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Mycoplasma bovis Mastitis

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A R T I C L E   I N F O

A B S T R A C T

Bovine mycoplasmoses, which is mostly caused by *Mycoplasma bovis*, is a significant problem in the dairy and beef industry. Mycoplasmal mastitis has a global occurrence with notable effects in the United States and Europe. The pathogen was first detected in a mastitis case in California, United States, and regarded as major contagious mastitis. It is highly contagious and resistant to antibiotics and lack cell wall rendering certain group of antibiotics ineffective. Outbreaks mostly originate from introduction of diseased dairy cows to a farm and poor hygienic practices that help to maintain cow to cow transmission. Rapid detection scheme is needed to be in place in dairy farms to devise preventive measures and stop future outbreaks. However; early detection is hampered by the fastidious growth of *M. bovis* and the need for specialized equipment and reagents in laboratory settings. Intramammary *Mycoplasma bovis* infections cause elevation in milk somatic cell count which is one of the important factors to determine milk quality for grading and hence dictates milk price. There are multiple attributes of *M. bovis* regarded as virulence factors such as adhesion to and invasion into host cells, avoidance of phagocytosis, resistance to killing by the alternative complement system, biofilm formation, and hydrogen peroxide production.

Nevertheless, there are still undetermined virulence factors that hamper the development of sustainable control tools such as effective vaccine. To date, most vaccine trials have failed, and there is no commercial *M. bovis* mastitis vaccine. *Mycoplasma bovis* has been shown to modulate both humoral and cellular immune response during bovine mastitis. In the future, research seeking new immunogenic and protective vaccine targets are highly recommended to control this important dairy cattle disease worldwide.

Abbreviations

PFGE	Pulsed field gel electrophoresis
LAMP	Loop-mediated isothermal amplification
LAG3	Lymphocyte Activation Gene 3
VspC	Variable Surface Lipoprotein C
VspF	Variable Surface Lipoproten F
IL-1β	interleukin 1 beta
IL-6	Interleukin 6
IL-8	Interleukin 8
TNF-α	Tumor necrosis factor-alpha
Th	T-helper cells
Th1, Th2, Th17	T helper 1, 2 and 17
Treg	Regulatory T cells
P81	membrane Lipoprotein P81
UgpB	Glycerol ABC transporter, glycerol binding protein
APHIS	Animal and plant health inspection service
CBPP	Contagious Bovine Pleuropneumonia
CCPP	Contagious Caprine pleuropneumonia
ELISA	Enzyme-linked immunosorbent assay
ENA-78	Epithelial Neutrophil-Activating Peptide 78
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
IFN	Interferon
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukin
IMI	Intramammary infection
MALDI-TOF	Matrix assisted laser desorption ionization-time of flight
MIP-1α	Macrophage inflammatory Protein-1 Alpha

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MIP-1β	Macrophage inflammatory Protein-1 Beta
M. bovis	*Mycoplasma bovis*
Mbp	Mega base pairs
NO	Nitric Oxide
OIE	Organization for Animal Health
PBMCs	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
qRT-PCR	Quantitative real time polymerase chain reaction
SCC	Somatic cell count
TNF	Tumor necrosis factor
U.S.	United States
USDA	United States Department of Agriculture

1. Introduction

Mycoplasmas cause some of the most important livestock diseases listed by the World Organization for Animal Health (OIE), including contagious bovine pleuropneumonia (CBPP), contagious caprine pleuropneumonia (CCPP), contagious agalactia, and avian mycoplasmosis (Nicholas et al., 2017 OIE, 2021;). *Mycoplasma bovis (M. bovis)* causes bovine mycoplasmoses worldwide manifested mainly as mastitis (Hale et al., 1962), pneumonia (Gourlay et al., 1976), endocarditis (Kanda et al., 2019), arthritis (Hananeh et al., 2018), and otitis (Foster et al., 2009). Mastitis due to *M. bovis* has a global occurrence causing significant economic losses and animal welfare concerns (Al-Farha et al., 2017 Hale et al., 1962; Junqueira et al., 2020; Lysnyansky et al., 2016;). In the United States, the disease was first reported in 1962 (Hale et al., 1962) and has since been distributed in the country (Fox, 2012). In other parts of the world, it was reported largely in the 1960s and 1970s, including in Israel, Spain, Australia, France, Britain, and Germany (Nicholas and Ayling, 2003). Notably, mastitis due to *M. bovis* is becoming common in ranched bison in the United States and Canada (Sweeney et al., 2013). Whether the disease existed in these animals and remained undiagnosed, or it is a recent event, and whether the *M. bovis* isolates are related to the cattle strains are still under investigation (Register et al., 2021).

Mycoplasmas belong to the family *Mycoplasmataceae* under the class Mollicutes and are devoid of cell walls. They have a smaller genome size (0.58–1.4 Mbp) than other bacterial species (spp.) and possess 23–24% G + C content (Razin et al., 1998 Vos et al., 2011;). It is a slow-growing organism requiring a long incubation period and specific media and growth conditions. Colonies exhibit a distinctive 'fried egg' appearance for most *Mycoplasma* spp. on agar-based medium when viewed under the light microscope (Parker et al., 2018). The gross morphology of 'fried egg' is because of the central portion of the colony embedding itself into the agar surrounded by a zone of surface growth (McVey *et al.*, 2013). However, differentiation by culture alone can result in a false *Mycoplasma* positive sample as *Mycoplasma* media support the growth of many *Acholeplasma* spp., an environmental contaminants with no documented pathogenicity (Parker et al., 2018). Digitonin sensitivity can be used as an additional step to distinguish *Mycoplasma* spp. from *Acholeplasma* spp. On a paper disk saturated with 1.5% digitonin, a large zone of inhibition will surround *Mycoplasma*, with a small to a non-existing zone of inhibition for *Acholeplasma* spp. (Boonyayatra et al., 2012). However, interpretation of digitonin sensitivity can be subjective (Parker et al., 2018). Molecular identification such as polymerase chain reaction (PCR) was developed in the 1990s to achieve more specificity and sensitivity and has widely been employed lately to detect *M. bovis* (Parker et al., 2017). Furthermore, an indirect enzyme-linked immunosorbent assay (ELISA) has also been used for the early detection of *M. bovis* in mastitis outbreaks (Byrne et al., 2000).

Introduction of diseased animals to herds and poor hygienic practices in dairy farms are the major factors in the occurrence and transmission of *M. bovis* mastitis (Fox et al., 2005). The most common way of *M. bovis* introduction into dairy herds is through the purchase of sub-clinically infected carrier non-lactating animals (calves, heifers, dry cows, or bulls). As there is no effective way of identifying *M. bovis* in such animals, they can pose a significant challenge to the prevention of the disease (Hazelton et al., 2018). Transmission of *M. bovis* from cows to calves can occur through milk consumption (Aebi et al., 2012). Co-rearing of dairy calves along with dairy cows contributed to high *Mycoplasma* infection, although it was not stated whether the former or latter served as a source of infection (Nicholas et al., 2016). According to Gille and co-workers, calving cows shed infectious amount of *M. bovis* around parturition which could potentially be a risk factor for spread of infection in a farm. The use of a separate calving pen is recommended as a protective measure (Gille et al., 2018). Furthermore, a study has shown that *M. bovis* strains follow a clonal epidemiological spread at the herd level. The same strain can persist in calves within the herd after clinical signs have disappeared (Arcangioli et al., 2012). Recently, semen has been shown to serve as a source of *M. bovis* mastitis which is suggestive of artificial insemination serving as a potential route of transmission (García-Galán et al., 2020 Haapala et al., 2018; Parker et al., 2017;). Similarly, the use of breeding bull has been associated with a high prevalence of *M. bovis* in dairy herds (Gille et al., 2018). Several *Mycoplasma* spp. can occur together in a mastitis case, thus leading to increased somatic cell count (SCC), altering milk protein percentage, decreased milk yield, fat percentage, and total milk solids (Al-Farha et al., 2017). In addition, co-infection of *M. bovis* with other mastitis pathogens is also reported (Timonen et al., 2017). The disease is severe in cows in early lactation and can eventually lead to agalactia (Pfützner and Sachse, 1996).

Mycoplasmas can be disseminated to different body sites through the hematogenous route (Biddle et al., 2005). This is clinically manifested as co-existence of arthritis along with mastitis or pneumonia (Houlihan et al., 2007). Furthermore, *M. bovis* which was inoculated in one quarter of cows, has been recovered from other three quarters, indicative of hematogenous spread (Bennett and Jasper, 1980). Inoculation of *M. bovis* into the udder results in frequent but intermittent shedding of the same strain from the nose, eye, rectum, vagina, and urine of the inoculated cows (Jain et al., 1969). The same authors also reported that *Mycoplasma* was isolated from lymph nodes, gastrointestinal contents, uterus, lung, liver, kidney, spleen, joint fluid, and fetus of the inoculated cows at postmortem examination. Similarly, Maeda and co-workers recovered *M. bovis* from different body parts such as ears, upper respiratory tract airways, lungs, lymph nodes, brain, and heart in a single animal following natural infection, which is indicative of the potential to spread to different predilection sites from the point of infection (Maeda et al., 2003). Furthermore, Biddle and co-workers examined *M. bovis* and *Mycoplasma californicum* isolates from numerous body sites of cattle associated with a single herd outbreak and found that isolates from the mammary gland frequently had identical pulsed field gel electrophoresis (PFGE) patterns to isolates from other body sites, suggesting internal transmission of a single strain (Biddle et al., 2005). Similarly, Parker *et al.*, 2016 observed identical genetic patterns amongst *M. bovis* isolates from lung, nasal, and milk samples from a single animal, which was consistent with previous findings and further reinforcing internal spread of the pathogen via blood stream.

Rapid detection of *M. bovis* in dairy farms is highly important for early treatment and prevention of future outbreaks. Microbial culture has long been employed to identify *Mycoplasma* spp. in cattle diseases (Parker et al., 2018). However, Andres and co-workers demonstrated that using quantitative real-time PCR (qRT-PCR) before culture improves isolation efficiency and saves time and resources (Andrés-Lasheras et al., 2020). Real-time PCR combined with a high resolution melting curve assay was proven effective to identify different mollicutes, including *M. bovis* in milk (Al-Farha et al., 2018). The *uvrC*, DNA repair gene, is the main target gene to detect *M. bovis* using PCR (Rossetti et al., 2010); however, assays targeting multiple genes such as loop-mediated isothermal amplification (LAMP) has been shown to be more sensitive (Appelt et al., 2019 Ashraf et al., 2018;). Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) method,

which utilizes proteins from mycoplasmas, is reported to assist species-level identification (Pereyre et al., 2013).

Although the prevalence of bacterial mastitis pathogens may vary with farms and geographical locations, *Streptococcus agalactiae, Staphylococcus aureus,* and *Mycoplasma* spp. are the major contagious mastitis pathogens (Wilson et al., 1997). *M. bovis* mastitis has been a significant problem in the dairy industry in the U.S. (APHIS-USDA, 2003, 2008). It is a highly contagious disease that is difficult to prevent (Fox et al., 2005) and becoming increasingly resistant to antibiotics (Liu et al., 2020). As treatments are not rewarding, replacement due to culling of infected animals entails a significant cost to farmers. Economic loss due to *M. bovis* mastitis is estimated to be more than $108 million annually in the U.S. (Nicholas and Ayling, 2003). Intramammary infection (IMI) causes an increase in SCC and reduction in milk yield and affects milk quality (Timonen et al., 2017), causing significant economic losses to the producers. The SCC is a measure of milk quality and general udder health. A failure to meet a low bulk tank SCC ($<$ 200,000 cells/mL of milk) or legal limit of SCC (ranging from 400,000 cells/mL to 750,000 cells/mL depending on the states) is a leading worry for dairy farmers in the U.S. (APHIS-USDA, 2019). Large herd size has been reported to be a risk factor for the *M. bovis* mastitis occurrence which is highly alarming for countries such as the U.S. where large-scale dairy farms exist (APHIS-USDA, 2008 Fox et al., 2003;). Yet, more than 60% of large dairy operations in the U.S. have not heard about *Mycoplasma* mastitis by 2002, although the contemporary status is researchable (McCluskey, 2003), highlighting a lack of awareness about the potential risks *M. bovis* mastitis can pose. Similarly, in 2014 United States Department of Agriculture (USDA) reported 25.2% of large operations (500+ cows) identified mycoplasmas (not specified as *M. bovis*) in milk, followed by 6.1% of medium operations (100 - 499 cows) and 0% in small dairy operations (30 - 99) (USDA, 2014). Unfortunately, 46.6% of dairy farm operations in the U.S. only heard of *Mycoplasma* mastitis. Still, they lack sound knowledge of its significance and transmission, which is believed to be a bottleneck for prevention and control of the disease (USDA, 2007). The significance of *M. bovis* mastitis also emanates from the fact that it usually affects more than one quarter, and even severely affected cows apparently appear normal, yet shedding the organism (Nicholas et al., 2016).

Studies on the prevalence of *M. bovis* mastitis are limited in the United States. The latest was conducted in 2003 (APHIS-USDA, 2003). This paper is a concise review of the current status of *M. bovis* mastitis and discusses important virulence factors of the *M. bovis* and associated host responses.

2. *M. bovis* virulence factors

The development of effective prevention and control strategies requires detailed knowledge of *M. bovis* virulence factors and pathogenesis mechanisms. Although mycoplasmas possess a limited number of metabolic pathways and require several growth nutrients *in vitro*, their mechanisms for successfully initiating natural infections are barely known (Sirand-Pugnet et al., 2007).

Adhesion of mycoplasmas to host cells is regarded as an important virulence mechanism in the *M. bovis* pathogenesis as non-adherent mutants are demonstrated to be non-pathogenic (Razin and Jacobs, 1992). Surface proteins such as variable surface lipoproteins C (VspC) and F (VspF) are believed to play this role as they are the first structures to be detected during *Mycoplasma*-host interaction (A. Thomas et al., 2003). James and co-workers have recently demonstrated that *M. bovis* membrane protein known as *Mycoplasma* immunogenic lipase A (MilA) is a multifunctional lipase with novel lipid and glycosaminoglycan binding activity. Additionally, MilA was shown to be an immunogenic protein that binds ATP and heparin. This study was one of the first to demonstrate the presence of a cell surface lipid-binding protein in a *Mycoplasma,* and such binding can be a prelude to import required nutrients (Adamu et al., 2020). The absence of cell-wall in mycoplasmas is

also believed to favor their direct and intimate fusion to the cytoplasmic membrane of the host cells (Rottem, 2003). Membrane proteins P81 and UgpB are highly immunogenic, and antibodies against them have inhibited *in vitro M. bovis* growth (Zhang et al., 2019). *M. bovis* also elaborates a membrane nuclease known as MnuA, which degrade and overcome the neutrophil extracellular traps; a system employed by neutrophils to trap and kill bacteria (Mitiku et al., 2018). *Mycoplasma bovis*-induced apoptosis has been reported widely in neutrophils, bovine mammary epithelial cells, and embryonic bovine lung cells (Jimbo et al., 2017; Liu et al., 2020a; Wu et al., 2021;). Transient neutropenia reported by Kauf et al. (2007) following IMI of dairy cows is in line with *M. bovis*-induced apoptosis of neutrophils.

Invasion into host cells is considered an important mechanism in *Mycoplasma* infection since it protects the *Mycoplasma* from host humoral immunity and antimicrobial agents (Rottem, 2003). *Mycoplasma bovis* has been shown to invade bronchiolar epithelial cells in experimentally infected calves (Nunoya et al., 2020). It has also been demonstrated intracellularly in the cytoplasm of peripheral blood mononuclear cells (PBMCs), hepatocytes, renal tubular epithelial cells, neutrophils, and macrophages (Maeda et al., 2003 van der Merwe et al., 2010;). Greater antigenic heterogeneity among *M. bovis* isolates allows them to evade the humoral adaptive immune system and is also one of the bottlenecks in vaccine development against *M. bovis* mastitis (Poumarat et al., 1994). The heterogeneity mainly lies in the diversity of the membrane surface proteins (Vsps) (Rosengarten et al., 1994). Under unfavorable conditions such as desiccation and antimicrobial pressure, *M. bovis* also synthesize a biofilm, a mass of bacteria attached to each other and surrounded by polysaccharide matrix (McAuliffe et al., 2006).

Resistance to killing by alternative complement system is regarded as one of the virulence mechanisms by *M. bovis* (Howard, 1980). Similarly, there is a report of *M. pulmonis* strains that synthesize certain types of variable surface antigen A (VsaA) and can escape the complement killing, although the details of the mechanism are unknown (Simmons and Dybvig, 2003). However, in the presence of antibodies, it has been demonstrated that *M. bovis* is susceptible to the classical complement (Zhang et al., 2019). On the other hand, the humoral immune response is considered ineffective in combating mycoplasmal infections (Nicholas et al., 2017), which could be attributed to humoral responses being mounted after host cells are invaded.

Mycoplasma bovis elaborates secondary metabolites such as hydrogen peroxide, which are associated with necrosis in lung tissues (Schott et al., 2014). It has been shown that killed/inactivated *M. bovis* antigens do not elicit cellular responses (Zbinden et al., 2015), which could be attributed to their failure to elaborate the secondary metabolites. In contrast, there is a report stating that killed *M. bovis* antigen could trigger enhanced cellular inflammation in the mammary gland (Boothby et al., 1988 Gondaira et al., 2018;). This could be due to different heat-killing procedures (95°C for 30 min *vs* 70°C for 5 min) employed by the authors.

Mycoplasma bovis is often involved in co-infections with other pathogens, which is believed to have a synergistic effect. A case of *M. bovis* mastitis and purulent inflammation of joints where *Trueperella pyogenes* and *Fusobacterium* spp. were isolated had also been reported (SAC, 2015). Similarly, *Trueperella pyogenes* were isolated along with *M. bovis* from suppurative otitis media and pneumonia in calves (Maeda et al., 2003). Examination of a bulk tank milk also revealed co-infection of *M. bovis* and other contagious mastitis pathogens such as *Staphylococcus aureus* and *Streptococcus agalactiae* (Olde Riekerink *et al.,* 2006). Furthermore, viral infections such as infectious bovine rhinotracheitis and bovine viral diarrhea have been associated with *M. bovis* mastitis and pneumonia outbreaks, respectively (Gourlay et al., 1974 Shahriar et al., 2002;).

3. Host responses to *M. bovis* intramammary infections

Upon interaction with the epithelial cells, *M. bovis* induces the

production of proinflammatory cytokines such as interleukin (IL) −1β, IL-6, IL-8, and tumor necrosis factor-alpha (TNF-α). This non-specific innate immune response can attack the invading pathogens as the first line of defense, resulting in a subsequent cascade of events, including antigen presentation by the dendritic cells, CD4 cells activation, and differentiation into T-helper cells Th1, Th2, Th17, and regulatory T cells (Treg) (Askar et al., 2021 Jimbo et al., 2017; Kauf et al., 2007; Zbinden et al., 2015;). During an intramammary challenge of cows with *M. bovis*, the initial reaction of the host was demonstrated to be increased SCC 66 h post-infection and synthesis of acute-phase proteins at 108 h post-infection (Kauf et al., 2007). Chemokines such as macrophage inflammatory protein-1 Alpha (MIP-1α), macrophage inflammatory protein-1 beta (MIP-1β), and epithelial neutrophil-activating peptide 78 (ENA-78/CXCL5) produced as the result of proinflammatory cytokines are reported to support the innate and adaptive immune responses in respiratory infections (Vanden Bush, 2003). The chronic nature of *M. bovis* mastitis could be associated with the high expression level of immune exhaustion related genes such as programmed cell death 1 (PD-1), programmed cell death ligand – 1 (PD-L1), lymphocyte activation gene 3 (LAG3), and cytotoxic T-lymphocyte-associated protein (CTLA4) in milk mononuclear cells (Gondaira et al., 2020).

3.1. Cellular responses

Cellular responses involve Th1 cells, which activate CD8 cytotoxic T-cells and macrophages through interferon-gamma (IFN-γ) and TNF-α to degrade the mycoplasmas or cause apoptosis (Askar et al., 2021). Interleukin 12, which mediates the differentiation of T-cells into Th1 was reported to be increased in mRNA profiling of *M. bovis* infected PBMCs (Askar et al., 2021 Gondaira et al., 2015;). Interferon-gamma (IFN-γ) and TNF-α have been shown to be elevated both *in vitro* in neutrophils and *in vivo* during intramammary *M. bovis* infection (Jimbo et al., 2017 Kauf et al., 2007;). In monocytes, however, Mulongo and coworkers reported that *M. bovis* inhibits the production of TNF-α and IFN-γ (Mulongo et al., 2014), which could be attributed to the difference in the cell lines. *Mycoplasma bovis* also triggers activation of CD4+ and CD8+ T cells *in vitro* in PBMCs and *in vivo* in immunized calves (Dudek and Bednarek, 2017 Vanden Bush and Rosenbusch, 2003;).

Th17 cells produce IL-17 and IL-22, which enhance the recruitment of neutrophils, also play a key role (Annunziato et al., 2015 Askar et al., 2021;). *Mycoplasma bovis*, however, causes persistent immunosuppression by repressing neutrophil activity, the first line of cellular defense against pathogens (Thomas et al., 1990, 1991). As reported by Jimbo and co-workers, this might be through reducing the ability of IL-17 and inhibiting the production of nitric oxide (NO). Interleukin 17 enhances neutrophil-induced destruction of *M. bovis*, whereas NO augments cytotoxic reactive oxygen species produced by neutrophils against pathogens (Jimbo et al., 2017).

3.2. Humoral responses

Type 2 immunity, which is coordinated by Th2 cells, involves the activation of B cells through IL-4 and IL-5 to differentiate into plasma cells, thus increasing antibody production (Askar et al., 2021). Although there are no reports on type 2 response associated cytokines in cows during IMI, an increase in IL-4 has been demonstrated in *M. bovis* experimental infection in calves (Dudek et al., 2013, 2018). The elevated level of IgG1 was reported during IMI of dairy cows (Boothby et al., 1987) and in dairy calves immunized with *M. bovis* bacterin sub-cutaneously (Maunsell et al., 2009). Similarly, intranasal inoculation of protein-based vaccines in feedlot calves revealed increased IgG1 (Prysliak et al., 2017, 2013 Vanden Bush and Rosenbusch, 2003;) and minimal IgG2 production (Vanden Bush and Rosenbusch, 2003). The antibodies believed to play an important role in localizing the mycoplasmal infection at a mucosal level and facilitating opsonization and subsequent phagocytosis by macrophages (Askar et al., 2021). Various

reports from experimental infections indicated production of IgG1 predominantly and also IgG2 (Boothby et al., 1987 Maunsell et al., 2009; Prysliak et al., 2013; Vanden Bush, 2003;). There is wider presence of the antibodies on mucosal surfaces, which is believed to limit the infection from spreading to the udder and other organ systems (Askar et al., 2021). However, it is poorly understood whether it is the humoral or cellular or both (synergistic) that is effective in clearing mycoplasmal infections and yet to be determined.

4. Prevention and control

Screening new dairy cows for *M. bovis* IMI before introduction to a farm is the most effective way to prevent the disease (APHIS-USDA, 2003). Even though serology is helpful to screen animals for biosecurity risk assessment, still more information about seroconversion, antibody longevity, sensitivity, and specificity of test diagnostic are required to establish its appropriate use for biosecurity purposes (Hazelton et al., 2018). Once *M. bovis* mastitis is diagnosed in a herd, identification, and segregation of infected animals are of great importance (Fox et al., 2005). This could be achieved through bulk tank milk monitoring and further tracing of cows shedding the pathogen through individual cow sampling (Nicholas et al., 2016). A study, however, reported low prevalence of IMI following *M. bovis* mastitis outbreak in herds and suggested identification of clinically mastitic cows after outbreaks contribute minimally to control and eradicate *M. bovis* (Hazelton et al., 2020).

Udder infection mostly results from the progressive colonization of pathogens through the teat canal upward, indicating where control measures should be directed (Pfützner and Sachse, 1996). Hence, hygienic milking practice and milking suspected cows at the end of each milking session is recommended (Brown et al., 1990). Culling has long been a practice to stop the spread of *M. bovis* mastitis (Pfützner and Sachse, 1996). However, it was reported that such practice may not be an essential disease control measure (Punyapornwithaya et al., 2012). In areas where artificial insemination is highly practiced, monitoring semen microbial quality could be worthwhile since studies have reported *M. bovis* in semen (García-Galán et al., 2020 Haapala et al., 2018; Parker et al., 2017;).

Mycoplasma bovis induced mastitis is becoming increasingly resistant to antibiotics (Liu et al., 2020a). Resistance to certain groups of antibiotics is partly due to its lack of cell wall, which narrows the margin of treatment options. Cephalosporins and tetracyclines are commonly used to treat mastitis in the U.S. (USDA, 2008); however, *M. bovis* has been shown to be resistant to the majority of these drugs (Thomas et al., 2003). Enrofloxacin and doxycycline have been shown to reduce the viability of *M. bovis* isolates from Angus bulls semen (García-Galán et al., 2020). Drugs targeting protein and DNA synthesis, such as tiamulin and fluoroquinolones, respectively, are shown to be effective against *M. bovis* isolates from herds with recurrent respiratory problems (Thomas et al., 2003). Despite the fact that these isolates were not directly from cases of mastitis, it is important to test efficacy of these antimicrobials on isolates from *Mycoplasma bovis* mastitis since these isolates also believed to cuase mastitis through hematogenous spread. This also provides more information on differences in suscepitlity of *M. bovis* isolates from different body sites against these antimicrobials as well as if there is strain variation in predilection sites. Although there are multiple trials to develop an effective vaccine against MBM, there is no commercial vaccine to date to control the disease.

4.1. Prospect for MBM vaccine development

Significant economic losses and increased resistance to drugs have prompted a global vaccine search to control this important dairy cattle disease. Although several vaccine trials have been underway, protective antigens are rarely known (Mulongo et al., 2013 Perez-Casal et al., 2017; Prysliak et al., 2013). Moreover, the involvement of various *M. bovis* isolates in different outbreaks renders vaccine development difficult

(Aebi et al., 2012). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) protein has been shown to induce immune response in beef cattle (Perez-Casal and Prysliak, 2007). However, a chimeric Gap-I protein composed of *M. bovis* GAPDH and the host-defense peptide indolicidin was shown to be not a suitable antigen due to failure to protect immunized animals against challenge (Prysliak et al., 2013). This could be attributed to GAPDH activating a strong humoral response but a weak cellular response which is indicative of humoral response being less successful in the elimination of *M. bovis* infections. In general, this strongly suggests that highly immunogenic products of *M. bovis* which can elicit a protective immune response, are yet to be found. Compared to bacterin vaccines which have limited protection and adverse reaction, specific products (subunit vaccines) are highly preferred as a vaccine entity (Perez-Casal et al., 2017).

5. Conclusion and future direction

Mycoplasma bovis mastitis is recently regarded as an important emerging dairy cattle disease due to surge in prevalence in several countries and significant economic losses in the dairy farms. The introduction of sub-clinically infected cows, is considered to contribute to the MBM outbreaks; hence the purchase of new dairy heifers should be made with caution and strict screening. Once occurred, there is a high chance of the disease spreading among dairy cows as it only causes subclinical mastitis and remains undetected at the onset stage. This necessitates regular monitoring followed by segregation and culling of infected animals. Research-driven use of antibiotics against MBM should be exercised in dairy farms for effective outcomes and to mitigate antibiotic resistance problems. With effective vaccine development hanging on the discovery of immunogenic products and there is no definite time to identify protective immunogenic antigens, prevention measures remain the only available options to reduce the incidence of MBM in dairy farms.

Availability of data and material statement

Not applicable

CRediT authorship contribution statement

Aga E. Gelgie: Writing – original draft, Writing – review & editing. **Mesula G. Korsa:** Writing – original draft, Writing – review & editing. **Oudessa Kerro Dego:** Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors have no potential conflicts of interests.

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