“The Problem with 291”

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Class of 2022

Clinicopathologic Conference

July 2nd, 2021

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Introduction:

Mycoplasma bovis is a bacteria in the class Mollicutes.[9,12,13] This bacterium's ability to cause pneumonia, arthritis, otitis media, mastitis, keratoconjunctivitis, and reproductive disease leads to *Mycoplasma bovis* being the most important *Mycoplasma* species affecting cattle in North America and Europe.[9] Infection with *Mycoplasma bovis* is a common and costly cause of morbidity and mortality in cattle worldwide.[3,14] Its role as one of the bacterial agents in the bovine respiratory disease complex (BRDC) lends to its importance specifically in the feedlot and stocker industries.[4] Due to the absence of a cell wall decreasing its antibiotic susceptibility, lack of available effective vaccines, and studies showing increasing signs of antibiotic resistance, *Mycoplasma bovis* infection can be challenging to treat and prevent.[9]

Mycoplasma bovis as a respiratory pathogen typically occurs due to a weakened immune system following a viral infection.[14] Common viral infections that preface respiratory infection with *Mycoplasma bovis* include but are not limited to Bovine Respiratory Syncytial Virus (BRSV), Bovine Parainfluenza 3 (PI3), Bovine Viral Diarrhea Virus (BVDV), and Infectious Bovine Rhinotracheitis (IBR).[3,14] The spread of *Mycoplasma bovis* from lung tissue to joint structures and other body systems following a respiratory infection via a hematogenous route has also recently been reported.[4,7]

History and Presentation:

Number 291, an approximately 6-month-old Angus-cross heifer, presented to the MSU-CVM Food Animal Service on the afternoon of 11/26/2020 due to a grade 3/5 lameness and swelling of the left elbow and shoulder. On 10/26/2020, 291 had been purchased at an auction market to be used in a research trial. She had been vaccinated with a modified live 5-way

respiratory virus vaccination (brand name unknown) and a clostridial 8-way vaccination (Covexin 8). She was also dewormed with fenbendazole (brand name unknown) and doramectin (Dectomax) the week following purchase, and a liver biopsy was taken for the research trial at this time. Clinical signs of bovine respiratory disease and a DART score of 3 out of 4 were noted the week of 11/16/2020. A DART score is from a scoring system that evaluates cattle based on depression, appetite, respiratory index, and temperature; a 3 out of 4 DART score would support that 291 was dealing with a respiratory infection. Number 291 was treated with an injection of ceftiofur crystalline free acid (Excede) in the fat pad behind the ear during the week of 11/16/2020 and placed in a barn with one other heifer. It was also reported that 291 was not observed to eat or drink since being placed in the barn, and she had been treated with an intravenous injection of 7.6 mL (2.2 mg/kg) of flunixin meglumine (Banamine) and a subcutaneous injection of 4.5 mL (2.5 mg/kg) of tulathromycin (Draxxin) before presentation.

Upon presentation, 291 was bright, alert, and responsive. She had a body condition score of 4/9 and weighed 380 pounds. A grade 3/5 lameness and notable soft tissue swelling surrounding her left humeroradial joint were observed. She was estimated to be 5% dehydrated with a notable skin tent. A rectal temperature of 103.8 °F (reference range for calves: 101-103 °F) and palpable ruminal mat were also noted. A heart rate and respiratory rate were not taken at the time of presentation. Due to 291's presentation on Thanksgiving Day, it was elected that she be further evaluated the next day.

On 11/27/2020, the swelling and lameness had remained static, but bilateral mucopurulent nasal discharge was seen. She was noted to eat Bermuda grass hay but did not drink water. An ultrasound of 291's left elbow was performed, and a pocket of anechoic fluid

measuring approximately 1 cm was visible dorsal to the olecranon. Other findings from the ultrasound included a circular structure (suspected to be joint effusion) filled with anechoic fluid and echogenic material that was approximately 2 cm in diameter located cranial and lateral to the olecranon and dorsal to the humeroradial joint. Soft tissue swelling of the area was visible. Due to her previous dehydration, 291 was administered 5 gallons of water via oroesophageal intubation, and the swollen left elbow was hosed with cold water for 10 minutes twice. She was given 3.8 mL (1.1 mg/kg) of flunixin meglumine (Banamine) intravenously following the ultrasound.

On 11/28/2020, the lameness appeared to have improved to a grade 2/5. Number 291 remained mildly dehydrated (less than 5%) but drank some water. She was noted to eat some of the offered grain and continued to eat a portion of the provided Bermuda grass hay. On physical examination, a heart rate of 100 beats per minute, which is the high end of normal, (reference range for calves: 70-100 beats per minute) and increased temperature (103.3 °F, normal is 100.5-102.5 °F) were found. Coughing was noted, and harsh lung sounds that were loudest ventrally were heard on auscultation. From 11/29-11/30/2020, the lameness remained unchanged, and the left elbow remained swollen, firm, and warm. Number 291 continued to have an elevated transrectal temperature and audible coughing.

Diagnostics:

Due to the non-specific nature of the clinical signs associated with *Mycoplasma bovis*, diagnosis cannot be obtained strictly based on clinical signs.[6] Although clinical signs cannot be used to diagnose this, a thorough history and physical examination are beneficial in creating a list of differential diagnoses. Available diagnostic methods include culture, ELISA, conventional

PCR, and real-time PCR, as well as many others.[6] Samples such as milk in the case of suspected mastitis, joint fluid, bronchoalveolar lavage fluid, swabs from various locations, and serum are used to diagnose *Mycoplasma bovis*. Culture of *Mycoplasma bovis* has been heralded as the gold standard for diagnosis.[3,6] Due to the slow growth and specific necessary conditions such as media with antimicrobials to decrease the likelihood of overgrowth of other pathogens and media that can support the growth of *Mycoplasma bovis* since it cannot synthesize amino acids, this diagnostic may be challenging and time consuming.[3,6,13] Also, culture results could be impacted by use of antibiotics before sampling, concurrent infections with organisms that more readily grow, inappropriate sample care, and errors in processing.[3] Culture medias available for *Mycoplasma bovis* culture are Hayflick's, modified PPLO, and Eaton's.[6] ELISA assays performed on serum, plasma, and milk samples detect antibodies to *Mycoplasma bovis*, but questions of cross-reactivity to other bacteria are present.[13] PCR appears to be the current choice for diagnosis of *Mycoplasma bovis*. It was shown that when testing calves for shedding *Mycoplasma bovis*, PCR and culture provided the same result when accounting for the farm, meaning that if one calf from a farm tested positive via culture at least one calf from that farm tested positive via PCR.[18] Although culture results for this study identified more animals shedding this bacteria than PCR, PCR was confirmed to be a quicker and still accurate test to confirm the presence of calves shedding *Mycoplasma bovis* on a farm.[18] A 2010 study by Clothier et al. found that real-time PCR "is a rapid accurate assay that is adaptable to a variety of PCR platforms and can provide reliable results on an array of clinical samples."[5] In "A Review of Mycoplasma Diagnostics in Cattle," PCR was confirmed to be a more efficient, specific, and sensitive diagnostic than culture.[13] Findings of a sensitivity and specificity comparable and even

greater than culture with faster results leads to this test being a more practical option for diagnosis of this pathogen.[5,13]

Pathogenesis:

Mycoplasma bovis is one of the bacterial pathogens included in BRDC.[14] BRDC includes Bovine Viral Diarrhea Virus, Bovine Respiratory Syncytial Virus, Parainfluenza Type-3 Virus, and Infectious Bovine Rhinotracheitis (Bovine Herpesvirus 1) as the viral pathogens and *Pasteurella multocida, Mannheimia haemolytica, Histophilus somni*, as well as *Mycoplasma bovis* as the bacterial pathogens.[3,14] The bacteria listed are often considered to be secondary pathogens after a viral infection has occurred.[14] Infection most commonly occurs after a stressful situation such as processing, weaning, selling, and shipping calves.[14] The immune system is not as effective at protecting animals as a result of this stress, providing an opportunity for respiratory infection.[14]

Mycoplasma bovis may be found in the upper respiratory tract of healthy animals, but invasion of viral and bacterial pathogens due to stress allows the relocation from upper respiratory tract to lower respiratory tract.[14] Though this bacteria can remain in the upper respiratory tract without the animal showing clinical signs, it is not in fact a normal flora of bovines.[10] Seemingly healthy cattle, known as carriers, that are shedding *Mycoplasma bovis* are considered to be the main source of infection for previously uninfected herds.[10] Routes of infection from respiratory secretions may include direct contact, aerosol, or indirect contact with areas of common usage such as water and feed troughs.[10] Calves that show signs of infection have also been shown to have a history of drinking infected milk from and nursing teats on cows with mastitis caused by *Mycoplasma bovis*.[4,10] Once an animal has been infected with

Mycoplasma bovis, the bacterium may be shed for months to years; when not in sunlight, *Mycoplasma bovis* may survive in the environment for months.[4,10] Higher rates of bacterial shedding occur during stress-inducing situations.[10] Clinical infection two weeks after exposure has been reported, but clinical arthritis and pneumonia have occurred 8-10 days after exposure experimentally.[4]

Clinical signs associated with respiratory infection may be increased respiratory rate, cough, fever, dyspnea, reduced feed intake, weight loss, depression, lethargy, and nasal discharge.[4,10] Due to hematogenous spread of *Mycoplasma bovis* from lung tissue, clinical signs of arthritis may be seen following a respiratory infection.[4,10,11] Clinical signs from arthritis due to *Mycoplasma bovis* may include lameness, swollen joints, increased temperature, and decreased feed intake and debilitation with severe infections.[12] Joints that are typically infected may be the elbow, hock, stifle, and carpus.[1,10] The effect of *Mycoplasma bovis* on the immune system with its ability to elude natural defenses that would eradicate it allows for chronic infections to form.[2] Although not pathognomonic, a lack of response to treatments coupled with chronic infection and arthritis may be more suggestive of *Mycoplasma bovis* than acute signs of disease such as coughing and high fever.[4]

On the cellular level, it has been found that *Mycoplasma bovis* increases neutrophil and lymphocyte apoptosis and encourages production of pro-inflammatory cytokines.[8,11,17] Research has linked variable surface proteins on the surface of *Mycoplasma bovis* with evasion of the immune system.[4,11,14] Lymphocyte proliferation is hindered by some strains although the cause of this has not been identified.[11] The full relationship between the host immune system and *Mycoplasma bovis* is not yet known.[11]

On necropsy, gross findings may include a caseonecrotic bronchopneumonia of the cranioventral lung lobes characterized by microabscessation of the tissue and fibrous or fibrinous attachments to the pleura. [4,7,10,16] Increased interlobular septae size due to edema and fibrin may also be noted.[16] According to the Smith et al., "peribronchiolar cuffing with lymphocytes and mononuclear cells," may be seen on histopathology with neutrophils present in airways.[16] Also on histopathology, areas of eosinophilic coagulative necrosis may be present.[16]

Treatment and Control:

Identifying cattle with clinical disease early in the infection is important to be able to begin therapy with antibiotics.[9] Within veterinary medicine, antibiotic use must be judicious, and antibiotics must be used to treat those diseases for which they are labeled. Due to the lack of a cell wall, some antibiotics such as Beta-lactams are not effective against *Mycoplasma bovis*.[15] Typical antibiotic classes used to treat suspected *Mycoplasma bovis* pneumonia include fluoroquinolones, tetracyclines, phenicols, and macrolides.[9] Macrolides, due to their ability to concentrate in compromised lung tissue, are a preferred antibiotic to treat *Mycoplasma bovis*.[9] Treatment with antibiotics for at least 10-14 days is recommended to maximize efficacy.[1,16] Studies have shown that resistance marked by increases in MIC_{50} for multiple antibiotic classes including macrolides, tetracyclines, and fluoroquinolones has developed.[9]

Due to the often ineffectiveness of treating *Mycoplasma bovis* infection and increased antibiotic resistance, attention must be turned towards prevention and vaccine development.[15] For the producer, maintaining a closed herd is ideal to avoid bringing *Mycoplasma bovis* into a herd.[3,10] For producers purchasing cattle, testing before purchase for antibodies with ELISA assays and separating cattle for a period of time (at least 3 weeks) prior to introduction are good

strategies.[3] An area to isolate sick cattle should also be available.[3] Practices to decrease stress and allow for maximum immune function should be set in place.[10,12] Calves to be sold should be processed (castration, dehorning, weaning) a minimum of 30 days prior to sale to decrease stress.[4] Vaccinating for other respiratory pathogens before sale is also beneficial.[4] Practicing metaphylactic antibiotic use, treating cattle that are suspected to be subclinical or are most at risk of developing respiratory disease to decrease clinical infections upon entrance to feedlots and facilities, has also been recommended to decrease signs of disease.[4,10] Two bacterin vaccines (MpB Guard and Myco-Bac-B) are available in the USA.[15] These vaccines are not widely in use however because of limited efficacy.[15] Further research into this subject needs to be performed in order to grasp the cellular interactions by which *Mycoplasma bovis* interacts with the immune system.[15] This research could lead to more efficacious vaccines being produced.[15]

Case Outcome:

At approximately 4 am on 11/30/2020, 291 was found in lateral recumbency. When prompted, she would move to sternal recumbency but would not stand. A physical examination was performed at this time, and a heart rate of 92 beats per minute and a temperature of 105.1 °F were noted. At 8 am, wheezing on pulmonary auscultation and increased respiratory effort were noted. She was given 4.0 mL (1.1 mg/kg) flunixin meglumine (Banamine) intravenously and 4.5 mL (2.5 mg/kg) tulathromycin (Draxxin) subcutaneously. Ultrasound of the left elbow and the thorax were performed. The findings of the ultrasound for the left elbow were highly suspect for septic arthritis. Multiple comet tails and circular structures filled with anechoic fluid were found in the cranioventral pleura as well as irregular echogenicity of the lungs.

Due to the poor prognosis associated with the lung pathology found on ultrasound and the highly suspected septic joint, 291 was humanely euthanized by captive bolt gun and infusion of intravenous potassium chloride. A necropsy was performed, and the findings were consistent with infection with *Mycoplasma bovis*.

On gross necropsy, the left humeroradial joint was noted to be swollen medially and laterally, and an ulceration had formed at the level of the olecranon. There was hyperemia of the musculature and bursa on cut surface, and the musculature and bursa appeared to be distended due to the presence of fluid. There was hyperemia of the synovium of the joint.

On gross necropsy of the thorax, hyperemia of the tracheal mucosa was noted, and there were softball sized bullae bilaterally at the caudal dorsal lungs. Atelectasis and discoloration of the lung to dark purple and red were noted in the cranioventral lung fields. There was a mass of caseous material measuring 8 cm x 8 cm x 5 cm located in the left cranioventral lung. Also, fibrinous connections between the cranioventral lung and thoracic wall were found. In general, dehydration was noted due to the tackiness and dryness of subcutaneous tissue. These findings led to the diagnosis of a chronic, severe bronchopneumonia.

The histopathologic findings consisted of neutrophils, plugs of fibrin, and eosinophilic fluid inside the alveoli. There were fibrin and neutrophils in dilated bronchioles with some bronchioles being eliminated and replaced with eosinophilic cellular debris. There were large amounts of lymphocytes and plasma cells encircling remaining bronchioles, and the epithelium of many of the remaining bronchioles appeared hyperplasic. Edema and fibrin were found to enlarge the interlobular septae with multiple lymphatic vessels of the septae filled with fibrin clots.

While the necropsy was being performed, samples were taken for aerobic culture of the lung and affected joint and for real-time PCR of the lung. Although culture of the joint did show faint growth, the bacteria that was grown was regarded as a contaminant. Due to this fact, a definitive diagnosis for the bursitis and synovitis was not identified. The lung culture showed no growth of respiratory bacteria. The real-time PCR of the lungs was found to be positive for Bovine Respiratory Syncytial Virus (BRSV) and *Mycoplasma bovis*.

Conclusion:

In conclusion, *Mycoplasma bovis* is the most important *Mycoplasma* species in cattle in North America and Europe.[9] It can cause pneumonia, arthritis, otitis media, mastitis, keratoconjunctivitis, and reproductive diseases.[9] *Mycoplasma bovis,* one of the bacterial pathogens in BRDC, causes significant economic losses annually in feedlot and stocker operations.[12] Diagnosis can be made via culture, conventional PCR, real-time PCR, or ELISA.[6] Due to its lack of a cell wall, evidence of antibiotic resistance occurring, and immune modulating abilities, infection from this bacterium remains difficult to treat with control and prevention being more applicable.[9] Future research to identify the mechanism of action by which *Mycoplasma bovis* impacts immune function needs to be performed to produce more effective vaccines.[15]

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