle. Most reports of bovine middle otitis in the previous 20 years include *M. bovis* as the most frequently isolated bacterium. This condition often appears in calves under four months of age, mostly from dairy systems.

In feedlots, we found only three scientific publications on otitis in cattle, but pneumonia-arthritis syndrome is by far more commonly reported (11). Several investigations revealed that roughly 74.2% of calves with *M. bovis* otitis also have pneumonia caused by this same organism (10, 15, 16, 27). According to the literature, *M. bovis*-associated otitis can be unilateral (47.5%) or bilateral (42.5%) (2, 14, 15, 27).

Table 1. A brief review of the epidemiology and etiology of otitis media or media/interna in cattle according to the literature.

Year of study	Country	Production system	Cases	Age	Bilateral otitis	Etiology of otitis* (bacteriologic culture)		Pneumonia	Reference
						Mycoplasmas	Aerobic		
1979-1982	USA (Wyoming)	Beef cattle	86	5-18 m	Yes (20/86)	ND	<i>P. multocida</i> (12/46) <i>C. pseudotuberculosis</i> (13/46) <i>Streptococcus</i> spp. (6/46)	NA	14
1983	Canada (Alberta)	Beef cattle	2	6-8 m	NA	ND	<i>H. somni</i> (2/2)	NA	22
1983	Canada (Ontario)	Dairy herd	1	Heifer	No	ND	<i>H. somni</i> (1/1)	No	19
1987-2002	Canada (Quebec)	Dairy herd	15	1-4 m	NA	<i>M. bovis</i> (5/6) <i>Mycoplasma</i> spp. (1/6)	<i>P. multocida</i> (1/15).	Yes (11/15)	10
1992-2004	USA (California)	Dairy herd	61	1-3 m	Yes (46/61)	<i>M. bovis</i> (51/54), <i>M. bovirhinis</i> (1/54), <i>M. alkalescens</i> (2/54).	<i>Pasteurella</i> spp. (5/61) <i>Trueperella</i> spp. (3/61)	Yes (47/61)	15
1997	USA (Michigan)	Dairy herd	5	< 1 m	Yes (2/5)	<i>M. bovis</i> (5/5)	<i>P. multocida</i> (1/5)	Yes (1/5)	27
1997-1999	Israel	Dairy herd	64	1-14 m (73% of case < 6m)	NA	ND	<i>Staphylococcus</i> spp. (46/64) <i>P. haemolytica</i> (21/64) <i>P. multocida</i> (20/64)	NA	28
2000-2001	Japan	Beef cattle	8	1-3 m	NA	<i>M. bovis</i> (8/8)	Negative	Yes (7/8)	16
2004	Israel	Dairy herd	1	Adult (cow)	Yes	ND	<i>T. pyogenes</i> and <i>E. coli</i>	No	29
2003-2010	Canada (Quebec)	Dairy herd	29	1-6 m	NA	<i>M. bovis</i> (10/12) <i>M. arginini</i> (1/12) <i>Mycoplasma</i> spp. (1/12)	<i>Staphylococcus</i> spp. (3/12) <i>T. pyogenes</i> (2/12)	NA	13
2009	United Kingdom	Dairy herd	4	< 1 m	Yes	<i>M. bovis</i> (4/4) <i>M. arginini</i> (1/4) <i>M. bovirhinis</i> (1/4)	<i>T. pyogenes</i> (1/4)	NA	9
2012	France	Dairy herd	5	< 1 m	NA	<i>M. bovis</i> (4/4) <i>M. arginini</i> (1/4)	<i>Staphylococcus</i> spp. (2/3) <i>T. pyogenes</i> (2/3)	NA	1
2013-2014	Italy	Dairy herd	22	< 3 m	Yes (6/22)	<i>M. bovis</i> (20/22)	<i>P. multocida</i> . (5/22) <i>Streptococcus</i> spp. (1/22) <i>Staphylococcus</i> spp. (1/22)	NA	2
2016	Italy	Beef cattle	2	12 m	NA	<i>M. bovis</i> (2/2) <i>M. agalactiae</i> (2/2)	Negative	Yes (2/2)	6

* Only the three most frequent were included in the scientific publications reporting four or more bacteria were recorded. ND: not done. NA: not available

Discussion

This report describes a case of otitis media/interna with facial paralysis caused by *M. bovis* infection in Argentina for the first time. The intralésional antigen by IHC in the ear and lungs confirmed that the causative agent was *M. bovis*. Seroconversion in affected and healthy animals confirmed an active circulation of *M. bovis* in these animals and further indicated that this organism was responsible for this outbreak. In addition, bacteriological cultures did not yield any aerobic and microaerobic bacteria relevant to bovine respiratory infections like *Mannheimia haemolytica*, *Histophilus somni*, *Pasteurella multocida*, or *Trueperella pyogenes*. These negative bacteriological findings further support that *M. bovis* was the putative pathogen causing bronchopneumonia and otitis. Since no cytopathic effect was detected in cell cultures and lung tissue tested negative for BVDV, it is unlikely that this virus was involved in the pathogenesis of otitis or pneumonia.

M. bovis is an emerging pathogen in cattle worldwide (5, 12), and there is mounting evidence that some strains are resistant to the antimicrobials most used in feedlots (5). A study in the USA (15) showed that *M. bovis* is the most common agent isolated from young calves with otitis media and internal. Some authors suggest this condition is more frequent in summer (15), as reported here. There are few reports of *M. bovis* infections in South American cattle, particularly in Brazil and Argentina, but they are typically associated with pneumonia (4,18,24) and mastitis (7). The gross and microscopic findings in the middle and inner ear were like those previously reported in other countries (15, 16, 27).

Our report illustrates that *M. bovis* should be investigated in cattle with facial paralysis. This organism should be added to the other etiologies described to cause facial neuritis or neuropathy in cattle, such as other bovine bacteria (*T. pyogenes*, *P. multocida*), parasites (8), *Prosopis* spp. poisoning (20), traumatic head injuries (21), cerebral listeriosis (3), and facial and vestibular paralysis syndrome of unknown etiology (17,23,25). In Argentina, this last condition is relatively common in beef (23) and dairy (25) calves and is clinically very similar to otitis caused by *M. bovis*. In the veterinary diagnosis service of INTA Marcos Juárez (unpublished information), we have had four reports of this syndrome in the last ten years. It should be noted, however, that a proper diagnosis of facial nerve neuritis requires a comprehensive examination of the middle and inner ears and the branches of facial and trigeminal nerves emerging from the brain stem. Our study also proved that IHC in paraffin-embedded blocks so widely used for diagnosing *M. bovis* pneumonia could detect infections in the middle and inner ear and facial nerves, even after the acid decalcification of bones. Future studies should investigate if decalcification methods or decalcifying solutions can affect the detection or sensitivity of *M. bovis* in osteomyelitis.

The type of exudate and pyogranulomatous inflammatory response in the auditive tube and surrounding bone was remarkably similar to that caused by *M. bovis* in the lung

(Figs 1E and 1H). It consists of a rim of proliferating fibroblasts, macrophages, and lymphocytes containing abundant hypereosinophilic granules at the center of the lesion (5,11, 12,26). The pathogenesis of *M. bovis* otitis must be adequately elucidated as there are still three possible portal entries: i. ascending infection via the Eustachian tube; ii. descending from the external ear canal; and iii. hematogenous dissemination from a distant *M. bovis* infection such as pneumonia or arthritis (1). However, extrapolating from human medicine, it is most likely *M. bovis* extends via the Eustachian tube from the nasopharynx across the tympanic membrane into the inner ear, followed by osteomyelitis in the most severe cases (15). The cranial nerve neuritis and associated facial paralysis are likely simple extensions of the adjacent osteomyelitis.

Finally, this report should alert veterinarians and diagnosticians to the importance of *M. bovis* as a cause of otitis and facial paralysis in cattle. Early detection and proper treatment are essential since *M. bovis* infection causes severe and often irreversible damage to the lungs, ear, and facial nerves. Delayed treatment or treatment during the advanced stages of *M. bovis* infection is generally ineffective.

Conflict of Interest

The authors declare no competing interests.

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