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Bovine Respiratory Disease Complex with Special Reference to *Mycoplasma bovis* in Egypt

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ABSTRACT

Mycoplasma bovis-related diseases in cattle are a global issue that negatively affects cattle husbandry in terms of both animal welfare and the economy. Many clinical symptoms, such as mastitis, pneumonia, infertility and abortion, otitis media, keratoconjunctivitis, and arthritis. To find gaps in our understanding of the causative organism regarding disease pathology, diagnosis, and control techniques, we examine and analyze the available data on diagnosis and control. The following are the main factors to consider there are no commercially available vaccines; antimicrobial resistance is rising; diagnostic and antimicrobial sensitivity testing need to be improved; and a pen-side test would enable quicker diagnosis and antimicrobial treatment implementation. It is necessary to gather more information on immune response, stress variables, infectious dosage levels, and host susceptibility. Further research is required to understand the effects of a symptomatic carriers, on the survival of *M. bovis* in the environment. In order to accelerate the development of vaccines, additional genomic study of *M. bovis* is necessary, its pathogenic mechanisms, which include variable surface proteins, and repeatable disease models.

Keywords: Cattle, Diagnostics, Disease Control, *Mycoplasma Bovis*, Research requirements.

INTRODUCTION

Bovine respiratory disease complex is a condition produced by many factors, such as environmental conditions, pathogen exposure and animal management which play a role in development of acute respiratory illness in cattle (Horwood et al., 2014).

There are numerous biological, chemical, and physical agents that can cause pneumonia. The biological agents include bacteria, protozoa, fungus, viruses, *Mycoplasma* (M), and parasites (Taylor et al., 2010).

A group of diseases that are known to have an effect on cattle's respiratory systems collectively known by the name "bovine respiratory disease" (BRD) complex (Apley, 2006). These comprise hemorrhagic syndrome, mucosal diseases, acute respiratory distress syndrome, atypical interstitial pneumonia and shipping fever syndrome. Bovine respiratory illnesses are caused by a complex combination of bacterial and viral infections, management factors, environmental variables and animal health status (Nickell & White, 2010).

It is a serious respiratory disease that affects cattle worldwide, including dairy and feedlot cattle. It is also known to cause high rates of morbidity and mortality, as well as significant financial losses from increased labor costs, veterinary and medication expenses, and decreased productivity (Gagea et al., 2006). Clinical indications of the BRD complex differ depending on the animal's condition, degree of stress, treatment methods, and extent of pathogen exposure (Snowder, 2009).

Viruses have a significant role in occurrence of BRD complex because they damage the respiratory mucosa directly, making the animal more susceptible to bacterial infection, and they also prevent the animal from fighting off commensal bacteria in the upper respiratory tract (Taylor et al., 2010). Bovine herpesvirus-1 that causes Infectious Bovine rhinotracheitis, bovine respiratory syncytial virus and parainfluenza virus-3 (Härtel et al., 2004) and bovine viral diarrhea virus are the main viruses that cause this disease complex (Griffin et al., 2010). The bovine viral diarrhea virus is known to be a significant pathogenic partner involved in the occurrence of the BRD complex. Furthermore, it has been determined which viral infections, such as the adenovirus, influenza-A virus, and bovine respiratory coronavirus, are responsible for the creation of the BRD complex (Gay & Barnouin, 2009).

Numerous bacterial pathogens, including *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni, and Mycoplasma bovis,* are included in the creation of the BRD complex (Grissett et al., 2015). Other bacterial infections associated with the BRD complex include *Bibersteinia trehalosi, Arcanobacterium pyogenes,* various types of *Pasteurella* and *Mycoplasma* (Griffin et al., 2010).

Mycoplasma species including *M. bovis, M. mycoides subsp. mycoides, M. agalactiae, M. dispar, M. californicum, M. alkalescens, M. canis, M. arginini, M. bovirhinis, M. bovigenitalium,* and *M. bovoculi* are responsible for economic losses and problems with cattle (Maunsell & Donovan, 2009). Even so, *M. bovis, M. bovigenitalium, M. dispar* and *M. bovirhinus* are the ones that cause pneumonia. They usually exist as part of the upper respiratory tract's normal flora (Arcangioli et al., 2008).

Mycoplasma bovis is difficult to identify and manage because of the variation in how the disease expresses itself, how therapies and immunizations impact it, and the substantial deficiencies in our understanding of these illnesses' biology and epidemiology (Maunsell et al., 2011).

Diseases caused by *M. bovis* have a significant negative impact on global trade, health, and welfare. *M. bovis* has become a global problem through decreased production, higher mortality rates, early culling of diseased animals, treatment costs, labour costs. Controlling Mycoplasma is difficult due to its antimicrobial resistance, there are no appropriate vaccinations and treatments (Dudek et al., 2020).

1. Mycoplasma bovis

The *Mollicutes* family includes *M. bovis*, which has a genome that can range in size from 948,121 base pairs to 1,038,531 base pairs and is characterized by cell wall absence. pleomorphic forms of cells are caused by the absence of a cell wall, which renders several standard antimicrobial treatments ineffective, including penicillin and other β-lactams (Horwood et al., 2014).

The *Mollicutes* are small self-replicating prokaryotic cells; they are actually simple, depend on cholesterol for growth, have a short genome that prevents them from carrying out a wide range of metabolic functions; and need to obtain their lipids, nucleic acid precursors and amino acids from outside sources. *M. bovis* is dependent on organic acids like lactate and pyruvate for nutrition because it does not hydrolyze arginine or ferment glucose (Caswell & Archambault, 1996; Khan et

al., 2005; Pitcher & Nicholas, 2005). Moreover, it ranks as one of the major pathogens causing mastitis, arthritis, otitis media and many other problems globally. It is the second-highest pathogenic *Mycoplasma* behind Mmm (Fox, 2012).

Numerous kinds of *Mycoplasma* that have different degrees of significance when it affects cattle. These types include *M. bovigenitalium, M. leachii, M. bovirhinis, M. bovoculi, Mycoplasma arginini* and *M. californicum* (Manso-Silván et al., 2012).

Because *M. bovis* in the lab requires the addition of nucleic acid, amino acids, precursors, and other nutrients, PPLO media need DNA, cholesterol, and serum. The organisms prefer a capnophilic surroundings with a high level of humidity, but they can develop weakly in air at 37 °C. Under a stereo microscope, colonies on solid agar resemble fried egg colonies, and on the surface of solid media, film and spot development are visible when lipolytic activity is present (Thorns & Boughton, 1978).

Although biological substances (milk, discharges, etc.) can greatly reduce standard disinfectants' efficacy, *Mycoplasma* is often resistant to them. For general disinfection purposes, formalin, iodofores, and peracetic acid have all been proven to be highly efficient. Therefore, they can be used for teat dipping. Mycoplasmas are widely believed to be particularly sensitive to a wide range of environmental factors, including high temperatures and dryness (Pfutzner, H. & Schimmel, 1985).

***2. Mycoplasma bovis* as a cause of BRD in Egypt**

The available literature which described *M. bovis* as a cause of BRD in Egypt are summarized in the following table. The table illustrates the reference, locality, Animal data, sampling, method of diagnosis, and the results.

Locality	Animals	Steps	Results	Sample	Reference
	Balady cattle Friesian cattle	MIC	MIC: *M. bovis* is highly sensitive to Enrofloxacin, Norfloxacin, Tiamulin and Ciprofloxacin.	Lung tissues	(El Shabiny et al., 1999)
Sharkia Governorate	Egyptian cattle	MIC	MIC*: Mycoplasma* isolates were sensitive to tilmicosin, tylosin, tulathromycin, spiramycin, and spectinomycin	Lung tissues	(Ammar et al., 2022)
Menofia Governorate	calves (6–12 months old)	1-Isolation 2- Biochemical identification 3- Molecular Confirmation by PCR	*M. bovis* isolated from five cases (8.33%), *M. bovigenitalium* (5%).	Deep nasal swabs	(Hashem et al., 2022)
Menofia Governorate	Calves (6–12 months old)	Sequence analysis of *M. bovis*	*M. bovis* isolates showed a high nucleotide sequence similarity with two *M. bovis* strains isolated from Canada with accession numbers CP069057 and CP022593, and with five *M. bovis* isolates	Deep nasal swabs	(Hashem et al., 2022)

			from Belgium with accession numbers CP058503, CP058464, CP058514, CP0558473, and CP058463.		
El- basatien abattoir Cairo	Buffalo calves (3 months to 1 year suffering from pneumonia	1-*Mycoplasma* isolation 2-positive samples confirmed by PCR using16S common gene for *Mycoplasma*.	*Mycoplasma bovis* isolation % (18.9%). all isolated strains from lung samples belonged to the class *Mollicutes* and give positive with PCR	buffalo lungs	(Emran et al, 2013)
Cairo	apparently healthy and diseased cattle	Isolation	*1- M. bovis* was the most prevalent *Mycoplasma* spp. in an incidence of 13.8% *2- M. arginine* in an incidence of 9.2% *3- M. bovirhinis* in an incidence of 3.7,4.6% from nasal and buccal swabs	Nasal, buccal, ocular swabs from	(Mahdy et al., 2015)
		Preparation of two bivalent autogenous vaccines (saponised and formalized vaccines) able to protect against M. bovis and M. bovigenitalium.	1-Saponised vaccine was safe and more potent than formalized vaccine. 2- Experimental work had shown that saponised vaccine can protect in the face of a large *Mycoplasma* challenge and was highly immunogenic.		(El-Jakee et al., 2011)
Menofia Governorate	cattle	Isolation	*Mycoplasma was* isolated in percentage of (8%, and 6%in both nasal swabs and lung tissues respectively. MIC revealed that *Mycoplasma bovis isolates were sensitive to Tulthromycin (Draxxin)* and *Ciprofloxacin*	nasal swabs and lung tissues	ElAhl et al., 2014)
El-basatien abattoir Cairo	buffalo calves aging from 3 months to 1 year	1-Immuno histochemistry 2- histopathology	The histopathology Showed bronchopneumonia - necrosis of the bronchial epithelia with necrotic	buffalo lungs	(Emran et al, 2013)

	old suffering from pneumonia		exudates filling the bronchial lumen. Lung alveoli showed grades of hepatization, edema and emphysema. 2- immunohistochemistry on different lung tissues revealed *M.bovis* antigen was detected within the epithelial lining the bronchi and bronchioles		
Sharkia Governorate	Feeder calves	Isolation	*Mycoplasma bovis* isolation % (31.66 %)	lung samples	(Fawkia, 1995)
	Balady cattle &Friesian cattle	Isolation	Isolation: 30 *Mycoplasma* isolates (17.6 %). (5) *Mycoplasma bovis* isolated from the balady cattle (4) *Mycoplasma bovis* isolated from Friesian cattle	lung samples from Balady cattle and from Friesian cattle	(El Shabiny et al., 1999)
Sharkia Governorate	Egyptian cattle	PCR	PCR: *M. bovis*(61%), *M. bovirhinis* (15%)	lung tissues	(Ammar et al., 2022)
Aswan Governorate	Cattle with pneumonia	1-PM examination 2- Isolation	The lungs showed characteristic thickening and fibrosis of the interlobular septa with caseated purulent exudate in lungs. All The culturally tested lungs were *Mycoplasma* positive.	. Lung tissues	(Kounour et al., 2023)
Sohag Governorate	Lung tissues	1-Isolation 2- Biochemical identification 3- PCR	All culturally examined lungs (50 cases) were *Mycoplasma* positive. (90 %) of the isolated *Mycoplasma* strains were glucose and arginine negative with production of film and spots. The PCR tested strains were *Mycoplasma bovis* infection.	. Lung tissues	(Kounour et al., 2023)

3. Clinical signs of *M. bovis*

BRD in calves can be solely produced by *M. bovis*, but the illness is typically multifactorial and involves a number of different bacteria and viruses. These include bovine respiratory syncytial virus, parainfluenza 3, adenovirus, *Histophilus somni, Trueperella pyogenes,*

and infectious bovine rhinotracheitis virus (Taylor et al., 2010). Moreover, *Histophilus somni and M. bovis* have been often detected together in younger calves, and co-infection with *Pasteurella multocida* is also frequent (Fulton et al., 2009 and López & Martinson, 2017).

Mild to persistent coughing, hyperpnea, nasal discharge, appetite loss, watery eyes, depression, and low-grade fever are a few of the symptoms that come with an *M. bovis* infection that induces pneumonia. One clinical sign of *M. bovis* infection is pneumonia, however it can also coexist with other clinical symptoms such mastitis in dairy cows, polyarthritis, and otitis media in calves (Maunsell et al., 2011; Gondaira et al., 2017)

Mycoplasma bovis causes pneumonia in cattle of all ages, including dairy and beef calves, cattle on feedlots, and adults. The clinical signs appear as fever, coughing, dyspnea, tachypnea, and nasal discharge. Animals with a chronic condition gain less weight. Otitis media, arthritis, lameness, and joint stiffness may all be related to *Mycoplasma* pneumonia (Stipkovits et al., 2000)

With an increase in clinical disease at 10-15 days and a 10% death rate due to severe serofibrinous pneumonia in calves. The symptoms seen in the calves that survived included fever, hyperpnea, dyspnea, nasal secretions, moderate to chronic coughing, and decreased appetite (Nicholas et al., 2000). *M. bovis* also cause chronic illness, inability to survive, gain weight (Shahriar et al., 2002).

M. bovis can cause arthritis in cattle of any age, but it usually affects pre-weaned calves and is linked to respiratory infections. Fever, edema, lameness, and joint discomfort are some signs of the acute phase. Another crucial aspect of the disease is a poor response to antibiotic therapy. Tenosynovitis and necrotizing fibrinosuppurative arthritis are two conditions that can cause joint lesions. When a chronic disease is present, yellow-white fibrous or caseous substances is present in the afflicted joint capsules (Maunsell et al., 2011; Bras et al., 2017).

Arthritis, lethargy, joint swelling, frequently accompanied by a slight fever, decreased feed intake and weakness. Infected herds have also reported keratoconjunctivitis, which can result in blindness, and otitis media, which frequently occurs with respiratory illnesses (Nicholas et al., 2008).

4. Postmortem investigation

The postmortem investigation showed distinct coagulative necrosis. Furthermore, proliferation of peribronchial lymphoid tissue associated with chronic infections results in constriction of the Lumina airway and compression and collapse of the adjacent pulmonary parenchyma. Moreover, numerous necrotic foci packed with dry, yellowish caseous substances are commonly seen in injured lungs. The interlobular septae contain necrotic lesions. It is usual to have severe fibrosis and necrotic sequestra. Acute fibrinous pleuritis or persistent fibrosing pleuritis can occasionally develop (Caswell & Archambault, 1996). Also, some slaughtered calves' lungs exhibited ulceration on lung surface and scattered caseous nodules on their surface, giving them a marble appearance (Hamad et al., 2019).

Multifocal nodules of caseous necrosis, circular, elevated, yellowish nodules containing dry, foci of caseous material have been found inside the lesions of cranioventral bronchopneumonia in the lung lesions connected to *M. bovis* infection. The liquid purulent material seen in lung abscesses and lesions resulting from persistent undifferentiated bacterial bronchopneumonia is not associated with lung lesions attributed to *M. bovis* (Gagea et al., 2006).

5. Mode of transmission

Calf pneumonia is primarily caused by *M. bovis*, it is also isolated from cattle, as well as occasionally from

buffaloes and small ruminants (Pfützner & Sachse, 1996). Furthermore, *M. bovis* affects all cattle sectors and all cattle ages (prewean, post wean, neonatal, and adult) and all cattle sectors (Nicholas et al., 2008).

The most frequent routes of transmission for the highly contagious *Mycoplasma bovis* are thought to be aerosol, nose-to-nose contact, or indirectly through food, drink, housing, or other environmental factors. However, if calves are given milk from sick cows, they are in serious danger. According to experimental studies, *M. bovis* can enter the fetus through the teat canal and vaginal tract of an infected mother (Nicholas et al., 2002). *Mycoplasma* is thought to shed with greater frequency in sick animals during the beginning stages of a disease. The mucosa of the upper respiratory system is where *M. bovis* colonizes most frequently. Infected cattle spread *Mycoplasma* throughout their respiratory systems over a period of months or maybe years, serving as disease reservoirs (Nicholas et al., 2002). Animals that are persistently and subclinically infected act as reservoirs, intermittent release of the disease through milk or mucosal secretions from the genital or upper respiratory tract (Pitcher & Nicholas, 2005 and Nguyen & Truong, 2015).

Cattle stress from climatic changes, overcrowding and treatment with dexamethasone is thought to raise the probability of a disease outbreak in the animals. Moreover, dexamethasone therapy led to increased pathogen shedding (Alabdullah et al., 2014).

6. Incubation period

The duration of the *M. bovis* infection's incubation period is unpredictable due to a variety of circumstances, including Stress levels in the animals, herd management techniques, the existence of coexisting illnesses, the pathological and clinical consequences of the infection, the infectious dose, the age of the sick animal, and the degree of virulence of field isolates are all factors to consider. Mastitis has a shorter incubation period in experimental infections than pneumonia, which can take up to seven days. *M. bovis* sheds irregularly, making it challenging to identify an animal by checking for the bacterium inside. As a result, herd diagnosis, especially when using serological techniques, may be more accurate for chronic disorders. Finding *M. bovis* is affected by the procedures used to collect the samples, transport them to the lab, and store them there (Calcutt et al., 2018).

7. *Pathogenesis*

Host cell invasion, host immune system modification, generation of secondary metabolites, adhesion, and biofilm formation are some of *M. bovis's* virulence features. One of the early stages of infection that permits lung colonization by *M. bovis* is its adhesion to the tracheobronchial epithelial cells of cattle. The membrane proteins that mediate it include Vsps as well as unrelated proteins including P26 and pMB67 (Buchenau et al., 2010).

Because *M. bovis* can colonize, penetrate tissues, and withstand powerful immune responses, it continues to exist in disease areas. Antigenic variation, Adhesion, invasion, toxic metabolites, biofilm development, and immunomodulation are all aspects of pathogenesis. Moreover, Hydrogen peroxide is one of the secondary metabolites produced by *M. bovis*. distinct strains producing hydrogen peroxide at varying amounts (Khan et al., 2005).

In the presence of an immune response and prolonged antibiotic therapy, *M. bovis's* ability to undergo antigenic variation through phenotypic modification of immunodominant surface lipoproteins and the maintenance of host immune response regulation will be important for *M. bovis* survival and the development of chronic infection (Gagea et al., 2006).

8. Isolation and Identification of M.bovis

As *M. bovis* does not exhibit pathognomonic clinical symptoms, laboratory confirmation is essential for an accurate diagnosis. When all other infections have been ruled out or the animals are still not responding to the first round of antibiotic treatment, *M. bovis* is not taken into consideration. The main means of identifying *M. bovis* are culture detection, molecular detection, and serological detection (Calcutt et al., 2018).

Microbial culture has been used to identify and diagnose *Mycoplasma* infections. The application of PCR to identify *Mycoplasma* species in different bovine samples has recently attracted more attention. In comparison to traditional culture-based approaches, PCR has improved efficacy, specificity, and sensitivity for laboratory confirmation (Parker et al., 2018).

Various different media types, including PPLO, Eaton's and Hay flick's media, are usually used in the confirmation of *M. bovis* infection Broth cultures are incubated in an aerobic environment at 37 °C and Usually, the bacteria start to grow after 48 hours. Before determining whether the sample is negative, it is recommended to incubate it for up to ten days. Agar plates are incubated at 37 °C ambient conditions with 5 to 10% CO_2 until visible colonies develop (2-4 days). When *M. bovis* colonies are studied under a stereomicroscope, they have a typical fried egg appearance and range in diameter from 0.1 to 0.5 mm (Dudek et al., 2020).

The growth mediums for *M. bovis* must contain cholesterol, serum, and DNA. Amino acids, precursors to nucleic acids, and other nutrients are required. Despite needing a high humidity environment, the organisms only develop slowly at 37 °C. *Mycoplasma* colonies resemble fried egg colonies (Rosenbusch et al, 1994)

On modified PPLO solid medium, *M. bovis* formed fried egg colonies. It showed negative results in biochemical assays for arginine hydrolysis, glucose fermentation and serum digestion. Even so, the organism passed the phosphatase test, the tetrazolium reduction test, and the disc growth inhibition test favorably, demonstrating *M. bovis* unique characteristics (Behera et al., 2018).

Mycoplasma bovis was discovered in 18% of the lung tissue of cattle in the Republic of Ireland (from April 1995 and December 1998) as a result of fatal pneumonia cases. In 66% of the cases where *M. bovis* was positive, infectious bovine rhinotracheitis, *Pasteurella species* and Parainfluenza 3 virus were the most frequently discovered respiratory diseases (Byrne et al., 2001).

Samples (nasal swabs, lung tissues, tracheal swabs, bronchial lymph nodes, and vaginal swabs) from cattle and buffaloes were gathered from numerous Egyptian governorates. The prevalence of *M. bovigenitalium* was 13.3 and 10%, respectively, *Mycoplasma* isolates found in the respiratory tracts of cattle and buffaloes. *M. bovis* was typed as *M. bovis* (2.7 and 1.7%, respectively) and other *Mycoplasma* species (10.8 and 4.2%, respectively) (Marouf et al., 2011).

In southeast Spain, 23 feedlot calves were examined between 2016 and 2019 that showed respiratory signs but had not responded to treatment. Histology, immunohistochemistry, and bacteriology (cultivation followed by PCR) were employed in the search for *M. bovis*. In 86.9% of the calves, the pathogen was discovered, mostly in the lungs (77.26%) (Garcia et al., 2021).

In one research of calf populations in the Netherlands, *Mycoplasma bovis* was detected in 20% of pneumonic lungs from fattening herds, although it was only detected in a small percentage of seemingly healthy calves. In 1994, (13% to 23%) of pneumonic lung cases in the North and South of Ireland tested positive for *Mycoplasma bovis*. Moreover, in 2001, 30 % of calf herds had isolates of *Mycoplasma bovis* in France. Approximately 20% to 25% of pneumonic herds in Britain during that time contained

animals with antibodies against *M. bovis* (Nicholas & Ayling, 2003).

Even though culture is the main technique for detecting *Mycoplasma*, it is difficult, takes a long time, and necessitates very precise feeding of the organism. The results may be affected by the following: the existence of a polymicrobial disease with organisms that proliferate more quickly; the treatment of animals with antibiotics prior to sampling; improper sample handling, processing, or storage; and other circumstances (Gille et al., 2018).

9. Serodiagnosis

Various ELISA techniques are employed to find *anti-M. Bovis* antibodies *or M. bovis* antigen in milk, and serum tissue samples. It is believed that the *anti-M. bovis* antibody detection ELISA is specific and is supported by the evolutionary separation of *M. bovis* from most other bovine *Mycoplasma* species. The detection of *anti-M. bovis* antibodies is typically employed for herd assessment, as titers may not always be highly correlated with illness or infection in individual animals (Maunsell et al., 2011).

Moreover, immunological tests only show previous exposure to the infection then they become ineffective before an animal seroconverts. Even while nasal carriage by itself might result in seroconversion without any obvious indications of disease, it's crucial to identify these carrier animals in a herd as possible carriers of disease that could later affect other people (Pfutzner & Schimmel, 1985). All the same, it has been demonstrated that ELISAs that identify *anti-M. bovis* antibodies are helpful both as a herd test and in proving an absence of infection (O'Farrell et al., 2001).

10. Molecular diagnosis

Many molecular techniques based on nucleic acids have been developed to solve challenges related to culture (Maunsell et al., 2009). PCR is more effective, specific, and sensitive than other methods for detecting *Mycoplasma* species in a range of sample types when used for laboratory diagnosis (Sachse et al., 1993).

The PCR methodology was discovered to be a good direct method for diagnosing *Mycoplasma*, and specifically *M. bovis*, from pneumonic lungs without the necessity for microbe cultivation. This approach is efficient and will save you time and work. In addition to *Mycoplasma* culture, PCR was advised for usage in numerous international investigations (Hamad et al., 2019).

Mycoplasma bovis can be identified using a species-specific PCR, which offers an innovative approach without the potential limitations associated with conventional diagnostic techniques. They talked about modifying and using PCR in the lab, and as a result PCR use in diagnostic labs became widespread. Quick isolate identification is made possible by the requirement for few organisms, and potential issues with some serological identification techniques may be avoided. A dependable and useful method for guaranteeing the accurate identification of *Mycoplasma* isolates is PCR (Ayling et al., 1997).

The target organism must be present in the sample and have undamaged DNA in order for amplification to take place, as PCR analysis necessitates amplifying the organism's DNA. Notably, unlike in cultivation, the organism does not have to be alive in order to be detected if the goal is just viable organisms. Some assays have been developed to identify many *Mycoplasma* species, followed by post-PCR speciation, whereas others have been established to detect specific *Mycoplasma* species. Targeting the 16S rRNA gene, traditional PCR techniques for *M. bovis* detection were developed in the 1990s (Hotzel, 1996).

One of the most widely used genes for bacterial identification is the 16S rRNA gene, which is found in all bacteria and whose function has not changed throughout time. Because it can be found in a large number of different bacterial

species, the 16S rRNA gene, a tiny subunit of prokaryotic ribosomes, is useful for identifying bacteria. It contains areas that are subject to change and may be species-specific, as well as portions that are mostly maintained (Kolbert & Persing, 1999)

11. Prevention and Control

Control can be increased by applying effective farming techniques and making sure that animal housing has enough ventilation. It also includes routine animal monitoring with a focus on early disease detection, sanitation and disinfection, not giving infectious milk, and only introducing tested animals. Isolated pens for sick animals, possibly even their execution. It has been suggested that dairy farms and calf fattening units should be kept apart and calves of different ages not be mixed together (Nicholas et al., 2016). Until *M. bovis* vaccinations are widely available, the only options to try to manage *M. bovis* infections are through sanitary preventative techniques and antibiotic therapy (Lysnyansky & Ayling, 2016).

12. Treatment of BRD and Mycoplasma bovis

Different short- and long-acting antibiotics are utilized for the treatment of BRD in cattle. Macrolides, phenicols, tetracyclines, (fluoro) quinolones and penicillin are frequently used for the treatment of respiratory disease in cattle. Tulathromycin exhibits antimicrobial effects against the major bacterial BRD pathogens (*M. haemolytica*, *P. multocida*, *H. somni* and *M. bovis*) and is approved as a single SC administration (2.5 mg/kg) (De Koster et al., 2022).

Recently, anti-inflammatory and antibiotic medications have been used in combination to treat BRD-associated inflammation and infection in clinical instances. While NSAIDs are available as adjunct therapy for BRD in single-substance form, some combination products (such as florfenicol and meloxicam, florfenicol and flunixin) or multiple administrations (such as ceftiofur and ketoprofen) have been approved, offering the benefit of combining antibiotic and anti-inflammatory treatment in a single administration. (De Koster et al., 2022).

The two main strategies to deal with *Mycoplasma bovis* infections, either as a preventative strategy or during the early stages of the disease, continue to be hygienic measures and antibiotic treatment. For the treatment or prevention of BRD, fluoroquinolones, long-acting macrolides like florfenicol, tildipirosin, tulathromycin, broad-spectrum cephalosporins and gamithromycin are frequently used (Khalil et al., 2016).

Treating symptoms linked to the disease with antibiotics and providing suitable housing is advised. While antimicrobial treatment for pneumonia has shown some promising results, antibiotic therapy for mastitis has a poor track record and may increase financial losses (Rosenbusch et al., 2005).

Since mycoplasmas don't produce folic acid and have no cell wall, they are inherently resistant to sulphonamides and β-lactam antibiotics. Mycoplasmas frequently exhibit resistance to antibiotics (fluoroquinolones) that stop the synthesis of proteins or nucleic acids, including tetracyclines, lincosamides, macrolides, and phenicols (Maunsell et al., 2011).

Tetracyclines, macrolides, and certain fluoroquinolones are among the antimicrobials that must be used in the early identification and treatment of *M. bovis* infection. Very few antimicrobials are specifically licensed for treating *M. bovis* in calves, with the exception of aminoglycosides, which are mycoplasmacidal at high concentrations, and fluoroquinolones, which are mycoplasmacidal at low doses. The remaining antimicrobials are *Mycoplasma* static and typically inhibit protein synthesis. The usual tetracyclines and doxycycline bind to the 30 S ribosomal

subunit to prevent protein synthesis in the ribosome (Bryskier, 2005).

Early antimicrobial treatment with specific drugs like macrolides, tetracyclines, aminoglycosides, chloramphenicols, and fluoroquinolones is crucial for controlling mycoplasma infections, but often ineffective in chronic respiratory disorders. Globally, antimicrobials like macrolides, tetracyclines, lincosamides, aminocyclitols, and fluoroquinolones have shown ineffectiveness against *M. bovis* due to increased MIC values (Lysnyansky & Ayling, 2016).

Uncontrolled treatment with antibiotics in the livestock industry has led to *M. bovis* developing resistance to many antimicrobial classes, including macrolides, tetracycline, and fluoroquinolones. This has resulted in financial losses because of the drugs' limited options for treatment (Lysnyansky & Ayling, 2016).

Mycoplasma species without a cell wall, making them resistant to several commonly used treatments, making the treatment of *Mycoplasma* disorders difficult. After exposure to five antibiotics, including erythromycin, gentamicin, tetracycline, streptomycin, and lincomycin, comparison of *M. bovigenitalium* and *M. bovis* isolates to reference strains revealed that lincomycin, erythromycin, and streptomycin are more effective at treatment of *M. bovis* and *M. bovigenitalium* (marouf et al., 2011).

The preferred treatment for many clinical and veterinary diseases such as *M. bovis* infection in cattle, is fluoroquinolone therapy. Fluoroquinolones block DNA gyrase and topoisomerase IV, two enzymes necessary for DNA replication (Ammar et al., 2021).

13. Vaccination against M. bovis

Due to the ineffectiveness of antibiotics in treating bovine mycoplasmosis, vaccinations have come to spotlight as a more lasting and cost-effective treatment alternative with much lower risks of antimicrobial resistance. In the USA, cattle have received numerous vaccinations, but there is no published data demonstrating their efficacy (Nicholas, 2011).

The most common vaccination used in studies to stop *M. bovis* infections is an inactivated vaccine. On the other hand, it is generally accepted that inactivated vaccines have some drawbacks, such as high production costs resulting from the requirement to culture vast quantities of the antigen and the potential for strain-specific protein changes during subculture (Wang et al., 2020).

Before using the autogenous vaccine, accurate laboratory identification is necessary since this type of vaccination works best when *M. bovis* is the only or predominant pathogen causing respiratory illness in a herd. Additionally, these findings demonstrate that vaccinations are more effective when administered to newborn calves as soon as they arrive at the farm, resulting in lower rates of death and medical expenses. The multivalent vaccinations now used to treat respiratory infections should incorporate the *M. bovis* vaccine (Nicholas., 2019).

There are no mycoplasmosis vaccines on the market right now. Despite showing some signs of protection against a natural infection of respiratory disease, a quadrivalent inactivated vaccine including respiratory syncytial virus, Parainfluenza types 3 and 2 mycoplasmas, *M. dispar* and *M. bovis*, was not completely effective (Howard et al., 1987).

The pneumonia losses and treatment costs in newly introduced feedlot calves were reduced by a vaccination designed using *M. bovis* and *Pasteurella haemolytica* strains that were formalin-inactivated and obtained from the target herd (Urbaneck et al., 2000). Additionally, it was found that a saponized-inactivated vaccine was risk-free, highly immunogenic, and protected against a

potent *M. bovis* experimental challenge (Nicholas et al., 2000).

Keeping the immunological and respiratory systems in a peak state is essential for the prevention and management of *M. bovis*-associated disease in cattle. If high-risk animals are consciously given antibiotic treatments upon arrival or during a BRD outbreak, mycoplasma disease may be less frequent. If ill cattle are separated from new arrivals and the hospital pen is kept separate, high-risk animals may not be as exposed to *M. bovis.* When handling sick cattle, the likelihood of fomite-mediated *M. bovis* transmission could be reduced by following the right hygienic practices (Maunsell et al., 2011).

To protect against *M. bovis* infections, there is no reliable vaccination. Antibiotic therapy is rarely effective, and increased antimicrobial resistance is noted (Klein et al., 2017). Additionally, the vaccination against *M. bovis* is difficult because there is currently no effective commercial vaccine. Numerous have been sold, particularly in the USA, but not enough is known about them to assess their immunogenicity and protective characteristics (Nicholas et al., 2016).

Furthermore, it is of interest to develop subunit vaccines and attenuated live vaccination strains for *M. bovis* according to (Zhang et al., 2014), a multi-passage attenuated *M. bovis* strain protected calves from virulent infection. Other Canadian attempts to protect cattle against *M. bovis* using membrane fractions and recombinant proteins, have similarly failed (Prysliak et al., 2013).

14. Mycoplasma bovis metaphylaxis

The metaphylactic use of an antibiotic may protect some undiagnosed sick calves or some calves in the very initial phase of disease as well as reduce pulmonary bacterial pathogen load at the feedlot. Indeed, arrival mass metaphylaxis is a very common practice at commercial feedlots in order to control respiratory disease (Nickell & White, 2010).

The study presented here demonstrated that a single injection of 2.5 mg/kg tulathromycin 10% was highly effective in the treatment and prevention of clinical cases of BRD. Tulathromycin is a novel semisynthetic antimicrobial agent that differs from other macrolides by having a much longer duration of action. This property is partly a consequence of the three amine groups responsible for the designation of the subclass as the triamilides. The study presented here demonstrated that a single injection of 2.5 mg/kg tulathromycin 10% was highly effective in the treatment and prevention of clinical cases of BRD. Tulathromycin is a novel semisynthetic antimicrobial agent that differs from other macrolides by having a much longer duration of action.

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15. Gap analysis

14.1. Mycoplasma bovis

With the majority of the information available, it is hard to determine the true incidence of *M. bovis*. Serological as well as slaughterhouse surveys are expected to provide a more precise picture of its real prevalence. An in-depth examination of all cost aspects, including deaths, veterinary expenses, treatment, milk loss, and housing, has not been done in order to determine the true economic cost of the disease. There aren't any official limitations on the movement of animals, but many countries that import cattle are becoming more aware of the dangers of bringing in infected animals

and are requiring that the cattle be tested for *M. bovis.*

We need to learn more about *M. bovis*. This includes its requirements for growth during colonization, survival in the environment and inside the host, including a better comprehension of particular Vsp functions and other mechanisms that impair the host immune system, production of biofilms, and generation of antimicrobial resistance.

We still need more studies and genome sequences of high-virulence and low-virulence strains, but comparing full genome sequences of *M. bovis* and gathering data on the presence of different antigens and repetitive sequences may help us better understand the disease. By integrating this genomic data, the core and pan-genomes of the species can be identified, and the genome plasticity of the pathogen can be thoroughly examined. Genomic comparisons with different *Mycoplasma* species should provide insights on the host specificity of *Mycoplasma*. The growth of other sectors that are currently underrepresented will be aided by genomic knowledge in order to enhance *M. bovis* diagnosis, treatment and prevention.

15.2. Disease course

There is still no conclusive explanation for the variety of clinical symptoms caused by *M. bovis*, which include genital infections, arthritis, abortion, otitis media, keratoconjunctivitis, pneumonia, infertility, and mastitis. Although the underlying interactions of the co-infectants are poorly understood, they are probably more persistent and have a bigger effect on field instances of pneumonia. Variations in herd management and strain heterogeneity may have an impact on the duration of the incubation period, which makes this a largely unstudied aspect of the disease's control. Again, the function of the asymptomatic carrier in a herd outbreak is unknown. Research on variables that affect virulence in disease, like the role of changeable surface proteins or metabolites produced by *M. bovis*, still needs to be done in great detail. Additional research is required to determine whether infection pathways, infectious dosages, host susceptibilities, age, and breed differ from one another. It is essential to comprehend the ways in which stressors such as weather, relocation, and housing impact a host's vulnerability to disease. Pneumonia throughout the winter is certainly significantly influenced by poor housing conditions.

15.3. Transmission, incubation period

Aerosols from frequent, close contact, contaminated milk, milk clusters, or milkers' hands are the most direct ways of transmission. Mycoplasmas are occasionally shed, but it's unclear if this is due to the infection's progression over time, the animals' stress level, or another factor. It's critical to recognize the potential for transmission and shedding from animals that only acquire arthritis. Since the environmental mechanisms of infection are still unknown, more research is necessary to fully understand the infectious dose and incubation period.

15.4. Control

An in-depth understanding of the risk variables that lead to outbreaks is required to control them at the herd level. Consequently, it may be possible to develop and implement successful intervention strategies without suffering large financial losses or incurring excessive medical expenses. By forming biofilms, sequestering intracellularly, or locating in tissues with antimicrobial concentrations below what is considered therapeutic, *Mycoplasma bovis* seems to be able to evade the effects of antimicrobial treatment. We need to learn more about the mechanisms underlying *M. bovis* resistance because antimicrobials are becoming ineffective and antimicrobial resistance is increasing. This information should guide initiatives aimed at preventing or slowing down the emergence of resistance and speeding up the process

of identifying resistance so that effective therapies can be implemented. Studies on effective antimicrobial therapy approaches are still needed.

Because in vitro laboratory studies, such minimum inhibitory concentration and minimum mycoplasmacidal concentration assays, are not yet standardized, standard control strains are required. It is therefore impossible to compare the outcomes of different laboratories. To create new or alternative antimicrobials, a variety of compounds, plant extracts, and antimicrobial peptides must be screened. For any newly created medications, however, adequate consideration must be given to withdrawal period from meat and milk. Early disease detection and treatment are crucial; nevertheless, some data suggest that extended therapy or metaphylaxic dosage of all groups is necessary, which is contrary to suggestions to use fewer antibiotics.

15.5. Vaccination

It is clear that there is a pressing need for better vaccines. These vaccines should ideally be safe, effective against all disease manifestations, useful at every stage of animal production, effective against all strains of *M. bovis*, stable, ideally given as a single shot, included in multivalent BRD vaccines, provide long-lasting protective immunity, and (viii) be accessible worldwide.

CONCLUSION

M. bovis-related illness is a widespread disease that has a major economic effect on cow husbandry. It is a significant barrier to intensive production that affects the amount of milk produced in high-producing herds and intensive beef production, especially in feedlots.

The bovine respiratory disease complex has several contributing factors, including (i) lack of effective vaccines; (ii) difficulty diagnosing latent infections; (iii) difficulty identifying the underlying pathogen when multiple pathogens are involved; and (v) development of antimicrobial resistance in *M. bovis* to many of the antimicrobials currently in use.

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