Veterinary Integrative Sciences 2023; 21(2): 567 - 576 DOI; 10.12982/VIS.2023.040

Vet Integr Sci

Veterinary Integrative Sciences

ISSN; 2629-9968 (online)

Website; www.vet.cmu.ac.th/cmvj



Review article

Biofilm: a platform for the evaluation of antimicrobial treatments and prevention against *Mycoplasma bovis* infection

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Abstract

Mycoplasma bovis is an important pathogen in large ruminants that causes various clinical pathologies with great economic impact. Though mycoplasmas were thought to have gone reductive evolution that led to the loss of genetic materials leading to the marked absence of cell wall and canonical virulence factors, adaptation made by some *M. bovis* isolates include production of biofilms to survive, evade host immune response, persists in abiotic environment and may mount related responses that render chemotherapy ineffective. In this review, topics on biofilms commonly produced by *M. bovis* isolates were discussed to view its pathogenicity. Reports that describe the involvement of variable surface antigens in biofilm production of *M. bovis* are also presented. This information invites interest on biofilms anchored on studies where it can be developed to be utilized as platforms for the evaluation of antimicrobial treatments and for the identification of a preventive and control strategy against *Mycoplasma bovis* infection.

Keywords: Biofilms, Mycoplasma bovis, Pathogenicity, Ruminants, Virulence

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Article history; *received manuscript: 3 June 2022,*
revised manuscript: 29 December 2022,
accepted manuscript: 10 January 2022,
published online: 25 April 2023
Academic editor; *Korakot Nganvongpanit*

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INTRODUCTION

In vivo, microorganisms do not live or thrive in pure cultures of dispersed or planktonic state but *in vitro,* microorganisms live in polymicrobial aggregates in different forms such as films, mats, flocs, or sludge commonly known as "biofilms." Biofilms allow the microorganism to thrive in aggregative stages and manifest a different lifestyle compared to their planktonic counterparts.

Commonly, the main composition of biofilms includes microorganisms in less than 10% of the dry mass and over 90% comprise the matrix. Biofilm formers produce a matrix which is a conglomeration of different types of biopolymers known as extracellular polymeric substances (EPSs). EPSs reportedly function to support the viability of the biofilm to initiate infection in an animal they can come in contact with (Flemming and Wingender, 2010; Yiwen et al., 2021).

Mycoplasma bovis (*M. bovis*) is a pathogen of the mycoplasma group that infects large ruminants and its pathogenicity is reportedly linked to its prolific ability to produce biofilms (McAuliffe et al., 2006; Yiwen et al., 2021). *M. bovis* is an emerging important pathogen of feedlot cattle and dairy calves as it causes pneumonia, arthritis and tenosynovitis (Caswell and Archambault, 2008; Caswell et al., 2010). Furthermore, other infections such as mastitis, pneumonia, septicemia and eventual death of infected dairy cattle are reportedly related to *M. bovis* (Justice-Allen et al., 2009) and it is the inexhaustible biofilm former capability of the pathogen that favors its pathogenicity. Studies that extensively explore the connection between the pathogenicity and virulence of *M. bovis* in connection with its prolific biofilm-forming ability is often hampered by the difficulty in isolating the bacteria directly from milk and in standard milk cultures of infected cattle (Gonzalez et al., 1992; Wilson et al., 2007; Justice-Allen et al., 2009).

Effort to bring information that defines the participation of biofilm in the pathogenicity of the *M. bovis* is necessary to understand intractable *M. bovis* infections in large ruminants. Information generated from this review on biofilms intends to bring light on some points that underscore the potential use of biofilm in the assessment of treatments against of *M. bovis* and define a biofilm-based strategy for the development of an effective control and preventive measure against the biofilm producer pathogen.

M. BOVIS AND BIOFILM FORMATION

M. bovis like other mycoplasmas are reportedly characterized by their small genome size. This bacterial group has been speculated to have undergone reductive evolution that contributed to the significant loss of genes commonly found in more complex bacteria. The feature of this genetic loss is reportedly linked to the marked absence of cell walls and the ability of the pathogen to self-produce 20 amino acids that are responsible for enzymes that supports microbial processes during the citric acid cycle and other biosynthesis of genes (Razin et al., 1998; McAuliffe et al., 2006).

Some mycoplasma species produce biofilms and these probably enable them to survive adverse environmental conditions (Table 1). Mycoplasmas can reportedly survive and persists even with the considerable reduction of their

genome and evolve to acquire necessary products from their host. *M. bovis* as a biofilm former possesses a matrix which contains different types of biopolymers known as EPS which are made up of proteins, polysaccharides, DNA, RNA, lipids, and phospholipids (Flemming and Wingender, 2010) which altogether function to support the viability of the biofilm (Table 2). With this, the ability of mycoplasmas to produce biofilms makes them as ideal organisms that can be used in determining essential functioning of living microbial cells.

Table 1 Species of mycoplasma that form biofilms (McAuliffe et al., 2006)

Mycoplasma species	Biofilm formation in qualitative crystal violet assay	Viable Cell Counts	
		Biofilm State	Planktonic State
M. agalactiae	+++	6.2×10^6	1.5×10^8
M. bovis	+++	1.3×10^7	1.6×10^8
M. cotewii	+++	4.6×10^7	8.4×10^8
M. capricolum	+	2.6×10^3	2.5×10^9
M. ovipneumoniae	+	1.8×10^4	2.9×10^8
M. mycoides subsp. *mycoides* capri	++	5.5×10^5	1.8×10^9
M. mycoides subsp. *mycoides* LC	++	6.7×10^5	2.4×10^9
M. mycoides subsp. *mycoides* SC	-	2.1×10^1	9.5×10^9
M. putrefaciens	+++	8.7×10^6	5.8×10^9
M. yeatsii	+++	3.8×10^7	1.4×10^9
Mycoplasma sp. bovine group 7	+	2.8×10^3	3.4×10^9

Legend: -, no adherence; +, low-level adherence with only sparse staining and no line of biofilm formation at the air/liquid interface; ++, intermediate level of adherence with staining below the air/liquid interface but no defined line at the air/liquid interface; +++, high level of adherence with a clearly defined line of staining at the air/ liquid interface and staining across the lower half of the coverslip.

REGULATORY MECHANISMS FOR THE FORMATION AND DISPERSION OF BIOFILM IN PATHOGENIC *M. BOVIS*

The significant loss of genome may lack canonical virulence factors like toxins and 2-component regulatory systems, *M. bovis* isolates. However, strains of biofilm formers like *M. bovis* reportedly encodes six unique proteins that may mediate the pathogen's virulence thus facilitating evasion of the host immune responses (Perez-Casal, 2020). Biofilm formation in *M. bovis* is often associated with the expression of certain variable surface proteins (VSPs) that protects the pathogen from host defenses and chemotherapy (McAuliffe et al., 2006; Maunsell and Chase, 2019; Perez-Casal, 2020; Yiwen et al., 2021). The VSPs like VspB and VspO have been found to be upregulated in *M. bovis* biofilm isolates but have never been correlated with increased pathogenicity (Burki et al., 2015; Yiwen et al., 2021). The expressions of six unique proteins by *M. bovis* in biofilms, however, are not observed in planktonic forms of *M. bovis* (Chen et al., 2018). With this, experts argue that biofilm formation by *M. bovis* is only be possible in a living animal host (Maunsell and Chase, 2019) which reportedly needs further validation (Chen et al., 2018).

Biofilm formation is a vital feature of chronic disease-causing microorganisms (Maunsell and Chase, 2019) like *M. bovis*. Others report that most strains of *M. bovis* are prolific biofilm formers (McAuliffe et al., 2006) (Table 2) and this ability is reportedly established *in vitro* (Figure 1) (McAuliffe et al., 2006). Others describe that *M. bovis* is infectious in the biofilm state and may not need a large number of organisms to establish infection (Nicholas, 2010). This may cater to the colonization of the pathogen in the upper respiratory tract (Maunsell and Chase, 2019) that leads to chronic pneumonia which often progresses to incurable states in spite of the prolonged use of multiple antimicrobial classes (Caswell et al., 2010).

Table 2 Composition of extracellular polymeric substances in biofilms (Flemming and Wingender, 2010)

Function	Relevance for biofilms	Extracellular Polymeric Surfaces components involved
Adhesion	Allows the initial steps in colonization of abiotic and biotic surfaces by planktonic cells; and Long term attachment of whole biofilm to surfaces	Polysaccharides, proteins, DNA and amphiphilic molecules
Aggregation of bacterial cells	Enables bridging between cells; Temporary immobilization of bacterial populations; and The development of high cell densities and cell-cell recognition	Polysaccharides, proteins and DNA
Cohesion of biofilms	Forms a hydrated polymer network (the biofilm matrix); Mediating the mechanical stability biofilms (often in conjunction with multivalent cations) and through EPS structure (capsule, slime or sheath); and Determining biofilm architecture, as well as allowing cell-cell communication	Neutral and charged polysaccharides, proteins (such as emyloids and lectins) and DNA
Retention of Water	Maintains a highly hydrated microenvironment around biofilm organisms leading to their tolerance of desiccation in water-deficient environments	Hydrophilic polysaccharides and, possibly proteins
Protective Barriers	Confers resistance to non-specific and specific host defenses during infection, and confers tolerance to various antimicrobial agents (for example, disinfectants and antibiotics) as well as protecting cyanobacterial nitrogenase from the harmful effects of oxygen and protecting against some grazing protozoa	Polysaccharides and proteins
Sorption of organic compounds	Allows the accumulation of nutrients from environmental and the sorption of xenobiotics (contributing to environmental detoxification)	Charged or hydrophobic polysaccharides and proteins
Sorption of inorganic ions	Promotes polysaccharide gel formation, ion exchange, mineral formation and the accumulation of toxic metal ions (contributing to environmental detoxification)	Charged or hydrophobic polysaccharides and proteins, including inorganic substituents such as phosphate and sulphate
Enzymatic activity	Enables the digestion of exogenous macromolecules from nutrient acquisition and the degradation of structural EPS, allowing the release of cells from biofilms	Proteins
Nutrient source	Provides a source of carbon-, nitrogen- and phosphorus-containing compounds for utilization by the biofilm community	Potentially all EPS components
Exchange of genetic information	Facilitates horizontal gene transfer between biofilm cells	DNA
Electron donor or acceptor	Permits redox activity in the biofilm matrix	Proteins (for example, those forming pili and nanowires), and possibly, humic substances
Export of cell components	Releases cellular material as a result of metabolic turnover	Membrane vesicles containing nucleic acids, enzymes, lipopolysaccharides and phospholipids
Sink for excess energy	Stores excess carbon under unbalanced carbon to nitrogen ratios	Polysaccharides
Binding of enzymes	Results in the accumulation, retention and stabilization of enzymes through their interaction with polysaccharides	Polysaccharides and enzymes

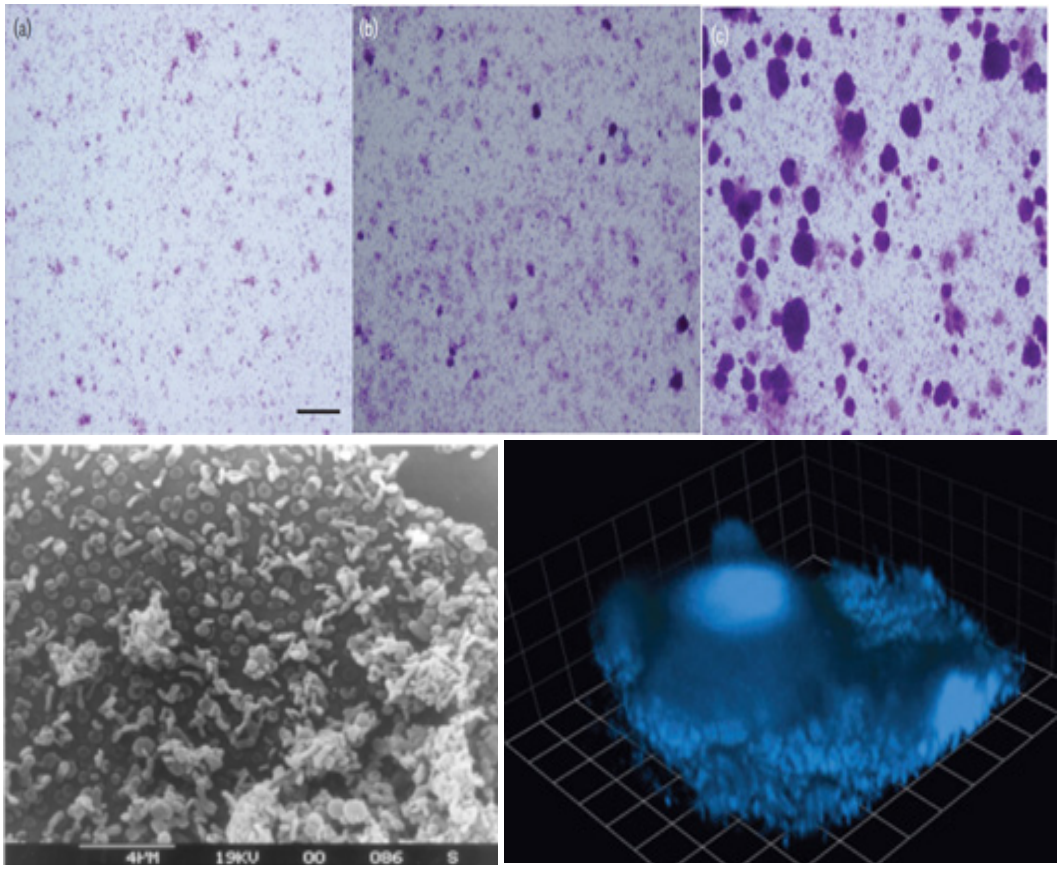

Figure 1 Biofilm formation of *M. bovis* reported in vitro at (a) 20 h, (b) 36 h, and (c) 48 h post-inoculation, Bar = 100 µm; (d) Biofilm analyzed under scanning electron microscope; and (e) Confocal imaging (in three-dimensional) which shows the thick layer of polysaccharide that covers stacks of cells. Bar = 20 µM (McAuliffe et al., 2006).

Adhesins in *M. bovis* is reported as a potentiator of biofilm formation making it as one of the virulence factors of *M. bovis*. Mycoplasma adhesins may initially play a great role in biofilm formation to facilitate adherence or adhesion of the pathogen as described (Rottem, 2003) to a host tissue or organ and initiate the vital steps of bacterial colonization to start an infection. Adhesion of *M. bovis* to its host cells is reportedly mediated by several proteins including VSPs, lipoproteins on the bacterial surface and, some unrelated proteins like pMB67 and p26 (Sachse et al., 1996; Thomas et al., 2003a; Thomas et al., 2003b; Caswell et al., 2010). As adhesion of *M. bovis* is reportedly mediated by various VSPs (Burki et al., 2015; Yiwen et al., 2021) and that biofilm-producing *M. bovis* isolates are correlated with different VSP species. Certain VSPs patterns in *M. bovis* isolates result in variable ability to produce biofilms and different adhesion capabilities (McAuliffe et al., 2006). Expression of VspB and VspO has been observed in biofilm-producing *M. bovis* isolates although it has not been associated or correlated with increased pathogenicity of these isolates (Burki et al., 2015). Other proteins for adhesion of *M. bovis* isolates are P26, Vsps, and VpmAX which actively participate in its pathogenesis (Zou et al., 2013; Burki et al., 2015). Studies that explore these adhesion molecules were strategies that would lead to knocking out the adhesion capability would reduce the pathogenicity of mycoplasma. This is an interesting area that needs investigation.

CONDITIONS THAT PROMOTE BIOFILM PRODUCTION IN PATHOGENIC *M. BOVIS*

Mycoplasmas are observed to be well adapted to survival on mucosal surfaces and are dependent on the hosts for the nutrients for survival and persistence (Caswell and Archambault, 2008). As *M. bovis* is a prolific biofilm former, these can adhere to mucosal surfaces and abiotic surfaces like sand, metal and beddings to effectively cause infection or as a possible source of infection. The viability of *Mycoplasma* spp. in recycled bedding sand relative to the pathogen's concentration is reportedly observed for eight months are associated with temperature, moisture, and growth of other bacteria (Justice-Allen et al., 2009). *Mycoplasma* spp. in previous negatively-culture recycled bedding reportedly re-appeared as abundant biofilms with the return of optimal growth conditions hastened by organic matter compared to clean sand and bedding incorporated with sand without organic matter as substrates (Godden et al., 2008). Successful cultivation of *M. bovis* obtained from a biofilm in the air has also been reported (Jasper et al., 1974) but has not been duplicated after monthly collection of air samples from veal raising farms (Soenhnlen et al., 2011). The ability of an air-borne *M. bovis* biofilm to form is reportedly linked as a function of adhesins that mediate adherence to a substrate which further corroborated with the result of Soenhnlen et al. (2011) who used aerosolized *M. bovis* (ATCC 25523) to effect formation biofilm and its collection from air. Growth factor-deficient environment is a trigger for mycoplasmas and other Gram-negative bacteria for the formation of biofilm as reaction for survival (Justice-Allen et al., 2009) and as a source of infection. Biofilm formation of *M. bovis* protects the pathogen from host defenses and chemotherapy (McAuliffe et al., 2006; Maunsell and Chase, 2019; Perez-Casal, 2020). *M. bovis* isolates that are capable of biofilm formation are more resistant to desiccation (state of extreme dryness) and extreme temperatures. The study of Justice-Allen et al. (2009) proved that *M. bovis* may persist in recycled sand bedding during dry summer months which demonstrates the additional protection accorded by high temperature to biofilm formation. McAuliffe et al. (2006) further reported that *M. bovis* isolates that form biofilms were more resistant to heat at 50ºC than their planktonic counterparts. Conversely, Justice-Allen et al. (2009) reported that *Mycoplasma* spp. growth is inhibited 24 h post-exposure to 60ºC and 48 h post-exposure to 36ºC. However, the addition of deionized tap water 48 h and 72 h post-exposure to 36ºC demonstrated a 7.2 % and 37.5 % survival rate, respectively. The results of Justice-Allen et al. (2009) further corroborated with the study of Butler et al. (2000) who described the loss of viabilityof *M. bovis* biofilms when subjected to 65°C for 2 min or 1 min at 70°C. Furthermore, Pfutzner (1984) reported that *M. bovis* isolates, if protected by sunlight, remain viable at 4°C in sponges or milk for 2 months and over 2 weeks in water. The study of Pfutzner (1984) described that at higher temperatures at 20°C and 37°C, the survival of *M. bovis* biofilm lowers to 1 from 2 weeks and 1 week, respectively. However, Nagatomo et al. (2001) reported that at 4 to 37°C, the growth and survival of *M. bovis* biofilm in liquid media is not affected for a period of 59 to 185 days. Other materials described in literature on which *M. bovis* biofilms survive include dry paper disks for 126 days and outdoor temperature (Nagatomo et al., 2001), 37 days in manure, 18 days in cotton, 13 days in straw, and 1 to 2 days in stainless steel (Ruffo et al., 1969).

Furthermore, researchers described that nitrogen and carbon contents play a role in the biofilm formation of *M. bovis.* In the study of Justice-Allen et al. (2009), 0.03 % nitrogen content and 1.33% carbon content were observed in recycled bedding sand that supported biofilm formation of *M. bovis*. This speculation hinges with the report of Godden et al. (2008) who discussed the presence of organic matters with nitrogen and carbon content of the recycled bedding sand. The formation of biofilms by *M. bovis* in inanimate substrates like recycled beddings and makes them viable for longer periods withstanding prolonged desiccation.

RISKS ASSOCIATED WITH *M. BOVIS* BIOFILMS

Some reports also exist that describe the risks associated with *M. bovis* biofilms. A biofilm of *M. bovis* with 8,000 colony forming units (CFU)/g mean cell density before incubation at 4ºC increased to a mean cell density of 131,000 CFU/g (Justice-Allen et al., 2009) after incubation for prolonged periods at the same temperature, a data which underscores the ability of *M. bovis* biofilms to survive at low temperature. This viability pattern of *M. bovis* biofilms is quite baffling as other studies described viable biofilms of *M. bovis* strain 43 after 36 h of drying and viable biofilms of *M. bovis* Strain 04 after 24 h of drying (McAuliffe et al., 2006). Moreover, another researcher (Nicholas, 2010) who reported low levels of viable *M. bovis* biofilms recovered after exposure to heat and desiccation remained more infectious even with a fewer number of viable cells. The work of Bennett and Jasper (1980) described that *M. bovis* biofilm at a low cell density of 70 CFU/g experimentally administered through teat diffusion resulted in a case of mastitis.

The biofilm state-mediated adherence can create a better opportunity for *M. bovis* to persist in biotic surfaces. This phenomenon is quite alarming as this may cater to the wider dissemination of infection from a biofilm source and more likely cater to the dispersion of biofilm-producing pathogens like *M. bovis* that may carry resistance to many antibiotics.

BIOFILMS LESSEN ANTIMICROBIAL ACTIVITY

Mycoplasmas are purportedly resistant to β-lactam antibiotics such as penicillin and cephalosporins (Caswell et al., 2010; Nicholas, 2010), a constitutive antimicrobial resistance phenomenon that can be linked to the absence of a cell wall but is compensated by the ability of the pathogen to self-produce the 20 amino acids for the citric acid cycle that biosynthesize genes and enzymes (Razin et al., 1998; McAuliffe et al., 2006). McAuliffe et al. (2006) and Caswell et al. (2010) speculated that biofilms may play a role in the development of antibiotic resistance of mycoplasma species. However, despite the supposed role of biofilm in antimicrobial resistance, the minimum inhibitory concentration (MIC) of *M. bovis* was not altered due to the biofilm in vitro (Burki et al., 2015, McAuliffe et al., 2006; Perez-Casal, 2020). However, oxytetracycline caused inhibition of polysaccharide film production in planktonic cells at concentrations far below the MIC value. In contrast, a higher concentration of oxytetracycline caused the inhibition in biofilm cells. However, this is phenotypic effects and does not reflect marked resistance

in biofilm cells compared to planktonic cells (McAuliffe et al., 2006). This phenomenon, that sub-inhibitory concentrations of antibiotics phenotypic effects on the production of polysaccharide and adhesion, has been previously reported on *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* (Rachid et al., 2000; Fonseca et al., 2004).

Nicholas et al. (2008) reported the increasing number of *M. bovis* strains that are becoming more resistant *in vitro* on commonly used antibiotics like fluoroquinolones. Hirose et al. (2003) reported that Japanese *M. bovis* isolates were highly susceptible to tiamulin, however, no antimicrobial activity of erythromycin was observed. Furthermore, *M. bovis* isolates were less susceptible to spiramycin, oxytetracycline, tylosin – suggesting resistance to these antimicrobials are observed in the field in Japan. Moreover, mycoplasmal diseases are often in co-infection with other respiratory pathogens such as the opportunistic pathogen*Histophilussomni* and the bacterial respiratory disease pathogen *Mannheimia haemolytica*. However, no literature has yet cited that biofilm potentiates or contributes to *M. bovis* antimicrobial resistance. There still needs to correlate antimicrobial *in vitro* testing to *in vivo* testing to provide a better guide to veterinarians (Nicholas, 2010).

CONCLUSIONS

M. bovis has VSPs known as VspA, VspB, VspE, and VspF which are reportedly mediate the adherence of *M. bovis* to colonize bovine respiratory tissues. The existence of advanced tools and technics in the medical sciences, the VSPs previously discussed can be exploited and manipulated as a vaccine that slows down the adherence of biofilms on tissues. Moreover, a preparation may likewise be made to effectively disrupt adhesion molecules that mediate the process of biofilm formation, which can be altogether undertaken and tested *in vitro* before its validation in *in vivo* studies. Moreover, research has more to offer in terms of the establishment of a biofilm-based platform for testing the *M. bovis* biofilms sensitivity and resistance of *M. bovis* to various antimicrobials. An effective and important protocol for the evaluation of antimicrobial treatments against *M. bovis* lay in tandem with a research-based approach for the prevention and control of mycoplasma infection are more likely to be achieved with biofilm as a platform for investigation.

ACKNOWLEDGEMENTS

The authors would like to thank the Central Luzon State University and the Philippine Carabao Center for the technical support to pursue this study.

AUTHOR CONTRIBUTIONS

GASP Tubalinal is the main author of this review article. He did the literature search and drafted the analytical write up. GG Garcia contributed in the analysis and final write-up. She serves as the academic adviser. CN Mingala served as the supervising technical adviser and contributed in the analysis and formatting of the manuscript.

REFERENCES

Bennett, R.H., Jasper, D.E., 1980. Bovine mycoplasmal mastitis from intramammary inoculations of small numbers of Mycoplasma bovis: Local and systemic antibody response. Am. J. Vet. Res. 41, 889-892.

Burki, S., Frey, J., Pilo, P., 2015. Virulence, persistence and dissemination of Mycoplasma bovis. Vet. Microbiol. 179(1-2), 15-22.

Butler, J.A., Sickles, S.A., Johanns, C.J., Rosenbusch, R.F., 2000. Pasteurization of discard mycoplasma mastitic milk used to feed calves: thermal effects on various mycoplasma. J. Dairy Sci. 83, 2285-2288.

Caswell, J.L., Archambault, M., 2008. Mycoplasma bovispneumonia in cattle. Anim. Health Res. Rev. 8(2), 161-186.

Caswell, J.L., Bateman, K.G., Cai, H.Y., Castillo-Alcala, F., 2010. Mycoplasma bovis in respiratory disease of feedlot cattle. Vet. Clin. North Am. Food Anim. Pract. 26(2), 365-79.

Chen, S., Hao, H., Zhao, P., Ji, W., Mingxia, L., Liu, Y., Chu, Y., 2018. Differential immunoreactivity to bovine convalescent serum between Mycoplasma bovis biofilms and planktonic cells revealed by comparative immunoproteomic analysis. Front. Microbiol. 9, 379.

Flemming, H.C., Wingender, J., 2010. The biofilm matrix. Nat. Rev. Microbiol. 8, 623-633.

Godden, S., Bey, R., Lorch, K., Fransworth, R., Rapnicki, P., 2008. Ability of organic and inorganic bedding materials to promote growth of environmental bacteria. J. Dairy Sci. 91, 151-159.

Gonzalez, R.N., Sears, P.M., Merrill, R.A., Hayes, G.L., 1992. Mastitis due to mycoplasma in the state of New York during the period 1972-1990. Cornell Vet. 82, 29-40.

Hirose, K., Kobayashi, H., Ito, N., Kawasaki, Y., Zako, M., Kotani, K., Ogawa, H., Sato, H., 2003. Isolation of Mycoplasmas from nasal swab of calves affected with respiratory diseases and antimicrobial susceptibility of their isolates. J. Vet. Med. B. Infect. Dis. Vet. Public Health. 50(7), 347-351.

Jasper, D.E., Al-Aubaidi, J., Fabricant, J., 1974. Epidemiological observations on mycoplasma mastitis. Cornell Vet. 64, 407-415.

Justice-Allen, A., Trujillo, J., Corbett, R., Harding, R., Goodell, G., Wilson, D., 2009. Survival and replication of Mycoplasma species in recycled bedding sand and association with mastitis on dairy farms in Utah. J. Dairy Sci. 93, 192-202.

Khan, F.A., Rasheed, M.A., Faisal, M., Menghwar, H., Zubair, M., Sadique, U., Chen, H., Guo, A., 2017. Proteomics analysis and its role in elucidation of functionally significant proteins in Mycoplasma bovis. Microb. Pathog. 111, 50-59.

Maunsell, F.P., Chase, C., 2019. Mycoplasma bovis interactions with the immune system and failure to generate an effective immune response. Vet. Clin. Food Anim. Pract. 35, 471-483.

McAuliffe, L., Ellis, R.J., Miles, K., Ayling, R.D., Nicholas, R.A.J., 2006. Biofilm formation by mycoplasma species and its role in environmental persistence and survival. Microbiol. 152, 913-922.

Nagatomo, H., Takegahara, Y., Sonoda, T., Yamaguchi, A., Uemura, R., Hagiwara, S., Seuyoshi, M., 2001. Comparative studies of the persistence of animal mycoplasmas under different environmental conditions. Vet. Microbiol. 82, 223-232.

Nicholas, R.A.J., Ayling, R.D., McAuliffe, L., 2008. Bovine respiratory disease. In: Mycoplasma disease of ruminants. CABI, United Kingdom, pp. 133-154.

Nicholas, R.A.J., 2010. The other M. bovis: Mycoplasma bovis. UK. Vet. Livest. 15(5), 32-34.

Perez-Casal, J., 2020. Pathogenesis and virulence of Mycoplasma bovis. Vet. Clin. North Am. Food Anim. Pract. 36(2), 269-278.

Pfutzner, H., 1984. The tenacity of Mycoplasma bovis. Zentralbl. Bakteriol. Mikrobiol. Hyg. A. 258(1), 38-41.

Razin, S., Yogev, D., Naot, Y., 1998. Molecular biology and pathogenicity of mycoplasmas. Microbiol. Mol. Biol. Rev. 62, 1094-1156.

Rottem, S., 2003. Interaction of mycoplasmas with host cells. Physiol. Rev. 83(2), 417-432.

Ruffo, G., Nani, S.P., Podesta, A., 1969. Survival of Mycoplasma agalactiae var. bovis in several materials and at different temperatures. Arch. Vet. Ital. 20, 459-464.

Sachse, K., Grajetzki, C., Rosengarten, R., Hanel, I., Heller, M., Pfutzner, H., 1996. Mechanisms and factors involved in Mycoplasma bovis adhesion to host cells. Zentralbl. Bakteriol. 284(1), 80-92.

Soenhnlen, M.K., Aydin, A., Murthy, K.S., Lengerich, E.J., Hattel, A.L., Houser, B.A., Fenton, G.D., Lysczek, H.R., Burns, C.M., Townsend, A.M., Brooks, J.W., Wolfgang, D.R., Jayarao, B.M., 2011. Epidemiology of Mycoplasma bovis in Pennsylvania veal calves. J. Dairy. Sci. 95, 247-254.

Thomas, A., Sachse, K., Dizier, I., Grajetzki, C., Farnir, F., Mainil, J.G., Linden, A., 2003a. Adherence to various host cell lines of Mycoplasma bovis strains differing in pathogenic and cultural features. Vet. Microbiol. 91(2-3), 101-113.

Thomas, A., Sachse, K., Farnir, F., Dizier, I., Mainil, J., Linden, A., 2003b. Adherence of Mycoplasma bovis to bovine bronchial epithelial cells. Microb. Pathog. 34(3), 141-148.

Wilson, D.J., Skirpstunas, R.T., Trujillo, J.D., Cavander, K.B., Bagley, C.V., Harding, R., 2007. Unusual history and initial clinical presentation of mycoplasma mastitis and arthritis in a closed commercial dairy herd. J. Am. Vet. Med. Asso. 230, 1519-1523.

Yiwen, C., Yueyue, W., Lianmei, Q., Cuiming, Z., Xiaoxing, Y., 2021. Infections strategies of mycoplasmas: Unravelling the panoply of virulence factors. Virulence. 12(1), 788-817.

Zou, X., Li, Y., Wang, Y., Zhou, Y., Liu, Y.,Xing, J., 2013. Molecular cloning and characterization of a surface-localized adhesion protein in Mycoplasma bovis Hubei-1 strain. PLoS One. 8(7), e69644.

How to cite this article;

Gabriel Alexis SP. Tubalinal, Gemerlyn G. Garcia and Claro N. Mingala. Biofilm: a platform for the evaluation of antimicrobial treatments and prevention against Mycoplasma bovis infection. Veterinary Integrative Sciences. 2023; 21(2): 567- 576.