Current challenges in controlling ruminant diseases associated with *Mycoplasma bovis*, *mycoides*, and *ovipneumoniae*

by

Brittany Ann Wilhite

B.S., Rutgers University, 2015

A REPORT

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Diagnostic Medicine/Pathology
College of Veterinary Medicine

KANSAS STATE UNIVERSITY
Olathe, Kansas

2019

Approved by:

Major Professor
Dr. Alison Paige Adams

Copyright

Abstract

Mycoplasma bacteria were discovered and have been explored in published literature for well over a century. Most notably, these organisms lack a cell wall and cause disease in animals and humans depending on the bacterial species. This report explores the characteristics of *Mycoplasma bovis*, *mycoides,* and *ovipneumoniae* and their associated challenges in controlling disease in ruminants, current strategies for mitigation, and possible utilization of vaccines for prevention. *Mycoplasma bovis,* one of the more pathogenic strains, affects cattle and manifests itself in causing mastitis, pneumonia, ocular signs, arthritis, and other symptoms. Economic losses from *Mycoplasma bovis* are incurred from the high morbidity rates. Laboratory diagnostics are necessary to confirm this causative agent. Treatment of *Mycoplasma bovis*, including the use of effective antimicrobials, is still debated. Sound herd management, as well as potential vaccination, are critical in the prevention of mycoplasmas within the United States. *Mycoplasma mycoides*, also affecting cattle, is the bacterium causing contagious bovine pleuro-pneumonia. Today, this condition primarily affects herds in Asia and Africa; however, American and British economies were affected by a suspected and primary diagnosis of this disease in the mid- to late- 1800s. Transmitted via aerosols, *Mycoplasma mycoides* causes primarily respiratory signs in adult cattle and arthritis in calves. Only a few different diagnostic tests can confirm *Mycoplasma mycoides,* but a confirmation, aside from clinical signs, is made post-mortem. There have been reports of antimicrobials demonstrating effectiveness with regard to combating *Mycoplasma mycoides*; however, there is no standard protocol for treatment at this time. Sanitary and medical prophylaxis is recommended to control and prevent *Mycoplasma mycoides* from infiltrating a herd. Finally, *Mycoplasma ovipneumoniae* has been implicated in domestic flocks of sheep and goats in addition to wildlife. Respiratory morbidities and high transmissibility of

Mycoplasma ovipneumoniae result in significant losses. A polymerase chain reaction (PCR) or competitive enzyme-linked immunosorbent assay (ELISA) can be used to diagnose the bacteria. Separating domestic flocks from wildlife is imperative in protecting both groups. Strategies to combat mycoplasmas include individual animal testing, herd surveys, and isolation of animals before exposure to the herd, among others. Vaccination strategies should be more aggressively investigated. In conclusion, there is much to be done in understanding and controlling this complex microorganism in ruminants.

Table of Contents

List of Tables

Acknowledgements

I would like to acknowledge and thank my major professor, Dr. Alison Paige Adams, for being a superb mentor throughout my academic program. She has gone above and beyond to encourage, support, and dedicate her time on this rigorous path of mine. Her overall mentorship has been invaluable, and I am forever grateful. Additionally, I would like to thank my graduate committee members, Drs. Gary Anderson and Justin Kastner, for their guidance and contributions. They have each served as a source of knowledge and sound advice during my program, for which I am immensely appreciative.

I cannot continue without thanking my loving parents, Billy Wilhite and Rosemary Madnick, and my family. Throughout my successes and tribulations, they have been my solid support system. Their love and empathy are unmatched, and they gave me hope when I doubted everything in life. I am the person I am today because of the strength instilled from my family. I owe them the world and more.

Dedication

I would like to dedicate this work to my brother, Joshua Madnick, and my sister, Tyler Madnick. You both encourage and inspire me to be a better person every day.

Chapter 1 - Introduction

With more than 120 species, mycoplasmas are the causative agent of a multitude of conditions in both humans and animals. This report aims to discuss three of the more pathogenic species of mycoplasma in ruminants: *Mycoplasma bovis*, *mycoides*, and *ovipneumoniae*. These three species were specifically selected due to the relevance in cattle and wildlife in Alaska, which aligns closely with the author's interests.

Background of Mycoplasma

Etymology

The term "mycoplasma" was first coined in a paper by German botanist, plant pathologist, and mycologist, Albert Bernhard Frank, in 1889. The term was also subsequently used by Swedish plant pathologist, mycologist, and taxonomist, Jakob Eriksson, in 1897. These scientists used the term to signify a close association between plant-invading fungi or other microorganisms and their host cells (Krass and Gardner, 1973). In 1929, Polish microbiologist and politician Julien Nowak used mycoplasma in a taxonomic context to describe the organism involved in bovine pleuro-pneumonia. Later, E. A. Freundt and D. G. Edward more recently used mycoplasma to classify organisms as the causative agents of pleuro-pneumonia in cattle (Krass and Gardner, 1973).

Classification

The genus Mycoplasma is within the Bacteria domain and Mollicutes class.

Properties

Mycoplasmas are prokaryotes and the smallest free-living, self-replicating microorganisms (Maglaras and Koenig, 2015). The defining characteristic of this class is that all members lack a protective cell wall. Due to this, they are easily damaged when they are outside of a host and can be difficult to stain in the laboratory using routine bacterial staining methods. Though mycoplasma species stain using Gram stain, and based on the phenotype, are considered Gram-negative, this is not due to the lack of peptidoglycans in their cell wall; as stated, this genus lacks a cell wall altogether. It is perhaps more correct to classify them as a Gram-positive bacterium that has lost the cell wall, and therefore, have lost the capacity to retain any crystal violet dye. The Giemsa stain is an alternative staining option. Mycoplasmas contain a small genome (ranging from 540-1300 kb) (Hardison 1997), and thus, have limited metabolic capability; this is due to a limited amount of proteins (enzymes) able to support growth. These microorganisms draw nutrients from mucosal surfaces after colonization. There are hemotropic (attracted to blood cells) and nonhemotropic forms (Maglaras and Koenig, 2015).

Chapter 2 - *Mycoplasma bovis*

Introduction to Disease

Mycoplasma bovis is a significant pathogen of cattle causing disease with impacts affecting the welfare of the animals as well as the economics of the farming community. In fact, *M. bovis* is regarded as one of the more pathogenic species of the genus. *M. bovis* was first detected in 1961 in the United States as a cause of bovine mastitis. Since the 1960s, *M. bovis* has been found in most countries around the world (Nicholas et al., 2003). True to the mollicute class, *M. bovis* is small and pleomorphic, meaning that this bacterial species can somewhat alter its characteristics to adapt to its environment. The genome size is 1,080 kbp (Nicholas et al., 2003), and it has a low GC ratio of about 29.3%, making it relatively unstable (Calcutt et al., 2018). Most mycoplasmas are fragile in the environment; however, *M. bovis* can survive at 4°C for about two weeks in sponges and milk and for longer than two weeks in water. Survival rates decrease significantly at higher temperatures (Nicholas et al., 2003). *M. bovis* is not a food safety risk for humans.

Economics

One published estimate of the economic impact of *M. bovis* in 1999 stated that the United States experiences approximately $108 million in losses as a result of the bacteria (Rosengarten and Citti, 1999). These losses are attributed more toward bovine mastitis than respiratory disease in dairy cattle, with infection rates of up to 70% in a herd (Rosengarten and Citti, 1999). A bulk milk tank culture over nine years in New York revealed that about 2.3% of tank samples were found positive for *M. bovis*. Samples retrieved from surveys in Iowa had similar results. Iowa herds with tank-positive cultures had culling rates of 30-70% of cows. Enzootic pneumonia

caused by *M. bovis* also affect veal calves and feedlot cattle. These losses are seen as loss of weight gain and loss in carcass values. Such losses, as of 1999, stood at approximately $32 million per year (Rosengarten and Citti, 1999). In all classes of cattle, *M. bovis* has been isolated from the genital tract as well as in aborted fetuses. Contaminated bovine semen is seen as a medium for transmission, which impacts the artificial insemination industry (Rosengarten and Citti, 1999). Other costs include general reduced production, drugs and treatment labor, death and culling losses, diagnostics, prevention strategies, control implementation, and costs related to decreases in animal welfare. Disease associated with *M. bovis* is typically chronic, and thus, costs per case are higher when compared to other pathogens in the bovine respiratory disease complex (Maunsell et al., 2011).

Transmission & Clinical Signs and Symptoms

M. bovis is highly contagious, and transmission was first thought to occur solely via aerosol. However, calves are at a high risk of infection when fed milk from infected cows. Some experimental studies have shown that the teat canal and genital tract can be means of infection passed from dam to fetus. Contaminated semen used for artificial insemination has been traced to female infections as well. Additionally, *M. bovis* has the capability to survive in bedding. Cattle are also most susceptible to infection given climatic changes, overcrowding conditions, or translocation (Calcutt et al., 2018). More details are described below with regard to common clinical disease manifestations associated with *M. bovis* infections.

Mastitis: The degree of mastitis in a herd can vary from endemic subclinical disease to severe outbreaks. In some subclinical infections, cows do not experience an increase in somatic cell

counts or a reduction in milk production, making it difficult to detect clinically. *M. bovis* does not discriminate, in that cows of any age or stage of lactation are susceptible. Clinical disease presents nonspecific signs. More than one quarter is typically affected, and there may be a dramatic decrease in milk production and/or mild signs of systemic ailments. The mammary gland itself could be swollen but not necessarily painful, and secretions can vary from minimally abnormal to gritty or purulent with a possible brownish hue. Clinical disease can last for several weeks, and antimicrobial resistance is common. Potential accompanying conditions include arthritis, synovitis, joint effusion, or respiratory disease. A return to homeostasis in the animal is possible, but it can be a slow process (Maunsell et al., 2011).

Pneumonia: Pneumonia caused by *M. bovis* can occur in any age of cattle. Clinical signs are nonspecific and may include fever, tachypnea, dyspnea, decreased appetite, and coughing with or without nasal discharge. Chronically affected animals will experience inadequate weight gain. Potential accompanying conditions include otitis media and arthritis. Chronic pneumonia and polyarthritis syndrome (CPPS) can occur in beef cattle several weeks after arrival to a feedlot (Maunsell et al., 2011).

Otitis media: Otitis media caused by *M. bovis* occurs in dairy or beef calves as either enzootic disease or as an outbreak. Clinical signs can be unilateral or bilateral. Calves experiencing early or mild cases of otitis media will maintain alertness and a sufficient appetite; however, disease progression will progress to fever and anorexia. Ear droop and ptosis are a result of ear pain and cranial nerve VII deficits. Calves with ear pain will also shake their head and scratch and/or rub their ears. Eyelid paresis can cause the secondary effects of epiphora and exposure keratitis. If

the tympanic membrane of the ear has been ruptured, purulent aural discharge can be evident. Accompanying conditions of pneumonia and/or arthritis are common. As sequelae to otitis media, otitis interna and vestibulocochlear nerve deficits can occur. A common clinical sign of otitis interna is head tilt, but certain animals will display nystagmus, circling, falling, or drifting toward the side of the lesion and vestibular ataxia. Meningitis can develop in advanced cases of otitis media and otitis interna. Additionally, spontaneous regurgitation, loss of pharyngeal tone, and dysphagia can occur. This is suggestive of glossopharyngeal nerve dysfunction with or without vagal nerve dysfunction (Maunsell et al., 2011).

Arthritis, synovitis, and periarticular infections: Arthritis caused by *M. bovis* can affect cattle of any age. Cases are typically sporadic and will accompany pneumonia or mastitis, and although most of these cases are reported in calves and dairy cows, CPPS is more likely to be reported in feedlot cattle. Clinical signs are typical of septic arthritis, including acute non-weight bearing lameness with joint swelling, pain, and heat upon palpation. Signs may also include a febrile and anorectic animal. Tendon sheaths and particular soft tissue involvement is common. Large rotator joints (hip, stifle, hock, shoulder, elbow, and carpal) are commonly affected. Other joints, such as the fetlock or even the atlantooccipital joint, can be involved. It is common for the animal to respond poorly to treatment (Maunsell et al., 2011).

Keratoconjunctivitis: *M. bovis* can be isolated from the conjunctiva of both healthy and diseased animals. The involvement in infectious keratoconjunctivitis is not well understood or reported, and it is mainly considered a predisposing or coinfecting agent. However, it was the only

pathogen isolated in an outbreak of infectious keratoconjunctivitis in calves where this ailment was followed by cases of pneumonia and arthritis (Maunsell et al., 2011).

Decubital abscesses: One report stated that 50 calves developed *M. bovis*-infected decubital abscesses over the brisket and joints. Some of these affected calves had *M. bovis*-associated pneumonia as well (Maunsell et al., 2011).

Cardiac disease: *M. bovis* was identified with the bacteria *Histophilus somni* in the hearts of 4 of 92 feeder calves dying from myocarditis. Additionally, a heifer with clinical signs of cardiac insufficiency was found to have mural and valvular endocarditis with *M. bovis* isolated from this case of chronic active fibrinopurulent endocarditis (Maunsell et al., 2011).

Genital disorders: There is scant evidence to support an important role for *M. bovis* in naturally occurring bovine reproductive disease. However, in isolated and mainly experimental cases, *M. bovis* has been linked to genital infections and abortions in cows and seminal vesiculitis in bulls (Maunsell et al., 2011).

Diagnosis

Laboratory diagnosis is necessary for pathogen identification as clinical and pathological signs are not unique to *M. bovis*. Thomas et al. (2002) noted that sampling by bronchoalveolar lavage was more indicative of lower respiratory airway pathogens, including *M. bovis*, than nasal swabs. *M. bovis* grows well in a variety of media and produces colonies within 3-5 days of incubation. In the correct medium, *M. bovis* produces films and spots, and gives the broth an

orange hue. Growth inhibition, film inhibition, fluorescent antibody, or metabolic inhibition tests can be used to identify mycoplasma using hyperimmune rabbit serum (Nicholas et al., 2003). A commercial sandwich enzyme-linked immunosorbent assay (ELISA) exists for *M. bovis* in which specific monoclonal antibodies fixed to a microplate capture the *M. bovis* antigen from the medium. *M. bovis* can be over grown by opportunistic mycoplasmas and sometimes antigenic variability of strains can make serological tests untrustworthy. In these conditions, polymerase chain reaction (PCR) can be more readily utilized. PCRs have been used to detect *M. bovis* from milk (even preservative-treated milk) and nasal swabs (Nicholas et al., 2003). Combined testing for both prior exposure (by detecting anti-*M. bovis* antibodies) and current infection (by detecting *M. bovis* antigen or nucleic acid) would provide the best control measures against new and potentially latent infections. However, if both tests yield a negative result, the "gold standard" would be to culture the organism (Calcutt et al., 2018).

Treatment

It was once considered that diseases and conditions resulting from *M. bovis* were resistant to any chemotherapy. However, antibiotics are typically prescribed to reduce secondary bacterial infections and do not effectively treat the mycoplasma. Evidence from Ayling et al. (2000) implies that European *M. bovis* strains are developing resistance to antibiotics traditionally used for mycoplasma treatment; more specifically, these antibiotics include oxytetracyclines, tilmicosin, and spectinomycin. On a scale of relative effectiveness, the fluoroquinolones are deemed effective, but their use in animals remains contentious (Nicholas et al., 2003).

Prevention & Control

The inability of chemotherapies to control these infections has caused a shift in focus to vaccination (Perez-Casal et al., 2017). Currently, there are no vaccines available in Europe. In the United States, there are a few commercially available vaccines (including autogenous and bacterin vaccines), although there is minimal data supporting their effectiveness (Calcutt et al., 2018). These United States vaccines are not licensed for use outside of the country (Calcutt et al., 2018).

Enforcing a closed herd policy can be critical in managing mycoplasma infections and disease. Additionally, confirming that animals are free of infection by testing for anti-*M. bovis* antibodies and appropriate quarantine measures with consistent health assessments are effective strategies for keeping a herd free of *M. bovis*. Good management practices and proper ventilation will also assist in control measures. Examples of good management practices include introduction of animals tested free of *M. bovis*, routine cleaning and disinfection of premises, prohibited feeding of infected milk, regular observation of animals, isolation of infected animals, and potential culling (Calcutt et al., 2018).

Chapter 3 - *Mycoplasma mycoides*

Introduction to Disease

As noted, mycoplasmas are significant bacterial livestock pathogens worldwide. *Mycoplasma mycoides* subsp. *mycoides* and *Mycoplasma capricolum* subsp. *capripneumoniae* are two members of the "*Mycoplasma mycoides* cluster" (Fischer et al., 2012). These bacteria are the causative agents of contagious bovine pleuro-pneumonia (CBPP) and contagious caprine pleuro-pneumonia. Both of these conditions cause profound losses in livestock, particularly in Africa and Asia as of today, and threaten re-introduction to disease-free countries such as Europe and the United States (Fischer et al., 2012). For the scope of this report, the focus will remain on *M. mycoides* affecting cattle and certain related bovids. Humans are not susceptible to CBPP, so there is no zoonotic risk (OIE 2019).

M. mycoides was characterized for the first time in 1898. CBPP was first noted in Europe and was introduced into Africa, North America, Australia, and New Zealand during colonial expeditions of livestock trade movements in the 18th and 19th centuries (Fischer et al., 2012).

M. mycoides does not survive for long in the environment under warm conditions; it can survive outside of its host for up to three days in tropical areas, and up to two weeks in temperate climates. If frozen, the bacteria can survive for more than ten years. With regard to temperature, it can be inactivated within one hour at 56°C and two minutes at 60°C. The bacteria can also be inactivated by both an acid and alkaline pH (OIE 2009).

Economics

Although economic impacts of *M. mycoides* occur in Africa and Asia at the present time, an example of how detrimental this threat of disease can be is seen when analyzing transatlantic trading between North America and Great Britain in the mid- to late-1800s.

As early as 1843, pleuro-pneumonia had been an issue between the United States and England as a result of U.S. Congress remaining lenient about tariffs on imports of breeding stock. A House report ("House Agriculture Committee Report on Cattle Disease") from the 36th Congress notes an infiltration of ruminant pleuro-pneumonia from England to Brooklyn, New York aboard the steamship *Washington*. Furthermore, when pleuro-pneumonia had found its way into New Jersey from Britain in 1847, it was contained as the concerning farmer slaughtered his infected herd. Pleuro-pneumonia was introduced once again to the U.S. in 1859, although this shipment of infected cattle originated from Holland enroute to Massachusetts. However, in the summer and fall of 1878, outbreaks of pleuro-pneumonia were evident in the District of Columbia, Maryland, Virginia, New Jersey, and New York (Kastner 2003).

The bulk of the story, as told by Kastner et al. (2005), begins in January 1879. At this time, the steamship *Ontario*, carrying a shipment of over 200 cattle from North America, was headed from Maine, U.S., to Liverpool, England. Meanwhile, veterinary authorities in both London and Liverpool, England, were planning the enforcement of a new Foreign Animals Order. This order, adopted under the Contagious Diseases (Animals) Act of 1878, stated that all livestock imports were to be slaughtered within ten days of arrival to port. This act included exempted countries, of which the U.S. and Canada were included. With these exemptions, animals from particular countries could be transported inland for further pre-harvest practices under the condition that they were deemed disease-free at port. There had been some reports by

certain veterinary professionals that the eastern U.S. harbored CBPP. This led to the question of how long the U.S. would remain exempt from the slaughter order at U.K. ports. These reports proved detrimental for those involved in the economics of North American cattle trading. Some businessmen from both the U.S. and the U.K. speculated that if livestock imports ceased, the supply of meat would decrease while prices would increase. When the *Ontario* arrived in England, there was just under 200 animals, as two of them had died. The Veterinary Inspector for the local authority of Liverpool did a post-mortem analysis of these two animals and suspected CBPP. The Inspector relayed these lung samples to the Veterinary Department of the Privy Council of London, who confirmed CBPP. There was a subsequent slaughter order for the entire shipment aboard the *Ontario*. Upon slaughter, the Privy Council veterinary authorities noted that many of the cargo had respiratory inflammation indicative of cold weather. Additionally, though, they noted the characteristic lesions of CBPP in twelve of the animals' lungs. The American exemption from the immediate slaughter order was retracted the next day. This proclamation wreaked havoc. The U.S. Treasury Secretary haphazardly created a program to inspect and certify live cattle; the U.S. Commissioner of Agriculture meanwhile had sent two men to inspect Chicago and New York stockyards. Few individuals, including a professor from a veterinary college in Edinburgh and a shipping mogul, questioned the final diagnosis of CBPP within the U.K. Thus, the "disease-free west" argument was born: that CBPP was in circulation in the eastern U.S. but was non-existent in the western states where the cattle originated (prior to being transported to Maine for shipment to England). Importers from Liverpool called in veterinary experts from around the U.K. to corroborate the Privy Council's diagnosis. Of the three consultants, two were definite in their diagnosis of CBPP, while one attributed the lesions to conventional bronchitis. This brought on debate within the transatlantic veterinary community,

which continued for some time. It is important to recognize that at this period of time, microbiological analysis was practically in its infancy. Thus, inspectors utilized gross visual analysis for diagnosis. The fact that CBPP was contagious was clear, but the specific causes of the disease (*M. mycoides*) would remain elusive until 1898. Without a solid program for inspecting and certifying cattle in the U.S., the U.K. had reason to deny claims and questions regarding CBPP. The issue resolved itself after 1900, when the demand for beef in an exponentially growing U.S. population took attention away from U.K. markets (Kastner et al., 2005). The most recent report of CBPP in Europe was in Portugal in 1999. Since then, Europe has been considered CBPP-free (CABI 2019). According to the USDA APHIS (U.S. Declaration of Disease Freedom), the United States has been declared CBPP-free since 1892 (USDA 2019).

Transmission & Clinical Signs and Symptoms

Incubation of CBPP is typically 1-4 months, but longer time periods have been observed. Transmission of *M. mycoides*/CBPP occurs through close, direct contact and inhalation of droplets from infected, coughing bovines; however, under particular atmospheric conditions including humidity and wind, the bacteria can be transmitted via aerosols over a longer distance (~200 meters). The bacteria can also be found in saliva, urine, fetal membranes, and uterine discharges. Transmission via the placenta is possible. Transporting cattle is usually a stressor in the spread of CBPP (OIE 2009).

Initial clinical signs in the adult may present as a depressed animal with inappetence and a moderate fever, to be followed by coughing, pain in the thoracic cavity, and an increased respiratory rate. As pneumonia progresses, the animal will develop labored respiration and dyspnea. The animal will also prefer to stand with the elbows abducted to relieve thoracic pain

and increase chest capacity. CBPP will often develop into a chronic disease demonstrating ill thrift and a recurrent low-grade fever that may be difficult to interpret as pneumonia. Forced exercise may encourage coughing. In the infected calf, clinical signs may present as arthritis and joint swelling instead of pulmonary tropism (OIE 2009).

Diagnosis

A clinician should suspect CBPP in a herd with pulmonary signs in adult cattle and arthritis in calves co-existing (OIE 2009). According to John Campbell of the Merck Veterinary Manual (2019), a diagnosis should be made based on clinical signs and gross characteristic lesions of the lungs. The OIE describes these lesions as often unilateral. The affected pulmonary parenchyma has no odor. The primary lesion is consolidation of individual lung lobules that become encased in widened interlobular septa (strands of connective tissue separating adjacent pulmonary acini – where the alveoli are located – and lobules), resulting in a marbled look. Interlobular septa become distended by edema, fibrin, and eventually, fibrosis. *M. mycoides* will produce a necrotizing toxin called galactan that will allow for the spread throughout the septa. A copious amount of yellow or turbid exudate in the pleural cavity will coagulate to form large clots. The pleura of the chest cavity will thicken and become inflamed, producing fibrous deposits. In recovered animals, there will be sequestrate with a fibrous capsule surrounding grey necrotic tissue (OIE 2009). In terms of diagnostic tests, the Merck Veterinary Manual notes that complement fixation (measuring the amount of complement available in serum to bind to an antibody-antigen complex), latex agglutination (measuring the number of antibodies or antigens in a sample of body fluid), or competitive ELISA tests can be used as an aid. Another confirmation can be made by isolating *M. mycoides* followed by growth inhibition or

immunofluorescence test using hyperimmune rabbit sera against *M. mycoides*. PCR is becoming increasingly popular as a diagnostic tool as well. Additionally, serologic reactions can be confirmed via immunoblotting test (searches for antibodies against *M. mycoides* proteins). Overall, it is strongly recommended that once an outbreak has occurred, or is suspected to have occurred, infected cattle should be slaughtered and submitted for necropsy for diagnosis (Campbell 2019).

Treatment

In Africa, there is one >60-year-old vaccine formulation (live T1/44) that appears to poorly protect animals, cause severe reactions if not administered properly, and has residual virulence. Moreover, a mathematical model has shown that even mass vaccination over a five-year period cannot eradicate CBPP unless other strategies are additionally used (Nicholas et al., 2012). The effectiveness of antibiotics is difficult to assess, and there is a need to reduce their use in a general, profession-wide effort to limit antimicrobial resistance. However, the harsh spread of CBPP throughout Africa (in addition to the lack of an effective vaccine) has caught the attention of official bodies, including the Food and Agriculture Organization of the United Nations (FAO), to reconsider the use of antibiotics (Nicholas et al., 2008).

Nicholas et al. (2012) recalls use of antimicrobials to treat an outbreak of CBPP in the Caprivi region of Namibia in Africa in 2003. This outbreak was severe with high mortality in cattle. Locals implemented vaccination policies to protect the naïve animals; however, animals continued to die. There was significant concern that this would endemically spread to other areas of Namibia with high cattle populations. Nicholas demonstrated in previous experiments that CBPP treatment with danofloxacin, a broad-spectrum fluoroquinolone used in treating

respiratory disease in a few varying species, could reduce transmission of *M. mycoides*. With this outbreak, Nicholas et al. (2012), therefore, treated all clinically affected or seropositive herds with danofloxacin according to the label. The amount of new cases fell instantaneously (only three animals died within the first three months after treatment) and no new cases developed afterwards. Subsequent monitoring continued for two years, and no new cases were reported. Vaccination was implemented once again following the last outbreak. Since this major outbreak and the use of antimicrobial treatment, there have been only minor outbreaks east of Caprivi. To provide some background information associated with this outbreak, the cattle infected there had not been treated earlier due to seasonal flooding of the Zambezi River. Most notably, there have been no reported cases in the primary cattle region southwest of Caprivi. This eradication treatment success was significant in that CBPP can normally persevere indefinitely in small populations. It is demonstrated that an antimicrobial with mycoplasmacidal properties can greatly reduce transmission to susceptible animals to a non-sustainable level in a herd (Nicholas et al., 2012). Furthermore, some evidence has suggested that antimicrobial treatment hurries the healing process by promoting fibrosis in the lung, thus helping to combat *M. mycoides* transmission (Nicholas et al., 2012).

In vitro antibiotic susceptibility testing of 41 isolates of the mycoplasma pathogen revealed that at least 10 antimicrobials demonstrated effectiveness (Nicholas et al., 2012); antimicrobial resistance in these treatments has not yet been apparent. Work still needs to be done to understand the current field conditions and to find appropriate, cost-effective antimicrobials with a proper dosing regimen. Monitoring resistance is also critical (Nicholas et al., 2012). Despite the evidence of Nicholas, the OIE (2009) does not recommend antibiotic treatment because it can cause a delay in disease recognition, potentially create chronic carriers,

and contribute to antimicrobial resistance. All in all, at this stage, there is no standard protocol for the treatment of CBPP caused by *M. mycoides*.

Prevention & Control

The OIE (2009) recommends both sanitary and medical prophylaxis. Within the realm of sanitary prophylaxis in disease-free areas, quarantine, control of cattle movement, serological screening, and culling of all positive and suspect animals is recommended. Contrary to the previous section with regard to antibiotics, the OIE recommends vaccination in control of CBPP (although they acknowledge the potential of severe adverse reactions). The OIE notes that current vaccine strains are made with live, attenuated *M. mycoides* strains whose efficacy is directly related to the virulence of the original strain in production. Additionally, they describe two strains used to prepare the CBPP vaccine: the T1/44 strain, which is naturally mild and isolated in 1951 in Tanzania (passaged 44 times through embryonated eggs), and the T1sr strain, which is evidently completely avirulent but has a shorter immunity than T1/44. In areas where CBPP is not prevalent, including Europe (in its proximity to Africa), vaccination is not recommended as it can interfere with screening surveillance serological tests (OIE 2009).

Chapter 4 - *Mycoplasma ovipneumoniae (M. ovi)*

Introduction to Disease

Mycoplasma ovipneumoniae, referred to in short as *M. ovi*, is a common respiratory pathogen affecting sheep and goats. According to the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) (2015), this bacterium was first isolated in 1974 in New Zealand; since then, it has been recovered from the respiratory tracts of both healthy and clinically affected sheep worldwide. Alone, *M. ovi* is not normally highly pathogenic, but it predisposes sheep to other respiratory infections, making the resulting pneumonia a polymicrobial infection (Besser et al., 2017). This occurs via interference with normal ciliary activity in the respiratory tract and by lymphocyte suppression (APHIS 2015).

In a study completed by the USDA's National Animal Health Monitoring System in 2011, 22 major sheep-producing states were evaluated for their health and management. This was the first study to estimate the prevalence of *M. ovi* within the United States. They discovered a high percentage of PCR- and ELISA-positive flocks, suggesting that *M. ovi* is a ubiquitous organism among national flocks. All herded-range flocks involved in the study tested positive for *M. ovi* carriage. Moreover, a higher percentage of medium and large flocks tested positive than small flocks. DNA fragment detection via nasal swab of ewes demonstrated carriage and possible shedding, but not necessarily that of an active infection. The ability of an animal to respond to potential exposure relies on many factors including, but not limited to, weather, flock movement, crowding, and/or nutritional status (APHIS 2015).

Economics

In United States sheep operations, respiratory diseases incur significant economic losses. According to APHIS, in 2009, 4.8% of nonpredator death losses in sheep and 12.6% of nonpredator death losses in lambs were caused by respiratory disease, such as pneumonia or shipping fever. Other economic losses based on the respiratory disease manifestation itself in sheep included poor growth rates, decreased feed-conversion efficiency, and increased labor and management costs (APHIS 2015). A 2011 estimate of economic impact due to *M. ovi* was determined to be $800 million annually in the United States (Highland, n.d.).

Transmission & Clinical Signs and Symptoms

In a study performed by Besser et al. (2017), they stated that *M. ovi* carried by domestic goats was transmitted to comingled bighorn sheep, and thus, this prompted the development of pneumonia. In this study, though, they found that the severity of disease was significantly milder than that seen in similar experiments with domestic sheep strains of *M. ovi*. Domestic goats (or sheep) may transmit other respiratory pathogens when contacting bighorn sheep (or other wildlife) on or near wild home ranges, due to stray animals from farms, when used for weed control, when commercial operations graze on public lands near wildlife ranges, or when used as pack animals for backcountry recreation. Sometimes wildlife will enter private lands as well (Besser et al., 2017).

In a separate study conducted by Besser et al. (2014), they concluded that *M. ovi* was most likely the principal epizootic agent of bighorn sheep pneumonia. The study objective was to better evaluate the outcome of experimental introduction of *M. ovi* into naïve bighorn sheep. Some of the most impactful findings in the study were the high transmissibility of *M. ovi* and the

consistent development of pneumonia that followed infection in bighorn sheep. Eight of nine bighorn sheep exposed to *M. ovi* via comingling with a challenged animal developed severe bronchopneumonia and three died, while all domestic sheep in the study remained healthy. The discussion states that they demonstrated that experimental inoculation of *M. ovi* infection in naïve bighorn sheep produces chronic, severe bronchopneumonia associated with multiple secondary bacterial infections. This infection was transmitted quickly to animals both within the same pen as well as to animals in adjacent pens. Altogether, respiratory disease, such as bronchopneumonia, can be expected with infection of *M. ovi*. Subsequent signs associated with respiratory disease can also be expected (e.g., ill thrift) (Besser et al., 2014).

Implications to Wildlife

Despite many early reports of the species tropism for sheep, *M. ovi* has been implicated in species beyond that of the subfamily *Caprinae*. Since *M. ovi*'s original isolation, there has been evidence supporting its connection with bighorn sheep pneumonia in western North America. Other reports of respiratory disease associated with *M. ovi* have included a few muskoxen as well as respiratory disease in Beira antelope in Qatar and at least 9 cattle in Colorado, United States (Highland et al., 2018). These studies could be considered by some to be somewhat questionable, though, as the methods used to identify *M. ovi* was limited to PCR testing, where no additional diagnostics or sequencing were performed. As stated by Highland et al. (2018), definitive claims of host range restrictions are absent from mycoplasma literature, because "assumptions about restricted host range of mycoplasmas, based on the host from which they were first or frequently isolated, are usually made in the context of nearly complete absence of representative sampling of the vast majority of potential vertebrate hosts." Additionally, the

particular and unpredictably culturable nature of *M. ovi* often requires molecular technologies for identification and confirmation. Highland et al. (2018) used such molecular techniques to analyze species from the subfamily *Capreolinae* for *M. ovi*.

M. ovi has recently infiltrated into the state of Alaska. The Alaska Department of Fish and Game (ADF&G) released a statement on their website of common questions and answers regarding *M. ovi*. To begin with, there is little known about the status of mycoplasmas, including *M. ovi*, in Alaska. The site states that *M. ovi* was first detected in Alaska's Dall's sheep and mountain goats in March 2018. In June 2018, *M. ovi* was found in caribou and moose.

During July 2017-January 2018, the U.S. Department of Agriculture Research Service in Pullman, Washington received nasal swab samples from 230 moose and 243 caribou from Alaska as well as 5 mule deer from Arizona. They also received one *M. ovi* isolate in February 2018 that had been cultured in Minnesota from the lung tissue of a white-tailed deer that had died during a pneumonia outbreak at a captive facility in the upper Midwest U.S. in 2016. They ultimately detected *M. ovi* in 6 moose (2.6%), including 3 from two of the captive facilities and 3 from free-ranging moose. *M. ovi* was detected in 5 free-ranging caribou (2.1%) and 2 of 5 mule deer, one of which was exhibiting a cough and nasal discharge during sample collection (Highland et al., 2018).

With regard to wildlife health status, finding *M. ovi* in wildlife does not necessarily mean the animal is "diseased," as there have been *M. ovi* isolates found in healthy Dall's sheep, mountain goats, caribou, and moose. The ADF&G website also notes that more research and monitoring is required to determine if any of the *M. ovi* strains in Alaska are similar to the continental U.S. strains associated with high mortality; thus far, there is no link. It is also unknown how *M. ovi* entered Alaska's wild sheep and wildlife population. However, the bacteria

have been detected in a small number of domestic flocks in Alaska. These flocks were not showing signs of illness. The estimated number of domestic sheep and goats in Alaska is approximately 1,500. At this point, almost 400 animals have been tested (blood and nasal swabs) and about 4% have been found to be positive for *M. ovi* (ADF&G). Farms participating in voluntary testing for the State of Alaska is continuing. Finally, the ADF&G website notes that in general, animals showing signs of respiratory disease should be safe to consume. It is recommended that affected areas should be trimmed off and the surrounding meat should be cooked thoroughly. However, despite not being a foodborne disease, it is recommended that wildlife hunters not harvest or consume animals that are visibly ill or are in poor condition as a general rule of thumb (ADF&G).

Diagnosis

According to Washington State University's Animal Disease Diagnostic Laboratory (WADDL) (2017), there are a few diagnostic methods to detect *M. ovi*. First, to obtain a sample for PCR detection in animals exhibiting pneumonia, the major bronchi can be swabbed and/or a sample of consolidated lung tissues can be submitted. If the lung tissue is unavailable, the nose, middle ears, or sinuses (especially if purulent exudate is present) can be swabbed instead. In healthy appearing or potential carrier animals, both nares of the nose can be deeply swabbed. It is advised to use swabs (tip and shaft) made of synthetic materials, and to avoid certain media as agar-based transport media can inhibit detection by PCR. If there are testing delays, the samples can be frozen for later analysis. Another method for detection of exposure is competitive enzyme-linked immunosorbent assay (cELISA). A cELISA test, developed at the WADDL, can

be used for *M. ovi* detection in both bighorn sheep and domestic sheep flocks. The test utilizes serum samples where either extracted serum or clotted whole blood may be submitted to the lab.

The WADDL also advises which diagnostic test(s) to use. Testing by cELISA is sensitive and used for determining if a population has had *M. ovi* exposure. Due to seroprevalence variance among bighorn and domestic sheep, the WADDL recommends sampling ten or more mountain sheep, 15 or more desert sheep, or 20 or more domestic sheep to reliably ascertain exposure status. They do not advise the cELISA for domestic goats, as this assay has not yet been validated. Alternately, PCR is utilized to determine the infection status of individual animals as well as identifying strain type. They also state that PCR is the most sensitive test for determining status in domestic sheep and/or goats because these species typically have a higher PCR prevalence than seroprevalence. For biosecurity purposes, if sampling domestic sheep to determine individual status, the WADDL recommends a minimum of two negative results from samples taken a week or more apart before confirming that the animal is overall negative and not infected.

Treatment

Scott (2011) claims that treatment is generally not necessary in domestic sheep because clinical signs are mild. Scott does, however, suggest that oxytetracycline (single intramuscular injection of a long-acting preparation at a dose rate of 20 mg/kg BW) should be administered to sick lambs demonstrating inappetence.

Prevention & Control

Improving building ventilation as well as reducing stocking density within a domestic herd can be used to reduce the occurrence of pneumonia. Additionally, newly incoming purchased lambs should be isolated and tested before introduction to the homebred stock (Scott 2011). Separating livestock from wild animals is critical for the protection of both groups.

Chapter 5 - Strategies for Controlling Mycoplasma Infection in Ruminants

It is clear that these bacterial species can cause significant damage in many areas, not only in terms of animal health and welfare, but also economically. To revisit the three mycoplasmas discussed in the previous chapters, please refer to Table 5-1 that provides an overview of transmission, clinical signs, diagnosis, and prevention/control. This chapter will also review current strategies (including vaccination) and future challenges for controlling this infection in ruminants.

Table 5-1: Comparison of the Three Species of Mycoplasma Affecting Ruminants

Bacteria	Transmission	Clinical Signs	Diagnosis	Prevention/Control
M. bovis	Aerosol, vertical, sexual, direct contact	Mastitis, pneumonia, otitis media, arthritis, keratoconjunctivitis, decubital abscesses, cardiac disease, genital infections	Growth inhibition, biofilm inhibition, fluorescent antibody, metabolic inhibition, PCR, ELISA, culture	Vaccinate (U.S.), closed herd policy, sound herd management, proper ventilation, isolation and culling
M. mycoides	Aerosol, direct contact	Depression, inappetence, moderate fever, coughing, pain, tachypnea, dyspnea, arthritis, joint swelling	Clinical signs, lesions on necropsy, complement fixation, latex agglutination, competitive ELISA, growth inhibition, immunofluorescence, PCR, immunoblotting	Quarantine, control cattle movement, serological screening, culling, vaccination in some regions
M. ovipneumoniae	Aerosol, direct contact	Bronchopneumonia with secondary bacterial infections	cELISA, PCR	Improving ventilation, reduce stocking density, isolation when infected or prior to herd introduction

Vaccination

The future of controlling mycoplasma infection in ruminants rests in the theoretical hands of vaccination research and development, implementation, and policies. In an era where antibiotic resistance is becoming an almost impending doom, reliance on vaccinations to prevent disease rather than antibiotics to treat disease is critical. Aggressive attention towards vaccine development is a must. Vaccination can be applied to reduce clinical signs as well as lesions, and also, to improve performance. Commercial vaccines have also been reported to reduce the number of mycoplasma organisms in the respiratory tract and infection level within a herd (Maes et al., 2018). Generally, the veterinary focuses for prevention is primarily on herd health rather than individual animal health in large animal (livestock) production operations. Unfortunately, there is little relevant information regarding vaccination technology for the three discussed species of mycoplasma.

There are, however, relatively recent reviews and studies completed covering these three mycoplasma species and vaccine development. The first is a review, published by Perez-Casal et al. (2017), noting the status of the development of a vaccine against *M. bovis*. The review covers various challenges toward creating a successful vaccine, to include colonization of young animals' upper respiratory tract, quality and variation of the host immune response, the role of other pathogens, such as bovine viral diarrhea virus (BVDV) and bovine herpes virus 1 (BHV-1), the need for an appropriate challenge model, and selecting proper adjuvant(s). Moreover, when discussing candidates, the review notes that due to a high level of *M. bovis* antigenic variation, key vaccine targets are most likely to be proteins that are conserved across strains, such as lipoproteins. When analyzing vaccine efficacy, there has been bacterin formulations; however, the studies of these formulations have flaws. For example, the efficacy of a bacterin may be

linked to the strain for production and not have broad application. Similarly, vaccine development based on recombinant proteins may not produce an ideal protective response. Perez-Casal et al. (2017) puts forth that more advanced approaches, such as reverse vaccinology, should be utilized to evaluate all potential antigens.

Next, a study completed by Nkando et al. (2016) covering recombinant *M. mycoides* proteins that elicit a protective immune response against CBPP discusses how new vaccine options are necessary. The paper mentions the T1/44 and T1sr vaccines only confer protection for less than one year, among other flaws. This study utilized reverse vaccinology from a previous study to test 38 bovine-related and 28 caprine-related *M. mycoides* proteins and their ability to promote protection against an experimental CBPP infection. Essentially, the results denote that immunization was beneficial and provided protection with some of the antigens, while others may have increased immune-related pathology. A future study offered in the discussion would be to compare a recombinant vaccine against the current T1/44 live vaccine.

Lastly, a study completed by Ziegler et al. (2014) tested the safety and immunogenicity of a *M. ovi* bacterin for domestic sheep. The study analyzed three separate immunization protocols as follows: 1) live *M. ovi* (50 μg protein); 2) killed *M. ovi* (50 μg whole cell protein) in oil adjuvant; and 3) killed *M. ovi* (250 μg whole cell protein) in oil adjuvant. The results found that the third protocol provided the most protection to ewes, who were then able to passively protect their lambs. Perhaps an intermediate antigenic mass could also produce protective effects.

Alternately, it seems as there has been more advances made in the vaccine technology for other mycoplasma species, particularly *Mycoplasma hyopneumoniae*; perhaps these efforts can be leveraged to approach vaccine development for species like those discussed in the previous chapters. In the case of *M. hyopneumoniae*, it solely affects swine worldwide, and causes a

pneumonia that is chronic, relatively clinically mild, and infectiously endemic within a herd. It is one of the primary pathogens in the porcine respiratory disease complex. Different vaccination strategies are implemented depending on the type of herd, production system, management practices, infection pattern, and preferences of the producer (Maes, 2018). However, the exact mechanisms of protection are not fully elucidated. Most commercial vaccines are comprised of inactivated, adjuvanted whole-cell preparations that are administered intramuscularly. For *M. hyopneumoniae*, bacterin (a suspension of killed or attenuated bacteria) formulations are commercially available. However, bacterin efficacy overall towards this species of mycoplasma as well as the other discussed species remains to be seen.

Finances relevant for both the consumer and manufacturer play a major role in determining investments in the necessary research to produce an efficacious vaccine. This is especially true in the realm of animal health, where financial support for vaccine development is only a fraction of what is available for human health. Certain regions of the world where species like *M. mycoides* flourish may experience financial difficulty developing a vaccine compared to the United States combating *M. bovis*, affecting a much larger number of animals across the country. One must also consider the complexity of providing vaccines to wildlife, as in the case of *M. ovi*. Prevention in wildlife would realistically fall upon domestic sheep and goat producers with herds that may come into contact with wild populations. Wildlife agencies and authorities could take on this role, but they are also limited by funding.

Vaccine technology is currently changing at a relatively rapid pace. We are moving beyond first-generation vaccines, such as whole microbial microorganisms that are killed or inactivated. Vaccine vector platforms, subunit vaccines, and DNA vaccines are becoming more attractive options in animal health that are more rationally designed based on an improved

understanding of the pathogenic nature of the organism. However, the cost for these vaccines must be relatively inexpensive for a herd production operation. Autogenous vaccines, or bacterial isolates from a single farm entity that are inactivated and then combined with an adjuvant, are also a possibility. However, it would be better if these diseases could be controlled or prevented on a greater scale. New vaccine platforms are among the technologies that could be investigated further. Microbial-based vaccine delivery systems are gaining traction, including bacterial vectors as vaccine platforms. The selection of a single or combination of adjuvants is also fundamental in order to stimulate a significant and appropriate immune response. Other technologies, such as DNA printing and Gibson assembly are newer approaches; here, a genome can be optimized, compartmentalized, and rapidly constructed for analysis. In the case of mycoplasma, for example, the genome/genetic makeup can be rearranged, and thus, the virulence of the organism can be attenuated. There is also the technology of the CRISPR Cas9, which allows for making changes to either a host or microbial genomes. Repairs/insertions can be made to the DNA, which can be done *in vivo* that appears to be a simple and versatile means of genome editing. One problem lies within the conflict of intellectual property ownership at this time and stage of development. Moreover, next generation sequencing seems to be the next "PCR" of the microbial world. This technology allows for the sequencing of the entire genome/microbiome of the organism for gene discovery; seemingly, the primary downside is that the amount of bioinformatic data produced can be daunting to complete and interpret in a timely manner. Finally, reverse genetics is another technology that can be used. As the name implies, one would work backwards from a genome and create/screen for antigenic characteristics based on computer-assisted design. Most likely these antigens could be used as

vaccine targets; however, it would be equally important to avoid selection of antigens that are too immunogenic, creating an intense immune response that adversely affects the animal.

Overall, mycoplasmas are complex microorganisms to analyze. There is much to be done in the area of prevention and control of mycoplasma species, especially those affecting ruminants.

Chapter 6 - Conclusions

Mycoplasma bacteria have been wreaking havoc over a multitude of species for what humans have been aware of for a century. This report focused on three species of mycoplasma bacteria affecting ruminants. To summarize, *Mycoplasma bovis*, a very highly pathogenic species, affects cattle and includes clinical signs such as mastitis, pneumonia, ocular signs, arthritis, and others. The economic implication of this bacterium makes combating it all the more challenging and yet equally important in developing prevention strategies. Laboratory diagnostics are necessary to confirm it as the causative agent. Effective treatment of *Mycoplasma bovis* is still unclear at this time, but sound herd management is essential for prevention. In the United States, there are some commercial vaccines; however, the options available may not be as desirable in efficacy as the animal health industry requires.

Mycoplasma mycoides, another species affecting cattle and small ruminants, causes contagious bovine pleuro-pneumonia. This disease primarily affects herds in Asia and Africa; however, there is concern that this disease will spread to current disease-free countries, like the U.S. and other countries, and cause major economic impacts. Previously, in the 1800s, American and British traders and consumers were severely affected economically by the threat of this disease. *Mycoplasma mycoides* causes respiratory signs in adult cattle and arthritis in calves and is transmitted via aerosol. Post-mortem confirmation is required to definitely identify CBPP. There is no standard protocol for treatment at this time for *M. mycoides*, although antimicrobial use has been reported. To prevent *M. mycoides* from entering a herd, sanitary and medical prophylaxis are highly recommended, where vaccines are limited.

Finally, *Mycoplasma ovipneumoniae*, affecting domestic sheep and goats as well as wildlife, has been increasing in prevalence in the western U.S. as of late. Significant losses are seen through respiratory morbidity and the efficiency of transmission. A PCR or cELISA can be used to diagnose the disease. To protect both domestic flocks and wildlife herds, domestic producers must create a barrier between the groups.

There are many strategies to combat mycoplasma infections and disease, including herd isolation, screening, improving sanitation and ventilation, among other methods. More vaccination possibilities and technologies should be explored aggressively, as this strategy could be one of the most effective ways in preventing the spread of these important ruminant diseases.

References

Alaska Department of Fish and Game. *Mycoplasma ovipneumoniae* (M. ovi) in Alaska Wildlife: Answers to Frequently Asked Questions. https://www.adfg.alaska.gov/index.cfm?adfg=hottopics.movi

APHIS USDA. *Mycoplasma ovipneumonia* on U.S. Sheep Operations. 2015. Veterinary Services Center for Epidemiology and Animal Health. https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/monitoring-and-surveillance/nahms/nahms_sheep_studies

Ayling RD, Baker SE, Peek ML, Simon AJ, Nicholas RA. Comparison of *in vitro* activity of danofloxacin, florfenicol, oxytetracycline, spectinomycin and tilmicosin against recent field isolates of *Mycoplasma bovis*. 2000 Jun 24. Vet Rec; 146(26):745-747.

Besser TE, Cassirer EF, Potter KA, Foreyt WJ. Exposure of bighorn sheep to domestic goats colonized with *Mycoplasma ovipneumoniae* induces sub-lethal pneumonia. 2017. PLoS One 12(6): e0178707.

Besser TE, Cassirer EF, Potter KA, Lahmers K, Oaks JL, Shanthalingam S, et al. Epizootic pneumonia of bighorn sheep following experimental exposure to *Mycoplasma ovipneumoniae*. 2014 Oct 1. PLoS One; 9(10):e110039.

Calcutt MJ, Lysnyansky I, Sachse K, Fox LK, Nicholas RAJ, Ayling RD. Gap analysis of *Mycoplasma bovis* disease, diagnosis and control: an aid to identify future development requirements. 2018. Transbound Emerg Dis; 65 (Suppl. 1):91–109.

Campbell J. Contagious Bovine Pleuropneumonia - Respiratory System. 2019. Merck Veterinary Manual. https://www.merckvetmanual.com/respiratory-system/respiratory-diseases-of-cattle/contagious-bovine-pleuropneumonia

Contagious Bovine Pleuropneumonia. 2019. CABI: Invasive Species Compendium. https://www.cabi.org/isc/datasheet/88090

Contagious Bovine Pleuropneumonia. 2009. World Organization for Animal Health (OIE). http://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/Disease_cards/CONTAGIOUS_BOVINE_PLEUROPNEUMONIA.pdf

Contagious Bovine Pleuropneumonia. 2019. World Organization for Animal Health (OIE). https://www.oie.int/en/animal-health-in-the-world/animal-diseases/Contagious-bovine-pleuropneumonia/

Fischer A, Shapiro B, Muriuki C, Heller M, Schnee C, et al. The origin of the '*Mycoplasma mycoides* Cluster' coincides with domestication of ruminants. 2012. PLoS One 7(4): e36150.

Hardison R. More Information about Mycoplasma. 1997. http://globin.cse.psu.edu/html/pip/more_myco.html

Highland, MA. Domestic Small Ruminants & Wild Sheep Respiratory Bacteria and Disease Research. (n.d.). https://dec.alaska.gov/media/7820/movi-presentation-highland-201708.pdf

Highland MA, Herndon DR, Bender SC, Hansen L, Gerlach RF, Beckmen KB. *Mycoplasma ovipneumoniae* in wildlife species beyond subfamily Caprinae. 2018 Dec. Emerg Infect Dis; 24(12):2384-2386.

Kastner J. Sanitary related international trade disputes: a multiple-factor analysis based on nineteenth century precedents [dissertation]. 2003. p. 32–3.

Kastner J, Powell D, Crowley T, Huff K. Scientific conviction amidst scientific controversy in the transatlantic livestock and meat trade. 2005. Endeavour; 29(2):78-83.

Krass CJ, Gardner MW. Etymology of the term mycoplasma. 1973. Int J Syst Bacteriol; 23(1):62-4.

Maes D, Sibila M, Kuhnert P, Segalés J, Haesebrouck F, Pieters M. Update on *Mycoplasma hyopneumoniae* infections in pigs: knowledge gaps for improved disease control. 2018 May. Transbound Emerg Dis; 65(S1):110-124.

Maglaras C, Koenig A. Chapter 92 - Mycoplasma, Actinomyces, and Nocardia. 2015. Small Animal Critical Care Medicine: Elsevier Inc. p. 481-487.

Maunsell FP, Woolums AR, Francoz D, Rosenbusch RF, Step DL, Wilson DJ, et al. *Mycoplasma bovis* Infections in cattle. 2011 Jul. J Vet Intern Med; 25(4):772-783.

Mycoplasma ovipneumoniae Diagnostics in Domestic and Wild Sheep and Goats. 2017. Washington Animal Disease Diagnostic Laboratory. https://waddl.vetmed.wsu.edu/animal-disease-faq/mycoplasma-ovipneumoniae-diagnostics-in-domestic-and-wild-sheep-and-goats

Nicholas RAJ, Ayling RD. *Mycoplasma bovis*: disease, diagnosis, and control. 2003. Research Vet Sci; 74(2):105-112.

Nicholas RAJ, Ayling RD, McAuliffe L. Mycoplasma diseases of ruminants. Wallingford: CABI; 2008.

Nicholas RAJ, Ayling RD, Tjipura-Zaire G, Rowan T. Treatment of contagious bovine pleuropneumonia. 2012. Vet Rec; 171(20):510.

Nkando I, Perez-Casal J, Mwirigi M, Prysliak T, Townsend H, Berberov E, et al. Recombinant *Mycoplasma mycoides* proteins elicit protective immune responses against contagious bovine pleuropneumonia. 2016 Mar. Vet Immunol Immunopathol; 171:103–14.

Perez-Casal J, Prysliak T, Maina T, Suleman M, Jimbo S. Status of the development of a vaccine against *Mycoplasma bovis*. 2017 May 19. Vaccine; 35(22):2902–7.

Rosengarten R, Citti C. Mycoplasmas of ruminants: pathogenicity, diagnostics, epidemiology and molecular genetics. The Role of Ruminant Mycoplasmas in Systemic Infection, eds L. Stipkovits, R. Rosengarten, and J. Frey. Brussels: European Commission. p. 14–17. 1999.

Scott PR. Treatment and control of respiratory disease in sheep. 2011 Mar. Veterinary Clinics of North America: Food Animal Practice; 27(1):175-186.

Thomas A., Dizier I., Trolin A. et al. Comparison of sampling procedures for isolating pulmonary mycoplasmas in cattle. 2002. Vet Res Commun. 26: 333.

USDA APHIS | Status of Reportable Diseases in the United States. 2019. https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/monitoring-and-surveillance/sa_nahss/status-reportable-disease-us

Ziegler JC, Lahmers KK, Barrington GM, Parish SM, Kilzer K, Baker K, et al. Safety and immunogenicity of a *Mycoplasma ovipneumoniae* bacterin for domestic sheep (*Ovis aries*). 2014. PLoS One. 9(4): e95698.