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OVERVIEW OF *Mycoplasma bovis* INFECTION IN DAIRY AND BEEF CATTLE

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Mycoplasma bovis is an economically important disease in dairy and feedlot cattle; the annual loss was calculated as 140 million USD in the USA. *Mycoplasma bovis* is a common bacterium found in mucous membranes in different animal species, including the respiratory tract, urogenital tract, and gastrointestinal tract. *Mycoplasma bovis* is a highly contagious disease with an over 70% infection rate. The disease prevalence of *M. bovis* infection is varied widely and high nasal prevalence was reported among young calves. The mortality were reported in 2-6 weeks of old calves while the peak clinical incidence was at the age of one month. *Mycoplasma bovis* is colonized in normal respiratory mucosae and tonsils. The clearance of the organism takes for a long period in cattle, it is for a couple of months to a number of years in the mammary gland. Mortality of *Mycoplasma bovis* infection is varied from herd to herd and location to location. *Mycoplasma bovis* has been isolated from milk, conjunctiva, semen, and vaginal secretions. The organism is excreted through milk either intermittently or continuously, even in sub-clinical infection. Airborne transmission is the main route of infection in the susceptible bovine host. An animal that sheds *M. bovis* is the main source of infection within a herd. The bacterium is excreted in colostrum, vaginal secretion and in respiratory secretion of infected cows. The pathogenesis of *Mycoplasma bovis* has been poorly understood or partly investigated in cattle. Both conventional and molecular methods are there to identify the organism with high analytical sensitivity and specificity. Both 16S rDNA and 23S rDNA targets are used to differentiate Mycoplasma from other possible microorganisms. The immunohistochemistry, in situ hybridization and histopathology, have also been optimized as diagnosis of *Mycoplasm bovis* infection in cattle. Serological diagnosis is also considered a cost-effective method to identify the seropositive animal. ELISA test has been optimized for field samples of both serum and milk. Three main mechanisms have been able to control or prevent *Mycoplasma bovis* infection in cattle farming such as sanitary control measures, antimicrobial therapy and vaccination.

Keywords: Cattle, Control, Epidemiology, *Mycoplasma bovis*

Mycoplasma bovis is an economically important disease in dairy cattle, the average annual loss was calculated as 140 million USD in the USA with 70% of infection rate in feedlot cattle (Khan et al., 2017). Mortality, reduced production, animal welfare and the cost of treatment are the main components of the cost of involvement in animal husbandry. It has been shown that estimated cost on treatment of bovine respiratory disease complex including *Mycoplasma bovis* was 23.60 USD per individual calf (Grissett, 2015). The eradication of *Mycoplasma bovis* is a very expensive task in

livestock; over 160 000 cattle were slaughtered in New Zealand with a value of 116 million Euros in 2020 (Aebi et al., 2015; Dudek, 2020).

Mycoplasma is a mollicute, a common inhabitant in respiratory tract, urogenital tract, and in gastrointestinal tract of livestock (Parker, 2018). The lack of cell wall is the main characteristic of this group of bacteria while it is also considered as the main survival mechanism against antimicrobials (Caswell, 2007). The genome size of *Mycoplasma bovis* is varied from 0.58 to 2.2 Mega bases (Maunsell, 2011). It is a small genome sized pathogen when comparing to rest of the pathogenic organism in livestock (Maunsell and Donovan, 2009). Mycoplasma bacterium consists of cytoplasm, cell membrane and nucleic material while other complex organelles are not found as a comparison to other pathogenic bacterial species in livestock (Dudek, 2020). An individual cell of Mycoplasma cannot synthesize its own requirement of amino acids and fatty acids due to the lack of complex internal structures; nutritional requirement depends on resources acquired from the host (Dudek, 2020).

Mycoplasma bovis causes mild to severe respiratory tract infections in calves and adults (Caswell, 2007). The following species of Mycoplasma were shown in varying degree of clinical importance in cattle as *Mycoplasma californicum, Mycoplasma bovigenitalium, Mycoplasma bovirhinis, Mycoplasma bovoculi, Mycoplasma leachii, Mycoplasma dispar, Mycoplasma canis, Mycoplasma canadense, Mycoplasma alkalescens, Mycoplasma arginini, and Mycoplasma wenyonii* (Maunsell, 2011; Parker, 2018). In addition, high clinical significance were shown in *Mycoplasma bovis*, *Mycoplasma mycoides mycoides*, *Mycoplasma bovigenitalium* and *Mycoplasma bovoculi* (Dudek, 2020). Bovine mycoplasmosis is either a primary or a secondary infection, the clinical outcome depends on factors related to the host, pathogen and environment (Parker, 2018). Therefore, identification of exact cause of the clinical infection is a challenging task in bovine herds. In addition, *Mycoplasma mycoides mycoides* is the causative agent for bovine pleuropneumonia in cattle (Dudek, 2020; Parker, 2018). Bovine pleuropneumonia is not a common disease in current bovine herds. Furthermore, this disease is limited to Sub Saharan countries as a result of continuous stamping out policies in many countries (Parker, 2018). *Mycoplasma bovigenitalium* is associated mainly with reproductive disorders while *Mycoplasma bovoculi* is found with keratoconjunctivitis in cattle (Dudek, 2020). *Mycoplasma bovis* causes a series of multiple clinical diseases such as mastitis, arthritis, pneumonia, otitis media and reproductive disorders in cattle especially in feedlot cattle (Caswell, 2007; Parker, 2018). The closely associated Genus of Acheloplasma has also been isolated in cattle, although clinical significance has not been proven (Caswell, 2007; Parker, 2018).

Mycoplasma bovis is an important member of bovine respiratory disease in cattle with a number of other bacterial pathogens such as *Mannheimia haemolytica, Mycoplasma bovis, Pasteurella multocida, and Histophilus somni,* as well as viral pathogens of bovine herpesvirus type 1 (BHV-1), parainfluenza- 3virus (PI3), bovine viral diarrhea virus (BVDV) and bovine respiratory syncytial virus (BRSV) (Grissett, 2015).

The mammary tissue infection or mastitis caused by *Mycoplasma bovis* is often shown in multiple quarters in cows and it was shown unresponsive against antimicrobial treatments frequently (Maunsell, 2009). Pneumonia and arthritis were observed common in adults and calves, while otitis media were limited to calves (Caswell, 2007). Although mycoplasmosis is a

contagious disease in cattle, reproductive diseases were considered under reported in the field due to inadequate laboratory facilities to identify the causative agent (Haapala et al., 2021). The vulvovaginitis, infertility, endometritis were common reproductive disroderd caused by *Mycoplasma bovis* (Caswell, 2007). In addition, early culling of dairy animals are often practiced due to the unresponsiveness of the diseases for long period (Haapala et al., 2021).

A limited literature is found on local herds and no extensive study has been performed in the country. Furthermore, *Mycoplasma bovis* has been recognized as a potential risk to the dairy industry as a mixed infection with BVD and Salmonella Dublin.

Epidemiology

Mycoplasma bovis was first reported in 1961, the USA as a consequent of a severe outbreak of bovine mastitis (Dudek, 2020). The disease was first recognized in Europe in mid-1970 as a consequent of international trade and bovine semen distribution (Dudek, 2020). Finally, the disease had been transmitted and reported in all over the World where dairy and beef industry is found (Dudek, 2020). The prevalence of *Mycoplasma bovis* infection is varied with geographic location in Europe. Currently, the disease prevalence was 1.5% and 5.4% in Belgium and Greese respectively (Nicholas et al., 2016). The disease prevalence was less than 1% in UK and France believing the infection was under-reported (Nicholas et al., 2016). The high prevalence of *Mycoplasma bovis* was observed in veal calve in Europe (Bokma et al., 2020). The situation is not known in developing countries due to the lack of disease surveillance on *Mycoplasma bovis*. In addition, high disease prevalence was reported in developing world. As an example, the prevalence of *Mycoplasma bovis* was 55%, 100% in Mexico and Iran respectively (Nicholas et al., 2016).

The isolation of the organism from nasal swabs is challenging although animals were clinically ill, correlation between isolation and the presence of clinical disease was limited (Caswell and Archambault, 1996). As an example, *Mycoplasma bovis* had been recoverd only in 4% of animals although clinical disease was high as 28-98% in Australia (Hazelton et al., 2020). Importantly, *Mycoplasma bovis* has been isolated from milk, conjunctiva, semen, and vaginal secretions (Caswell and Archambault, 1996). Furthermore, *Mycoplama bovis* had been isolated from joint fluid, eyes, sheath washings, urogenital tract and heart blood (Dudek, 2020). *M. bovis* can be isolated from conjunctiva of the cattle, *M. bovis* associated kerato-conjunctivitis also been reported (Dudek, 2020).The organism is excreted through milk either intermittently or continuously even in sub-clinical infection (Caswell, 2007).

The prevalence of *M. bovis* infection was high as 40-100% in feedlot cattle (Castillo-Alcala et al., 2012; Dudek, 2020). The peak clinical incidence was observed in one month old calves (Maunsell and Donovan, 2009). Young calves are more susceptible on the infection and high mortality was reported in 2-6 weeks of old calves (Maunsell and Donovan, 2009). The percentage of mortality is varied with multiple factors such as specific herd, severity of clinical disease, type of disease, environment and geographic location, it was 5-10% pneumonic calves in Italy (Calcutt, 2018). The reported morbidity were high as 35% in Europe (Calcutt, 2018). *M. bovis* is colonized in normal respiratory (upper respiratory tract and lower respiratory tract) mucosae of cattle, mammary gland and in tonsils (Maunsell, 2011).

Airborne transmission is the main route of infection from infected animal although little experimental evidence had been published (Dudek, 2020). *Mycoplasma bovis* shedder is the

key and main source of infection in a herd (Dudek, 2020). *Mycoplasma bovis* is excreted in colostrum, vaginal secretion, and respiratory secretion of infected cows, which were the main source of infection in newly born calves (Aebi et al., 2015; Dudek, 2020; Gille et al., 2020). The recovery rate of *Mycoplasma bovis* in colostrum is varied, the organism has not been isolated always from infected herds (Gille et al., 2020). However, lack of isolation in colostrum is not an indicator on *Mycoplasma bovis* free status in a herd (Gille et al., 2020). Conversely, more studies are required to understand the shedding of *Mycoplasma bovis* in colostrum (Gille et al., 2020). Although the frequency was observed low, congenital *Mycoplasma bovis* infection has been reported in cattle, other routes of infection were shown common (Caswell, 2007). The organism can be recovered in either nasal swabs or broncho-alveolar lavage of infected and healthy animals (Schibrowski et al., 2018).

Antibodies against *Mycoplasma bovis* are detected in 10-14 days post infection in cattle and detectable level of antibodies remain for several months (Sachse et al., 1993). The serum and bulk milk samples are used to detect antibodies in individuals and herds respectively (Sachse et al., 1993). Indirect ELISA has been developed such as BIO K302, BIO K 260, MilA ELISA, to detect *Mycoplasma bovis* antibodies in bulk milk and serum with over 94% sensitivity and specificity (Hazelton et al., 2020; Nielsen et al., 2015; Vähänikkilä et al., 2019). However, MilA ELISA is not suitable for testing serum from early stage of calf (<3 weeks) (Vähänikkilä et al., 2019).The determination of sero positivity for bovine mycoplasmosis depends on multiple factors such as stage of the clinical disease and efficacy of laboratory tests. The detection of the infected animals depends upon sensitivity & specificity of serological test, way of sample handling and factors related to laboratory skills (Dudek, 2020). Presence of asymptomatic animals is the vital component of reinfection in healthy bovine herds (Maunsell, 2011).

The clinical mastitis caused by *Mycoplasma bovis* is a highly contagious disease in cattle. *Mycoplasma bovis* causes a purulent mastitis in cattle and odourless content, presence of abnormal and disclosure secretion and lack of systemic signs were shown common (Nicholas et al., 2016). The clearance of the bacterium out of mammary gland is long as months to years (Nicholas et al., 2016). Importantly, many sub-clinical mastitis cases in *Mycoplasma bovis* were shown neither high somatic cell count nor reduced milk yield (Maunsell, 2011). Therefore, identification of sub-clinical mastitis caused by *Mycoplasma bovis* is challenging in the field. Importanly, sub clinical mastitis caused by *Mycoplasma bovis* has been shown in all stage of lactation and dry animals (Maunsell, 2011). Furthermore, all ages were susceptible for the sub-clinical mastitis (Maunsell, 2011). These asymptomatic animals shed the organism for many months to years and can act as reservoir for *Mycoplasma bovis* infection (Horwood, 2014; Khan et al., 2017).

In clinical mastitis, no specific sings are found in *Mycoplasma bovis* although more than one quarter of mammary gland are affected (Maunsell, 2011). Mild systemic infection, drastic reduction of milk production, swollen gland, non-painful infection, greety or purulent or purulent colored secretion from teat canal were observed *Mycoplasma bovis* infection (Maunsell, 2011). Although it is less common, severe udder disease had been reported in clinical mastitis caused by *Mycoplasma bovis* in cattle (Aebi et al., 2015; Nicholas et al., 2016). Mastitis remained for several weeks and very slow recovery time was reported (Maunsell, 2011). Combination of other systemic infection such as arthritis, synovitis were also shown with

lack of significant response for commonly used antimicrobial (Maunsell, 2011). A number of risk factors have been investigated on presence of mastitis in cattle by *Mycoplasma bovis* such as herd size, newly introduced cattle, dry period, feeding of waste milk or milk products, calves contact with old cows, shared pen water, adjoining pens with infected animals (Nicholas et al., 2016; Schibrowski et al., 2018).

Pneumonia caused by had been observed in any age of cattle including calves, dairy cows, beef cattle with other nonspecific clinical sings as fever, tachypnea, dyspnea, decreased appetite, with or without nasal discharge and cough (Maunsell, 2011). Poor weight gain is the common clinical signs in chronic mycoplasma infection in cattle (Maunsell, 2011). Otitis media caused by *Mycoplasma bovis* were shown in calves as an epizootic infection (Maunsell, 2011). Those calves were shown febrile illness, anorectic and sign of ear pain (Head shaking and rubbing of ear). The infection is either in unilateral or bilateral with purulent aural discharge, epiphora, and exposure keratitis (Maunsell, 2011). In severe cases, nystagmus, circling, falling and drifting movement were also shown (Maunsell, 2011). The concurrent mixed infection with other systemic microbial infection has been shown common in clinical *Mycopalsma bovis* in cattle (Dudek, 2020).

The seasonal variation had been observed in respiratory tract infection in calves caused by *Mycoplasma bovis* observed, high incidence rate were reported in colder months (Dudek, 2020). Therefore, environment plays a significant role in *Mycoplasma bovis* infection either directly or indirectly. In addition, calf pneumonia had been observed common in beef herds than dairy herds (Dudek, 2020). Mixed infection were shown common in cattle, *Mycoplama dispar*, *Mycoplama canis* and *Mycoplama arginine* have been isolated with *Mycoplasma bovis* in upper respiratory infection in calves (Chazel, 2010). In addition, *Mycoplama bovigenitalium*, *Mycoplama californicum* and *Mycoplama alkalescens* had been isolated from mastitis infection with *Mycoplasma bovis* (Mackie, 2000).

Several molecular typing methods have been optimized with variable degree of reproducibility, discrimination ability and cost effectively (Register et al., 2020). MLST, WGS, single nucleotide polymorphism has been widely used and optimized (Register et al., 2020). A multiple MLST scheme have been published, either targeting 8 or 11 genes in *Mycoplasma bovis* (Bell-Rogers et al., 2018). However, “PubMLST” has been targeted with 8 target genes with sufficient discriminating power and enough informative evidence to understand the diversity of the organism (Bell-Rogers et al., 2018; Register et al., 2020). In Belgium, five main clusters with one outlier have been shown with WGS approaching in molecular epidemiology (Bokma et al., 2020; Yair et al., 2020). In a similar approach, highly homogenous genome is found in Denmark ST 17 and ST-94 *adh*-1 were identified as predominant sequence types in the country (Bokma et al., 2020).

Pathogenesis and occurrence of clinical disease

The incubation period of *Mycoplasma bovis* infection depends upon several factors such as the infective dose, immune status, age, coinfectants, herd management, stress factors of the animal and the tissue of infected, which is few days (2-14 days) for mastitis and for pneumonia (7 days) (Calcutt, 2018). *Mycoplasma bovis* causes wide range of pathological conditions such as caseonecrotic pneumonia, mastitis, arthritis, keratoconjunctivitis, suppurative otitis media, meningitis, decubital abscesses, endocarditis and reproductive disorders in cattle (Houlihan et

al., 2007; Maeda et al., 2003). Differential diagnosis is a challenging task since coinfection with multiple respiratory pathogens were found common in bovine host (Dudek, 2020).

The pathogenicity of *Mycoplasma bovis* infection is multifactorial and coinfection with other bacterial, viral and fungal causes are found common which leads into the severe pathological lesion with poor prognosis (Grissett, 2015). As an example, *Pasteurella multocida*, *Mannheimia haemolytica*, *Histophilus somni*, bovine respiratory syncytial virus (BRSV), bovine herpes virus 1 (BHV-1), bovine viral diarrhea virus (BVDV) and parainfuenza virus type 3 (PIV-3) had been identified as main source of coinfection with *Mycoplasma bovis* in cattle (Caswell, 2007; Dudek, 2020; Gagea et al., 2006; Justice-Allen et al., 2011; Parker, 2018; Schibrowski et al., 2018).

Stress factors may play a vital role on pathogenesis, mycoplasmosis is common in herd with inadequate feeding, long term transportation or low environmental temperature (Aebi et al., 2015). Feeding of infected colostrum is considered as the main source of *Mycoplasma bovis* infection in calves (Dudek, 2020). This has been partly proven by isolation, identification phylogenetically closed or similar *Mycoplasma bovis* from both calves and cows (Dudek, 2020). Secondly, the importance of aerosol transmissions, direct or indirect contact with infected individual cannot be excluded. Furthermore, nose to nose contact, fomite route of infection and contaminated hand of farm personals need to be minimized at farms field (Haapala et al., 2021). *Mycoplasma bovis* spreads through hematogenous route in the body, which is the reason for simultaneous infection of arthritis and pneumonia in cattle (Gagea et al., 2006). However, the primary site of the infection is the lung and *Mycoplasma bovis* had disseminated into joint though hematogenous route (Gagea et al., 2006).

M. bovis is adapted to respiratory mucosae in cattle and the organism survives for prolonged period without causing any clinical disease (Maunsell and Donovan, 2009). The nonspecific clinical signs are found in the infected animals including depression, high fever, shaking of head and swelling of joints (Maunsell, 2009; Maunsell, 2011). In addition, respiratory sings are commonly found in both claves and adults such as nasal discharges, persistent cough, and dyspnea in affected animals (Maunsell, 2011). Swelling of joints in legs and lameness also been reported among affected animal population (Maunsell, 2011). Insufficient feeding of colostrum is considered as a main risk factor on susceptibility to mycoplasmosis resulting of poor immune response against common pathogens at the early stage of life (Fox et al., 2008). In addition, an outbreak of mastitis had been reported as a result of artificial insemination of contaminated semen (Dudek, 2020). Presence of multiple caseo-necrotic foci in lung is found common in infected herds (Hermeyer et al., 2012). Peribronchiolar lymphoid hyperplasia, acute/subacute suppurative bronchiolitis, thicken alveolar septae, atelectasis and foci of cougulative necrosis were also observed as pathological changes in the infected calves (Dudek, 2020; Hermeyer et al., 2012). Caseonecrotic broncho pneumonia, combination of caseonecrotic and fibrinosuppurative bronchopneumonia were also found in *Mycoplasma bovis* infection (Gagea et al., 2006). Necrotizing fibrinosuppurative arthritis or tenosynovitis were shown in joint infection by histological examination (Gagea et al., 2006). In otitis media, extensive fibrino- suppurative exudate in tympanic bullae were observed in calves (Ayling et al., 2005; Dudek, 2020). Furthermore, meningitis, suppurative otitis media/interna were diagnosed in the postmortem

examination of infected calves (Ayling et al., 2005; Dudek, 2020). In addition, necrosis in brain tissues and fibrinous heart lesion were found common in dead calves (Maeda et al., 2003). In some cases, lesions were observed in cerebellar, liver and kidney (Maeda et al., 2003).

The whole process of pathogenesis is not well understood in *Mycoplasma bovis* infection in cattle, primary cell adherence occurred by surface membrane protein of *Mycoplasma bovis* due to the lack of cell wall in the organism (Dudek, 2020). *Mycoplasma bovis* excretes phospholipase, hydrogen peroxide, superoxide radicals which damages host cells found in mucous membrane. In addition, *Mycoplasma bovis* is shown heat resistant with the ability of forming a biofilm around the cell (Dudek, 2021).

The role of maternal antibodies against *Mycoplasma bovis* in unknown (Petersen et al., 2018). Alveolar macrophages play a vital role in clearance of *Mycoplasma bovis* in respiratory tract infection. Detrimental inflammatory response activation by the organism also been shown by excessive TNF- alpha (Gondaira, 2018; Vähänikkilä et al., 2019). Excessive accumulation of neutrophils is facilitated by the activation of macrophages in mycoplasmosis in cattle (Dudek and Bednarek, 2017; Vähänikkilä et al., 2019). Therefore, amount of neutrophils correlate the degree of *Mycoplasma bovis* infection (Dudek and Bednarek, 2017). The adaptive cellular mechanism against mastitis is poorly understood *Mycoplasma bovis* and previously infected animal were shown either mild or subclinical mastitis in cattle (Dudek, 2020; Punyapornwithaya et al., 2010). In addition, chronic mastitis was observed in immunosuppressed or immunocompromised animals (Dudek, 2020; Punyapornwithaya et al., 2010). It has been shown that Mycoplasma secrete several immunomodulatory agents to induce bovine immune responses (Gondaira, 2018). The survival mechanism against host defense mechanism has not been thoroughly understood in *Mycoplasma bovis* (Dudek, 2020). Under experimental condition, calves had shown a high level of IgG1 and low level of IgG2 in a systemic infection while high amount of IgG and IgA for a local infection (Dudek, 2020). In pathogenesis, *Mycoplasma bovis* is engulfed by peripheral cells such as peripheral blood mononuclear cells (PBMCs), erythrocytes and turbinate cells (Gondaira, 2018). However, unique cellular changes and cytopathic effects have not been observed on *Mycoplams bovis* infection by these categories of cells, however, *Mycoplasma bovis* has capability to be survived in bovine macrophages (Gondaira, 2018).

In clinical mastitis, mammary epithelial cells may play a vital role in innate immune response to recognize pathogen associated molecular pattern by pattern recognition receptors in mammary epithelial cells (Gondaira, 2018). After the recognition, cells secrete chemokines, cytokines, and antimicrobial peptides, resulting in leukocyte recruitment and activation of further defense mechanism (Gondaira, 2018). Importantly, Gondaira el al,2018 suggested that innate cells responses in *Mycoplasma bovis* was different in *Mycoplasma bovis* which induced suppression of host immune response by bovine cells (Gondaira, 2018).

Diagnosis of the disease

Bacteremia ended up with a lung infection of *Mycoplasma bovis* infection, the organism can be recovered 9 days of post inoculation either in intranasal or intra tracheal inoculation in challenging experiments (Horwood, 2014). Bacteremia may lead to have septic arthritis, as well as other systemic infection in cattle (Horwood, 2014). The gross pathological lesion of caseonecrotic bronchopneumonia has been suggested of *Mycoplasm bovis* infection in cattle (Horwood, 2014). The cranioventral bronchopneumonia with multifocal nodules of

caseous necrosis is considered as the typical lesion for bovine *Mycoplasma bovis* infection (Horwood, 2014). Meanwhile, *Mycoplasma bovis* has been isolated and identified in caseonecrotic bronchopneumonia, caseonecrotic and fibrinosuppurative bronchopneumonia or fibrinosuppurative bronchopneumonia in cattle (Gagea et al., 2006). Although *Haemophilus somni* and *Manheninimia hemolytica* had been observed with non-raised, red or pink, irregular shaped non friable foci in lung tissue, Mycoplasma lesions were circular, raised white yellow nodules containing dry friable foci of caseous material in post mortem examination (Horwood, 2014). Mycoplasma is a cell surface associated organism, optimum sampling with vigorous handling of swabs is required to improve the sensitivity of the test (Gagea et al., 2006). Wooden shaft cottons are not recommended since which inhibit the growth of Mycoplasma while Mycoplasma selective transport media were given better recovery in the laboratory setting (Gagea et al., 2006). The swabs can be stored at 4℃ for couple of days before plating or putting into the specific medium. However, lung tissue cannot be kept for few hours since inhibitory factors are released from the tissue into the specific medium (Maunsell, 2011).

Diagnostic tests

Diagnosis of mycoplasmosis is based on isolation and identification of *M. bovis* organism, detection of genetic material & protein and serological testing for detection of *M. bovis* specific antibodies in the circulatory system. *M. bovis* infection is often underestimated due to the requirement special laboratory conditions and trained human resources on identification of the organism (Parker, 2018).

The isolation and identification of the organism is the gold standard on diagnosis of *Mycoplasma bovis* in cattle (Justice-Allen et al., 2011; Maunsell, 2011). However, long time duration under the general laboratory conditions is the limitation of the conventional tests (Dudek, 2020). Mycoplasma is a fastidious and slow growing bacteria and number of commercial specific media are found in the market such as Hayflikc's, Eaton's and modified PPLO (Dudek, 2020). Overgrowing of other fast-growing organism is the compromising factor in the isolation of Mycoplasma process (Maeda et al., 2003). Therefore, inhibitors such as thallium acetate or antimicrobials are being used to suppress other bacterial growth. The incubation period is 7-10 days (about 1 and a half weeks) at 37℃ with 5% CO_2 (Dudek and Bednarek, 2018; Dudek, 2020; Parker, 2018). Fried eggs shaped micro colonies appeared under the light or phase contrast microscopy. However, Acholeplasma also has similar colony morphology in the Mycoplasma specific medium (Dudek, 2020). The sensitivity to digitonin may be used to differentiate Mycoplasma from Acholeplasma in diagnostic laboratory (Dudek, 2020). The minimum limit of detection of *Mycoplasma bovis* in milk was 100 CFU/ml by the conventional methods (Haapala et al., 2021). Therefore, Centrifuged milk samples were shown high sensitivity by the traditional isolation methodology (Dudek, 2020). Lack of sophisticated instruments and low cost are the advantages of conventional methods in diagnosis of Mycoplasmosis in cattle (Behera et al., 2018). In addition, pooled samples were shown high sensitivity than individual samples due to high concentration of organism (Dudek, 2020). The collected sample need to be stored in a freezer when the sample are not processed within 2 days period, 1 or 2 log reductions were reported in the freezing (Parker, 2018). The recovery rate of Mycoplasma had reduced in samples stored in a freezer or fridge, while continuous freezing and thawing had been further reduced in *Mycoplasma bovis* (Parker, 2018). In addition, multiple sampling for couple

of days may improve the sensitivity of isolation of Mycoplasma in milk and other clinical samples (Cai et al., 2019; Dudek, 2020; Parker, 2018). Since intermitted shedding of the organism is reported in subclinical and chronic mycoplasma infections in cattle, multiple sampling may prevent detection of false negatives in diagnostic samples in laboratory (Parker, 2018).

Molecular techniques such as conventional and quantitative PCR are used common with high analytical sensitivity and specificity. The minimum detection limit was 4 x 10^2 CFU/ml in broth cultures and milk (Parker, 2018). Non-viable organism or DNA also he detected as positive sample by molecular methods (Behera et al., 2018; Justice-Allen et al., 2011; Parker, 2018). 16S rDNA and 23S rDNA targets also been used to identity and to differentiate Mycoplasma from other microorganisms (Parker, 2018). In addition, qPCR, LAMP, MOLDI TOF MS are the common techniques used in bacteriological laboratories worldwide (Bokma et al., 2019; Dudek, 2020; Parker, 2018). Furthermore, immunohistochemistry, in situ hybridization and histopathology are being used as common techniques in cattle (Dudek, 2020). The serological diagnosis is a cost-effective method the ELISA test has been optimized for field sample of both serum and milk (Behera et al., 2018; Parker, 2018). A less percentage of false positive animals were found in ELISA test in cattle (Nielsen et al., 2015).

Treatment and antimicrobial resistance

Mycoplasma bovis is intrinsically resistance to antimicrobials such as Fosfomycin, glycopeptides, β-lactams, Sulfonamide, first- generation quinolones, trimethoprim, polymyxins, and rifampicin (Bouchardon, 2018; Sulyok et al., 2017). Therefore, no clinical outcome can be expected treatment with those antimicrobials, clinicians need to be awarded on such classes of antimicrobials. Furthermore, macrolides and tetracyclines are the widely used antimicrobials against *Mycoplasma bovis* infection in cattle (Bouchardon, 2018; Gautier-Bouchardon et al., 2014). In addition, other antimicrobials such as lincosamide, pleuromutilin, fluoroquinolone, phenicol, spectinomycin, clindamycin, tetracycline, and azithromycin and aminoglycoside were also shown positive clinical outcome on *Mycoplasma bovis* infections in cattle (Bouchardon, 2018). Macrolides penetrate the host cells through cell membrane resulting effectivity against intracellular infections of *Mycoplasma bovis* (Dudek, 2020).

Antimicrobial resistance is an emerging problem in livestock, a threating antimicrobial resistance has been reported in *Mycoplasma bovis* by different studies (Bouchardon, 2018; Sulyok et al., 2017). Importantly, *Mycoplasma bovis* is a strong biofilm producer, hence, emergence of antimicrobial resistance is facilitated such artificial environment (Calcutt, 2018). However, antimicrobial resistance on macrolids and tetracyclines has been increasing reported in *Mycoplasma bovis* in several countries in Europe (Bouchardon, 2018). Furthermore, overall antimicrobial susceptibility of *Mycoplasma bovis* against commonly used antimicrobial in bovine practice had been reduced gradually last 30 years (Gautier-Bouchardon et al., 2014). Importantly, reduction on antimicrobial susceptibility had been observed on tylosin, tilmicosin, tulathromycin, spectinomycin, enrofloxacin, danofloxacin, marbofloxacin and oxytetracycline (Cai et al., 2019; Gautier- Bouchardon et al., 2014). In Europe, minimum inhibitory concentration of commercial antimicrobials on *Mycoplasma bovis* were shown high than rest of the regions in the world (Lysnyansky and Ayling, 2016). According to the Lysnyansky and Ayling, antimicrobial resistance for tetracyclines, macrolides, lincosamides, aminoglycosides,

chloramphenicol, and fluoroquinolones were increasing in trends in *Mycoplasma bovis* (Lysnyansky and Ayling, 2016). In addition, similar reduction of antimicrobial susceptibility was observed in North America (Cai et al., 2019). Resistance to spectinomycin, clindamycin, tetracycline and azithromycin also been reported in Canada (Bouchardon, 2018). Antimicrobial resistance is a major problem in feed lot cattle, resistance was shown high to tulathromycin, gamithromycin, tylosin and enrofloxacin (Jelinski et al., 2020). Furthermore, genetic mutation has been identified as the most common mechanism of resistance in *Mycoplasma bovis* (Jelinski et al., 2020).

Prevention of *Mycoplasma bovis* infection

Three main strategies are found on prevention of *Mycoplasma bovis* infection in cattle such as establishing sanitary control measures, antimicrobial therapy, and vaccination (Dudek, 2020; Haapala et al., 2021). Imposing trade restriction is a challenging task in current livestock business with turbinating political environments. In addition, culling of infected and contact animals is a burning issue in livestock farming with lack of compensation by the government. However, over 2000 of infected farms were culled in New Zealand due to the infection caused by *Mycoplasma bovis* and most of animals were not shown any clinical sign or gross pathology (Browning, 2019). Lack of hygienic practices, lack of surveillance mechanism and no early detection laboratory facilities, infected semen on artificial insemination, and low milk production were the main risk factors on *Mycoplasma bovis* infection in cattle (Haapala et al., 2021). Therefore, hygienic measures at milking, calf pens, feeding buckets, and teats has significantly reduced the *Mycoplasma bovis* infection (Haapala et al., 2021). Minimizing nose to nose contact of calves had reduced incidence of *Mycoplasma bovis* infection (Haapala et al., 2021). Separation of infected or test positive animals is practical methods to prevent further within a specific herd of cattle (Dudek, 2021). Furthermore, milking animals need to be tested continuously to identify the positive animal (Dudek, 2021). As a clarification, 1 X 10^6 cfu/ml of organism were found in infected milk from mastitis, therefore, screening on bulk milk tank is a sound method to control *Mycoplasm bovis* infection in cattle (Dudek, 2021). Pasteurization of colostrum and raw milk is also recommended to destroy *Mycoplasma bovis* in milk, discarding infected colostrum and raw milk is also suggested (Gille et al., 2020). Screening of milk sample from sub clinical mastitis for *Mycoplama bovis* has been recommended to control he infection in dairy herds. However, *Mycoplasma bovis* has been reported from animals with low somatic counts or clinical sings (Kauf et al., 2007). Continuous monitoring of bulk milk tank for *Mycoplasma bovis* in highly recommended (Nicholas et al., 2016). Screening on newly introduced animals is an important strategy to control incidence of mycoplasmosis in a dairy farm (Nicholas et al., 2016).

Vaccination

Lack of a successful vaccine against *Mycoplasma bovis* is still an overwhelming issue in cattle husbandry (Dudek, 2021). In addition, effective commercial vaccine is found to control *Mycoplasm bovis* infection (Tardy et al., 2020). Several killed bacterin have been registered in USA such as “Myco- Bac-B”, Pulmo-Guard™MpB, Mycomune® “Mycoplasma Bovis bacterin” (texasvetlab.com) with variable success. All these vaccines are focused on respiratory tract infections and no vaccine targeting reduction of clinical mastitis caused by *Mycoplasma bovis* in cattle (Dudek, 2020).

Although inactivated vaccines are popular to control *Mycoplasma bovis* infection in bovine host, cost of production was shown high than production of live *Mycoplasma bovis* vaccine (Nicholas et al., 2002). However, killed vaccine has proven on reducing clinical disease, minimum colonization in upper respiratory tract, reduced lung lesions and high antibody titters of post vaccination in cattle (Perez-Casal et al., 2017). In addition, satisfactory mucosal immune response were also observed after the killed vaccination (Perez-Casal et al., 2017). However, nasal shedding of *Mycoplasma bovis* was shown continue with homologous and heterologous *Mycoplasma bovis* vaccination (Dudek, 2021). Adjuvant is the limiting factor in new vaccine formulation in livestock, vaccine which formulated with commercial adjuvants and polyinosinic:polycytidylic acid (poly I:C) , cationic innate defense regulator (IDR) peptide 1002 were shown high efficacy against *Mycoplasma bovis* infection in cattle (Prysliak et al., 2017).

The live vaccine is another kind of vaccine to be used on prevention of *Mycoplasma bovis* infection in calves and cows. The efficacious protection has been observed with live attenuated vaccine used in China, developed early immune response early as 7 days and persisted till 30 days post vaccination (Han et al., 2015; Zhang et al., 2014). The live vaccine was shown positive effect on IgG, IFN Beta and TNF alpha while no different in IgA and TNF alpha under experimental condition in calves (Zhang et al., 2014). The vaccine was not shown success on shedding of the organism though mucous membrane (Dudek, 2021). In addition, subunit vaccine, recombinant based vaccine also been investigating, inducing cell mediated immune responses is the remain challenge although high antibody mediated responses were observed (Zhang et al., 2014). Furthermore, more research is needed to identify recombinant protein that confer protection against *Mycoplasma bovis.* Therefore, combination of vaccination and inducing host immune response is considered as effective methodology for controlling the *Mycoplasma bovis* infection in cattle (Tardy et al., 2020). Sero-surveillance, culling of infected and contact animals were done to eradicate *Mycoplasma bovis* infection in cattle, similar practiced was done in recent past in New Zealand (Dudek, 2020). In USA, autovaccinations are practiced produced from field isolates of the same farm premises. Three auto vaccines have been studied by Nicholas, 2019 and concluded low mortality and high body weight in calves of vaccinated although clinical incidence were not reduced (Nicholas, 2016).The significant level of protection against *Mycoplasma bovis* was shown with saphonin inactivated *Mycoplasm bovis* vaccine in cattle with low incidence of pneumonia, low number of lung lesions and internal organs (Nicholas et al., 2002). In addition, naval vaccine containing saphonin and lysozyme dimers has effectively stimulated cell mediated immune responses in calves (Dudek and Bednarek, 2017). Satisfactory humoral responses also been shown with experimental recombinant vaccine of *Mycoplasma bovis* (Prysliak et al., 2017). In addition, differentiation of vaccinated antibodies against antibodies of post infection is also possible in *Mycoplasma bovis* infection (Han et al., 2015). Therefore, a dream of effective vaccine against *Mycoplasma bovis* infection is not such far in cattle industry.

The culling of infected herd is a proven alternative to eradicate a contagious disease with the limitation on diagnostic tests. As an example, New Zealand became the last cattle rearing country who were infected with *Mycoplasma bovis* (Browning, 2019; Dudek, 2021). Conversely, the culling strategies are challenging task in developing countries due to the scarcity for funding on compensation.

***Mycoplasma bovis* in Sri Lanka**

No detail studies on *Mycoplasma bovis* had been carried out in the country and scarcity of the literature is the throwback to know the status of *Mycolasma bovis* infection in cattle. Therefore, more studies are required to know the exact prevalence of *Mycoplam bovis* in cattle, Sri Lanka. Importantly, diagnostic facilities on *Mycoplsam bovis* has not been established either in peripheral or central veterinary laboratories. Establishment and maintenance of diagnostic facilities are required together with serological screening of suspected herds. The screening programme needs to be targeted healthy animals, live animals with respiratory tract infections, milk collecting centers and slaughterhouse to control the infection in cattle.

CONCLUSION

Mycoplasma bovis is an intracellular pathogen in cattle, Sri Lanka due to the recent importation of animal from outside**.** The disease prevalence is not well known in the country and no extensive studies or surveillance programme has been established against *Mycoplasma bovis*. However, it is a difficult bacterium to control or eradicate from cattle and silent spreading is the key feature of this infection. The regular monitoring, improving biosecurity measures, quarantine regulations, hygienic practices in farming and vaccination are the most proven strategies of controlling disease mycoplasmosis in cattle.

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Authors contribution

Whole authors were contributed on writing the paper at different percentages as MARP (50%), GISP (25%) and NL (25%). The paper was edited by corresponding author, MARP.

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