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Mycoplasma bovis in North American bison (*Bison bison*): history, advances, and challenges

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North American bison (*Bison bison*) are keystone herbivores that shaped the ecology and evolution of North American prairies and peoples alike. Bison populations were pushed to near-extinction at the turn of the 20th century. Today, bison remain highly susceptible to newly introduced pathogens to which they have not evolved immunity, and *Mycoplasma bovis* is a significant threat to bison health. Although *M. bovis* is frequently associated with multifactorial bovine respiratory disease complex in its reservoir host, domestic cattle, *M. bovis* is a devastating primary pathogen in bison. As a fastidious, insidious, and rapidly mutating organism that lacks a cell wall, *M. bovis* is difficult to diagnose in an infected animal, and the lack of bison-specific knowledge and diagnostic tools further limits options for herd managers. Here we present a review of the current state of the field of *M. bovis* in bison, identify gaps in our understanding of bison physiology and *M. bovis* ecology, and we highlight the unique evolutionary differences of bison from domestic livestock. Dedicated bison research is urgently needed to improve prevention, surveillance, response, and management of *M. bovis* in this iconic North American wildlife species.

KEYWORDS

bison (*Bison bison*), disease, *Mycoplasma bovis*, conservation, epidemiology

1 Introduction

North American plains bison (*Bison bison*) are keystone herbivores that shaped the ecology and evolution of North American prairies and peoples alike (Olson and Janelle, 2022; Feir et al., 2022), and bison in some form have occupied North America for at least 300 thousand years (Shapiro et al., 2004; Froese et al., 2017). Modern plains bison once numbered in the 10s of millions, extending from what is today Mexico to the northern territories of Canada and occupying more than 51% of the continental landmass (Dodge, 1877). Over 70% of terrestrial North American species co-occurred with bison, and many depended upon bison grazing, stomping, wallowing, defecating, and dying for their own survival (Towne et al., 2005; Olson and Janelle, 2022).

With efforts to settle the western United States, bison populations and some grassland-associated species were nearly extinguished. Domestic livestock brought diseases, including brucellosis, malignant catarrhal fever, and others, and parasites including many species of strongyles and coccidia to which bison had not evolved immunity, although the population-level impact of these diseases is largely unknown and likely underestimated (Isenberg, 2020; Wobeser, 2002). More impactfully, coordinated efforts to commodify and later extinguish bison populations were nearly successful (Phippen, 2016; Isenberg, 2020). The last plains bison was shot in Canada in 1879, and by the late 1800s only a few hundred bison remained in small, scattered herds in the United States. Conservationists gathered small numbers of remaining bison to save the species by placing them in captivity, and by 1902 only 23 wild bison were left in the United States located within Yellowstone National Park (Hornaday, 2022; Meagher, 1973). Most estimates show that bison populations today originate from a founder population of only 30 to 50 individuals (Hartway et al., 2020). Additionally, many of the private bison herds where bison survived depopulation efforts were established as part of an effort to hybridize bison and European cattle (Coder, 1975). Although these historic hybridization efforts were largely unsuccessful, they resulted in cattle DNA likely being present in most North American bison herds, presenting an additional challenge for wild bison conservation (Hartway et al., 2020, Oppenheimer, in prep).

Today, bison numbers have rebounded to an estimated 400,000, with the vast majority managed for meat production purposes (USDA, 2016; Oyler-McCance et al., 2024). Whether managed for production or conservation, the genetic bottleneck followed by management of bison in small, isolated herds during the demographic recovery period over the last century has resulted in demonstrable genetic drift, loss of within-herd genetic diversity in some herds, along with the potential loss of wild traits due to captivity-based trait selection (Gates et al., 2010, Oppenheimer, in prep). Modern bison may therefore have reduced adaptive capacity, increased susceptibility to environmental stressors and may be more vulnerable to introduced diseases, with overall lower ability to adapt and evolve (Allendorf and Leary, 1986; Ballou and Ralls, 1982; Franklin, 1980; Frankham et al., 1999; Mitton and Grant, 1984; Halbert et al. 2004; Halbert et al., 2005). How previous bottlenecks have specifically affected modern bison resilience is unknown; however, regular live animal translocation to mitigate drift by restoring gene flow has been identified as critical for long-term species preservation (Hartway et al., 2020; Oyler-McCance et al., 2024).

Live animal translocation is a management tool used for both bison conservation and production purposes. Herds primarily managed for commercial purposes often include intensive management practices such as higher densities, supplemental feeding, and intermingling and translocation of bison from multiple different sources (USDA, 2016). Bison have unique diets, behaviors, and microbiomes compared to domestic cattle (Bergmann et al., 2015; Fresno Rueda et al., 2023), and cattle-based intensive management practices thus have the potential to introduce or exacerbate infectious diseases when applied to bison. Intensive management practices may result in increased stress, changes to the microbiome, reduced adaptive capacity, or disease introduction associated with bison movement between herds as is commonly practiced in production-based systems (USDA, 2016; Bras et al., 2016).

Bison are often highly susceptible to newly introduced pathogens to which they have not evolved immunity (Jones et al., 2020). For example, ovine herpes viruses cause no clinical disease in the reservoir host of domestic sheep but are acutely fatal in bison (Cunha et al., 2012), and bovine intestinal parasites such as strongyles can cause significant disease in bison even when present at levels considered non-pathogenic in cattle (Avramenko et al., 2018; USDA, 2023). Similarly, *Mycoplasma bovis* has recently been identified as a paramount threat to bison health (USDA, 2013, USDA, 2023; Krus et al., 2025). Although *M. bovis* is considered an important component in the multifactorial bovine respiratory disease complex in its reservoir host, domestic cattle, it is commonly isolated from the respiratory tract of healthy cattle and appears to primarily cause disease in young and immunosuppressed animals in concert with environmental stressors, microbiome changes, and other pathogens (Chai et al., 2022). In contrast, *M. bovis* has been demonstrated to be an especially virulent primary pathogen in bison, causing mortality in over 30% of adult bison in an affected herd with no co-infecting pathogens identified (Dyer et al., 2008; Janardhan et al., 2010; USDA, 2013; Register et al., 2013). Here we review the history, advances, and needs in understanding *Mycoplasma bovis* as a causative agent of a devastating disease in bison.

2 The causative organism

Mycoplasma bovis (M. bovis), a member of the class *Mollicutes*, is a bacterial species best defined by the lack of a cell well, small genome, unique genetic code, and the lack of specific metabolic pathways (Razin et al., 1998). Like other *Mycoplasma* species, *M. bovis* lacks rigid peptidoglycan cell walls and intracytoplasmic membranes, instead being bordered by a plasma membrane, which subsequently confers resistance to beta-lactam antibiotics (Razin, 1978). *M. bovis* lacks a functional tricarboxylic acid cycle and instead primarily utilizes organic acids such as lactate and pyruvate as energy sources (Miles et al., 1988). Unlike other *mycoplasmas*, *M. bovis* is capable of prolonged survival outside of a host, with studies showing survival in mild and various materials at 4 C for several weeks to nearly two months, and under field conditions surviving in recycled bedding sand for eight months (Pfutzner and Sachse, 1996; Justice-Allen et al., 2010). Prolonged environmental survival of this pathogen is thought to be mediated through the formation of biofilms (Mcauliffe et al., 2006). Although the host-range for many *Mycoplasma* spp. is highly restricted, the number of species susceptible to *M. bovis* continues to increase, including cattle (*Bos taurus*), bison (*Bison bison*), pronghorn (*Antilocapra americana*), domestic goats (*Capra hircus*), white-tailed deer (*Odocoileus virginianus*), and mule deer (*Odocoileus hemionus*) (Hale et al., 1962; Dyer et al., 2004, Dyer et al., 2008; Kumar et al., 2020; Malmberg et al., 2020).

The minimal genomes of Mycoplasmas are the result of reductive evolution from gram positive bacteria with low GC content (Hutchison and Montague, 2002). The genome of PG45, the type-strain for *M. bovis*, is 1,003,404 bp with a G + C content of 29.3% and an estimated 826 protein coding genes including pseudogenes (Adamu et al., 2013), with the genomes of other sequenced *M. bovis* isolates sharing similar characteristics. *Mycoplasma* genomes are highly dynamic, undergoing frequent rearrangement via homologous recombination and horizontal gene transfer (Lysnyansky et al., 2009; Garcia-Galan et al., 2022). As *M. bovis* has been documented to infect multiple species (see discussion below), several studies have sought to identify host-specific genotypes or mutations. Multilocus sequence typing (MLST) has been employed to compare *M. bovis* genomes. Initially reported in 2015 (Register et al., 2015) and later revised in 2020 (Register et al., 2020) the current MLST scheme, comprised of seven housekeeping genes, has been used to characterize the genetic diversity of *M. bovis* isolates from bison and cattle, and to compare these isolates collected across a variety of geographic locations and with isolates from other affected species. The analysis by Kumar et al. (2020) found a greater number of unique sequence types (STs) identified in cattle (n=39), compared to bison (n=5), with only four STs being identified in both species (Register et al., 2019). Further genetic analysis of *M. bovis* genomes from multiple species and countries using single nucleotide polymorphism (SNP)-based phylogeny determined six different genetic clades for *M. bovis* (Kumar et al., 2020). The genomes from bison and cattle isolates populated four different clades, with two of the clades being comprised primarily of bison isolates. Further analysis identified gene clusters that were associated with different hosts including ten genes, mainly lipoproteins and hypothetical proteins associated with bovine isolates (Kumar et al., 2020).

M. bovis is noted for causing chronic infections in large ruminant species and can persist in the upper respiratory tract of apparently healthy animals for prolonged periods (Maunsell et al., 2011), Buttke et al., 2025). Evasion of the host immune response is crucial for bacterial persistence and *M. bovis* utilizes several mechanisms to escape detection and clearance (Burki et al., 2015a). The variable surface lipoproteins (Vsps) are a family of 13 genes encoding immunodominant lipoproteins that are involved in adhesion (Sachse et al., 1996, Sachse et al., 2000). These proteins undergo spontaneous changes in expression states by frequent DNA inversion within the *M. bovis* genome resulting in phase variation of surface antigens (Lysnyansky et al., 2001) contributing to immune evasion and persistence in the host. The immunoglobulin binding (MIB) and *mycoplasma* immunoglobulin protease (MIP) system has been shown to bind and destroy host IgG immunoglobulins (Arfi et al., 2016). The *M. bovis* genome is predicted to encode 3 and 2 copies of MIB and MIP genes, respectively, suggesting the MIB-MIP system acts in preventing antibody binding and immunoglobulin mediated bacterial clearance (Arfi et al., 2016, Arfi et al., 2021). A growing number of *M. bovis* proteins involved in adhesion to host cells have been identified, including proteins involved in metabolic pathways with moonlighting functions as adhesins (Xu et al., 2022). Several putative *M. bovis* proteins have been shown to bind fibronectin including methylenetetrahydrofolate tRNA–(uracil-5)–methyltransferase (TrmFO) and the hypothetical lipoprotein P27 (Guo et al., 2017; Chen et al., 2018). Plasminogen and heparin are additional targets for *M. bovis* adhesins, being bound by α-enolase and MilA, respectively (Song et al., 2012; Adamu et al., 2020b). Additionally, several adhesins have been found to bind multiple targets on the host cell including NADH oxidase (NOX), which binds amyloid precursor protein 2 and fibronectin, the leucine rich repeat lipoprotein MbfN, which binds fibronectin and heparin, and fructose-1,6-diphosphate aldolase (FBA) which binds fibronectin and plasminogen (Zhao et al., 2017; Gao et al., 2018; Huang et al., 2019; Adamu et al., 2020a).

M. bovis can also adhere to and invade host cells as a means to further evade and alter the host immune response (Burki et al., 2015a). *M. bovis* has been shown to invade and replicate in primary bovine cells and bovine cell lines derived from lung, nasal, and mammary epithelium (Burki et al., 2015b; Josi et al., 2018) as well as in peripheral blood mononuclear cells (PBMC) and erythrocytes (Van Der Merwe et al., 2010), with the infection of bovine monocytes and macrophages likely contributing to dysregulation of the host immune response (Arfi et al., 2021). *In vitro* infection of bovine monocytes has been shown to delay apoptosis and alter cytokine expression, reducing the production of pro-inflammatory cytokines IFN-ϒ and TNF-α and increasing the production of IL-10 (Mulongo et al., 2014). Bison *M. bovis* isolates were found to infect and inhibit the proliferation of bison PBMC and alveolar macrophages while also preventing macrophage apoptosis (Suleman et al., 2016). Conversely, the secreted P280 protein has been shown to induce macrophage apoptosis *in vitro*, suggesting *M. bovis* can simultaneously prevent apoptosis of infected macrophages while removing uninfected macrophages that could aid in bacterial clearance (Zhao et al., 2021). Several studies have observed the induction of the Programmed Death 1 (PD-1) receptor and Programmed Death Ligand 1 (PD-L1) on T-cells and macrophages during *M. bovis* infection (Goto et al., 2017; Suleman et al., 2018). PD-1 and PD-L1 expression is indicative of T-cell exhaustion, a reduced state of responsiveness and loss of effector functions of antigen specific T-cells, frequently observed in chronic infections and contributing to diminished pathogen clearance (Goto et al., 2017; Suleman et al., 2018).

M. bovis can evade multiple neutrophil effector functions including phagocytosis, degranulation, and the release of neutrophil extracellular traps (NETs), likely contributing to persistence of infection. *M. bovis* can infect bovine neutrophils *in vitro* resulting in the increased production of the pro-inflammatory cytokines IL-12 and TNF-α (Jimbo et al., 2017). Microarray analysis found increased expression of the proinflammatory cytokine genes IL-1β, IL-8, IL-12, TNF-α, and IFN-ϒ (Gondaira et al., 2021). Conversely, *M. bovis* infection reduced nitric oxide production (Jimbo et al., 2017), despite increasing expression of iNOS (Gondaira et al., 2021), and inhibited the respiratory burst of bovine neutrophils (Thomas et al., 1991). The *M. bovis* genome contains genes for three major membrane nucleases, with the mnuA nuclease shown to degrade NETs (Sharma et al., 2015), networks of

fibers comprised of chromatin and granules released into the extracellular environment by neutrophils to bind and kill pathogens (Brinkmann et al., 2004), suggesting nucleases are important for the persistence of *M. bovis* infection. Further, extracellular DNA, from neutrophils and other cells can lead to increased H_2O_2 production (Zhu et al., 2019) which is hypothesized to correlate with *Mycoplasma* virulence (Khan et al., 2005; Schott et al., 2014).

3 History of *Mycoplasma bovis* in North American bison

Mycoplasma bovis was first isolated from a dairy cow with mastitis in 1961 and has since been identified in every major cattle-producing country and production system, with over 70% prevalence in many cattle herds even without clinical signs (Hale et al., 1962; Maunsell et al., 2011; D. Buttke pers. comm.). Although significant gaps in our understanding of this pathogen exist, *M. bovis* has only recently been identified as a pathogen in bison, with a significantly higher mortality rate and different epidemiology than that seen in domestic cattle (USDA, 2013). The difficulty in detecting *M. bovis*, coupled with the lack of bison-specific diagnostic tools and health expertise, likely delayed recognition of its importance as a pathogen (USDA, 2013). While few published case reports can be found prior to 2008 (Dyer et al., 2008; Janardhan et al., 2010; USDA, 2013), anecdotal reports suggest *M. bovis* may have been causing disease outbreaks in bison in the late 1990s. In late 1999, *M. bovis* was involved in a mortality event with retropharyngeal abscesses and pneumonia in a Montana bison herd on pasture affecting primarily yearling and 2-year-old bison (Register et al., 2021a; T. Bragg, pers comm.). Possibly due to the lack of highly sensitive *Mycoplasma* diagnostic techniques available at that time, this outbreak was originally mis-diagnosed as a possible *Rhodococcus equi* infection due to the morphology of the pneumonia lesions observed at necropsy, delaying the identification of *Mycoplasma* until *Mycoplasma*-specific culture techniques were attempted and highlighting the challenges in studying *M. bovis*. In 2001, in an unpublished report from Canada, an outbreak of *M. bovis* was found in a bison herd in Saskatchewan with polyarthritis and pneumonia (Woodbury and Windeyer, 2012). Additional outbreaks occurred in a breeding herd in New Mexico in 2004 affecting adult breeding bison females (D. Hunter, pers comm) and a newly established breeding herd of yearling female bison with retropharyngeal abscesses in Nebraska in January 2006 (T. Bragg, pers comm.). A separate ranch in Nebraska experienced an outbreak of *M. bovis* affecting a group of yearling bison with significant death loss in late 2006 and early 2007 (T. Bragg, pers comm.). This herd received animals from the Montana herd that experienced the *M. bovis* outbreak in 1999 and has had repeated *M. bovis* mortality outbreaks approximately every 5–7 years in their groups of yearlings, which are segregated from the breeding herd at 9–10 months of age, with the most recent outbreak occurring in 2021-2022. On a third location in Nebraska, a *Mycoplasma* outbreak occurred in a bison herd in 2009, killing more than 300 animals across multiple age classes of bison (T. Bragg, pers comm.). Significantly, this herd had received bison in the previous 12 months from the New Mexico property that had suffered *M. bovis*-attributed losses approximately 4 years prior to the transfer of apparently healthy animals. Genomic work characterizing *M. bovis* isolates across these herds is ongoing. These outbreaks highlight the potential risk of disease introduction through the movements of asymptomatic carriers, as well as the potential for subclinical maintenance of disease within a herd between outbreaks.

The distinct differences in cattle and bison epidemiology and the myriad of symptoms reported in both species make *M. bovis* diagnosis challenging. Bison calves appear to be largely clinically unaffected by *M. bovis*, whereas young and neonatal domestic cattle experience some of the highest morbidity and mortality in this species (Bras et al., 2016; Buttke et al., 2025; Nicholas and Ayling, 2003; Maunsell et al., 2011). In contrast, adult cattle are rarely clinically affected by *M. bovis*-induced pneumonia even when *M. bovis* mastitis is circulating in a herd, while adult bison suffer the greatest *M. bovis* mortality rates of any age class from severe, caseonecrotic pneumonia, with mastitis as a potential component of disseminated, systemic disease in some cases (Buttke et al., 2025). As a result of these differences among clinical signs and disease impacts between cattle and bison, bison producers and their veterinarians with cattle experience may assume that *M. bovis* is a secondary, stress-induced pathogen associated with polymicrobial bovine respiratory disease complex, such that *M. bovis* infections in bison may be missed. Furthermore, lethargy and loss of condition are prominent clinical signs of *M. bovis* infection in bison (Dyer et al., 2008; Janardhan et al., 2010; USDA, 2013; Bras et al., 2016; Martin et al., 2025), which may have contributed to the common misunderstanding that *M. bovis* is secondary to presumed immunosuppressive nutritional deficiencies, poor management, or other respiratory infections, despite a lack of diagnostic evidence or scientific understanding of bison physiology to support these claims (T. Bragg, pers. comm). Finally, as a prey species, bison typically only show clinical signs late in a disease process, and many bison herd managers report sudden death as the first detected sign of *M. bovis* (Bras et al., 2016; Martin et al., 2025), making early diagnosis and intervention even more challenging in bison compared to cattle.

Additionally, *M. bovis* has been reported in some cases to cause necrotizing pharyngitis in bison in the absence of pneumonia or arthritis, which may manifest as a wasting disease as the pharyngeal lesions reduce feed intake (Dyer et al., 2013). Systemic infections in bison can also seed joint infections, mastitis, and can cause abortions, with reduced reproduction reported in surviving animals following a herd outbreak (Dyer et al., 2008; Register et al., 2013; Bras et al., 2016, 2017; Martin et al., 2025). Genital disease is reported in domestic cattle and *M. bovis*-infected semen is thought to be responsible for the importation of *M. bovis* to New Zealand (Haapala et al., 2018). However, the authors have not found any reports of similar investigations in bison and the potential transmission via semen is unknown.

As of this writing, few experimental infection studies of *M. bovis* have been conducted in bison. Register et al. (2018) compared the

development of clinical signs and lung lesions following experimental infection of bison and cattle in a 33-day study of acute infection. A quadrivalent inoculum of *M. bovis* isolates representing the two most prevalent multilocus sequence types (MLST) in bison was administered intranasally to presumptively naïve animals. While no clinical signs were observed in the Holstein calves, three of the five bison had elevated body temperatures compared to baseline on days 3–17 post-inoculation. Although no other clinical signs were noted in the bison after day 17, moderate to severe bronchopneumonia was observed in four of the seven adult bison at necropsy. However, findings in this study may not reflect the totality of acute disease presentation in naïve bison since 7 of the 8 bison were found to have been previously infected when *M. bovis* was cultured from nasal samples collected immediately prior to experimental infection. Additionally, Bras et al. (2016) found that clinical signs were first observed in bison herds 6–8 weeks after the introduction of new animals to herds that subsequently experienced *M. bovis* outbreaks, suggesting that longer study periods of at least 8 weeks may be necessary to observe clinical *M. bovis* disease in bison following natural infection.

Comparison of nasal and tissue swabs collected in the 2018 comparative experimental infection study demonstrated differences in the recovery of *M. bovis* from the lung between cattle and bison, with *M. bovis* being recovered from the lungs of 5 of 8 bison while no bacteria was recovered from Holstein lung samples (Register et al., 2018). Interestingly, the majority of nasal swab samples collected from both bison and cattle were positive for *M. bovis*, confirming the nasal cavities as primary colonization sites in both bovid species. The disparities in clinical signs and bacterial colonization between bison and cattle may include differences in intrinsic susceptibility, and observations in the field demonstrate that bison are significantly more susceptible to *M. bovis* compared to cattle. However, the inoculum in the 2018 experimental infection study was comprised of bison isolates which could have been better adapted to the bison respiratory tract compared to that of cattle, influencing the interpretation of species-specific disease impacts. Studies comparing the cellular attachment, tissue colonization, and persistence of different *M. bovis* isolates from each host species could better inform the underlying mechanisms of susceptibility in bison. While lower pathogenicity has been noted from repeated passage isolates (attributed to decreased expression of key variable surface protein-encoding genes) in cattle, no clear genetic patterns or isolates have been definitively associated with virulence profiles *in vivo* in bison to date (Rasheed et al., 2017; Calcutt et al., 2018).

Kaplan et al. (2024b) conducted a second experimental infection study in bison as part of a vaccine trial, inoculating nine bison (seven adults and two yearlings) with bovine herpes virus-1 followed four days later with a pentavalent *M. bovis* inoculum. In this study, the coinfection resulted in the euthanasia of two animals in the unvaccinated control group prior to the scheduled end of study (days 15 and 22 post inoculation) due to lameness, tachypnea, labored breathing, and inappetence. Interestingly, complete blood counts from these two animals found elevated blood neutrophil and macrophage levels compared to other bison in the study which is consistent with the presence of significant numbers of myeloid cells in lung lesions during *M. bovis* infections (Devi et al., 2014; Baquero et al., 2021). Lesions in the control group were characterized as multifocal caseonecrotic lesions similar to those described previously by Register et al. (2018), and histologic analysis identified similar lesion morphology characterized by concentric areas of hypereosinophilic caseonecrosis surrounded by neutrophils and macrophages, then lymphocytes and plasma cells. Together, these findings may suggest coinfection with BHV-1, and potentially other pathogens, may enhance or accelerate the manifestation of clinical disease in experimental *M. bovis* challenges in bison. However, the lack of *M. bovis*-naïve animals in the Register et al., 2018 study rather than BHV-1 coinfection, may also account for the differences noted in disease severity in the two experimental infection studies.

4 Challenges in *Mycoplasma bovis* detection

Detection and diagnosis of *M. bovis* in live animals is challenging and significantly hampers our understanding of *M. bovis* ecology and epidemiology in bison. *Mycoplasma* infections are difficult to detect due to their fastidious nature and multiple immune-evasion strategies, with variable shedding and prolonged infections a near-universal hallmark of mycoplasmosis (Burki et al., 2015a; Arfi et al., 2021). In contrast to cattle, where more *Mycoplasma* species and numbers are routinely recovered from the shallow nasal cavity compared to the deep nasopharyngeal cavity, and where *M. bovis* is present in over 90% of animals in affected herds (Stipkovits et al., 2000; McDaneld et al., 2018), *Mycoplasma* species are significantly less common in bison and are more likely found in the deep nasopharynx when recovered (Register et al., 2021b; Kaplan et al., 2024a; Schwartz et al., 2024). While several *Mycoplasma* species have been isolated from guarded deep nasopharyngeal swabs of bison, including *M. bovirhinis, bovoculi, arginini, dispar, alkalescens* (D Buttke pers. comm) and *bovis*, with *M. bovirhinis* isolated from nearly 30% of animals in one study (Register et al., 2021a), recent studies found overall relatively low agreement between shallow nasal swabs and guarded deep nasopharyngeal swabs in both cattle and bison (Pohjanvirta et al., 2021; Schwartz et al., 2024). Overall, *Mycoplasma* spp. were detected in more animals sampled with a guarded deep nasopharyngeal swab, but a portion of *M. bovis* positive bison were missed when sampled only with a guarded deep nasopharyngeal swab (Schwartz et al., 2024). *Mycoplasma* shedding patterns have been extensively studied in dairy cattle, where patterns of prolonged asymptomatic carriage and sporadic shedding in nasal discharges and milk have been well-established (Nicholas et al., 2008; Wilson et al., 2007). Similar sporadic shedding in sub-clinically infected bison may account for the apparent stochasticity observed in *M. bovis* antemortem testing of bison, but more work is needed to determine if intermittent shedding occurs in bison.

More recent work comparing shallow and deep nasopharyngeal swabs in bison suggests infection progression may influence swab

detection probability (Buttke et al., 2025). In a recent longitudinal study of a naturally infected juvenile bison cohort that compared shallow and deep nasopharyngeal swabs, *M. bovis* was generally first recovered from shallow nasal swabs of newly infected animals followed by continued detection only by deep nasopharyngeal swabs. Infection in the shallow nasal cavity appeared to be transient and may indicate transmission probability, while infection of the deep nasopharyngeal cavity lasted longer, up to several years in some cases, and was not associated with documented transmission (Buttke et al. under review). These findings are consistent with those found in cattle by Maunsell et al. (2012), where *M. bovis* was isolated from shallow nasal swabs in less than a quarter of experimentally inoculated calves, often at levels too low to be reliably detected using PCR techniques, while *M. bovis* was isolated from both palatine and pharyngeal tonsils in 100% of experimentally inoculated calves regardless of route of inoculation (oral or trans-tracheal). Unfortunately, while *M. bovis* has been routinely and reliably isolated from the tonsils of *M. bovis*-infected bison post-mortem, bison tonsillar swabs are logistically difficult to obtain antemortem, require specialized equipment, induce a high degree of stress on the animal, and are not feasible as a routine sampling strategy for most herd managers due to their time- and technique-intensive nature (pers. comm, D. Buttke). While more work is needed to identify factors that may affect shedding patterns, we suggest that deep nasopharyngeal swabbing techniques, particularly those that sample tonsillar-adjacent tissues, improve diagnostic sensitivity for *M. bovis* over nasal swab approaches in bison.

Anatomic sampling location is one critical aspect influencing diagnostic sensitivity; laboratory methods used to detect the organism is another. Several studies have evaluated PCR targets (Clothier et al., 2010; Register et al., 2018), molecular amplification techniques (Andrés-Lasheras et al., 2020), growth conditions, culture times, and identification methods (Bokma et al., 2019), but relatively few have focused on detection in bison specifically (Register et al., 2018, Menghwar et al., 2021). While it has been demonstrated that polymorphisms within the primer binding region of *uvrC* could result in false negatives (Register et al., 2018), the *uvrC* target has been reported as the most reliable PCR target when using a Ct of ≤36 (Schwartz et al., 2024). After optimizing cycling conditions (Rossetti et al., 2010, Johnson et al., 2022), targeting *uvrC* resulted in fewer indeterminant results as compared to targeted *oppD*, defined as those with Ct values of 36-37. A direct comparison performed on nasopharyngeal ($n = 21$) and nasal ($n = 15$) swab samples from bison revealed 100% agreement between the two target genes, with the *uvrC* target resulting in less variation across technical replicates and fewer indeterminant calls when triplicate Ct values were averaged, therefore reducing the need to repeat qPCR to obtain a diagnostic outcome (J. Malmberg et al. unpublished data). We also aimed to determine if calcium alginate fibers in swab tips inhibited PCR as has been reported for other pathogens (Wadowsky et al., 1994) but did not find a difference in PCR results when comparing to polyester-tipped swabs without calcium alginate fibers. Additional work is needed to further explore swab tip material, size, and texture, along with shaft length, shaft flexibility, and transport/preservation media to maximize *M. bovis* detection sensitivity in live bison.

5 Epidemiology of *Mycoplasma bovis* in bison

Previous efforts to epidemiologically characterize *M. bovis* in bison have relied heavily on diagnostic tools developed for cattle, along with surveys of managers of bison herds affected by the disease (Bras et al., 2016; Bras et al., 2017; Register et al., 2021b; Martin et al., 2025). As described above, improvements in direct *M. bovis* detection sensitivity have advanced our understanding of this disease in bison, but significant gaps remain in understanding the epidemiology of this disease. With bison experiencing high mortality from *M. bovis*, understanding previous exposure and immune response is critical to evaluating future risk for individual herds, as well as to wild bison conservation, ecological and cultural restoration efforts.

Previous studies suggest that enzyme-linked immunosorbent assays (ELISAs) developed for cattle sera may not be optimized for detection of *M. bovis* antibodies in bison sera, and a few bison-specific serology tests have subsequently been explored (Register et al., 2013; Bras et al., 2017; Kaplan et al., 2024a). While the in-house bison serology test developed by Bras et al. (2017) was not specific to *M. bovis* and likely detected other non-pathogenic *Mycoplasma* species, 8 of 11 herds with no history of *M. bovis*-associated disease were found to have at least 1 seropositive individual, suggesting either the potential for more widespread exposure to *M. bovis* than previously thought, or high prevalence of exposure to other *Mycoplasma* species. Using a commercially available ELISA developed for cattle, Register et al., 2021b reported seropositive bison sampled from a variety of herds across the United States and Canada from as long ago as the late 1980s; however, subsequent work pairing culture and PCR with additional samples compared to the same commercial ELISA revealed concerns about test specificity, and especially including potential cross-reactivity with *M. bovirhinis* (Register et al., 2021a). More recently, Krus et al. (in review) developed and evaluated a P48-based indirect ELISA for anti-*M. bovis* detection in bison and compared its diagnostic performance to the commercially available ELISA. While the P48 ELISA was not found to be superior to the commercial ELISA for either sensitivity or specificity, the authors identify significant challenges in serological diagnostics of *M. bovis* in bison due to cross-reactivity of both tests with other *Mycoplasma* species. Until more comprehensive studies are conducted, serologic studies of *M. bovis* should be interpreted with caution.

As recognition of cross-reactivity improves our understanding of the limitations of historic serological study interpretation, recent studies on the prevalence of, and transmission among, infected individual bison in *M. bovis* infected herds suggest that chronic carrier states interspersed with periods of recrudescence and shedding may better explain some outbreaks than new disease introduction (Buttke et al., 2025, T. Bragg, pers comm.). Nevertheless, several previous studies have correlated disease

outbreaks with introduction of new animals and/or vehicle movements (Bras et al., 2016, Martin et al., 2025). Combined with the correlation between previous history of *M. bovis* disease and currently having an *M. bovis*-infected animal, additional research is needed to better understand the introduction and recrudescence of this disease in naïve and in previously exposed herds (Schwartz et al., 2024).

Mycoplasma bovis has recently emerged as a primary pathogen in a few other free-ranging ungulate species including pronghorn (*Antilocapra americana*) and mule deer (*Odocoileus hemionus*). In pronghorn, *M. bovis* was first described in Wyoming in 2019 and has been responsible for several epizootics within the state, killing over 600 animals across three epizootic events (Malmberg et al., 2020; Johnson et al., 2022, Malmberg et al. in prep). Pronghorn that died of *M. bovis* had severe, acute, fibrinous pleuropneumonia, occasionally with fibrinous pericarditis and infrequently with fibrinosuppurative arthritis and conjunctivitis. The outbreaks observed thus far indicate that co-infecting pathogens are uncommon in pronghorn and are limited to occasional opportunistic bacteria, such as *Trueperella pyogenes* (Malmberg et al., 2020).

In a GPS-collared mule deer that shared a pasture with dairy cattle in Colorado, fibrinosuppurative pleuropneumonia was recently described as a result of *M. bovis* infection (Malmberg et al., 2025). In contrast to the regional epizootics observed in pronghorn, the small number of mule deer cases appear to be isolated individual cases and limited evidence suggests a more subacute to chronic disease timeline in mule deer. White-tailed deer are also known to be susceptible to *M. bovis* and may develop severe, subacute to chronic pneumonia, though detailed reports are so far limited to farmed deer (Dyer et al., 2004). Collectively, these observations indicate that the host range of *M. bovis* is broader than previously recognized and provide strong evidence for *M. bovis* as a multi-host pathogen warranting expanded research at the wildlife-livestock interface.

Genetic characterization of *M. bovis* isolates is another useful epidemiologic tool. While multi-locus sequencing typing (MLST) approaches are less sensitive than whole-genome sequencing approaches and can miss significant gene deletions or mutations (Register et al., 2015; Register et al., 2019; Register et al., 2021a; Kinnear et al., 2021), they have helped characterize *M. bovis* epidemiology at a broad scale. Register et al. (2019) noted that a larger proportion of the United States bison population had been sampled relative to the U.S. cattle industry and the majority of bison isolates had been collected more recently than the cattle-sourced isolates, such that direct comparison of bison and cattle isolates should be done with caution until more isolates are available. The over-representation of bison isolates compared to cattle isolates, particularly in recent years, supports the observed higher virulence of *M. bovis* disease in bison compared to cattle. The Register et al. (2019) study also highlighted that multiple bison isolates exist, and each was more closely related to known cattle isolate than to another bison isolate. Whole genome sequencing comparing four Canadian cattle and bison isolates supports these findings, with unique single nucleotide polymorphisms, limited diversity in gene content, and shared virulence genes across all isolates (Menghwar and Perez-Casal, 2022). This work collectively suggests that while more work is needed, multiple spillover events into bison have occurred and additional studies characterizing epidemiologic linkages of *M. bovis* isolates and hosts is needed. Future work utilizing whole-genome sequencing approaches is needed to understand the origin and mechanisms of spread of *M. bovis* in bison and other wild ungulate species.

6 Management strategies

Mycoplasma species are resistant to most antimicrobial therapies due to their lack of a cell wall, propensity to form biofilms, and increasingly reported acquired resistance to macrolides and fluroquinolones (Lysnyansky and Ayling, 2016; Bokma et al., 2021). Antimicrobial therapy is therefore largely ineffective for *M. bovis*; in fact, M. *bovis* carriage was found to be higher and overall microbial species diversity and richness lower in cattle with a history of antimicrobial use (McMullen et al., 2019), suggesting that antimicrobial therapy could provide an advantage to this pathogen. Restrictions in certain classes of antibiotics for use in food producing animals, the need for early and repeated antibiotic treatment of *Mycoplasma*-induced disease for efficacy, and the difficulty in identifying and handling recently infected bison together make antimicrobial therapy inappropriate to mitigate the impacts of *M. bovis* in bison.

Development of an effective vaccine has also been challenging, in large part due to the frequent antigenic changes *Mycoplasma* species make through the altered expression of variable surface proteins, combined with the lack of bison-specific facilities to support such vaccine work (see Perez-Casal et al., 2017, for review). Some vaccine studies in cattle reported increased mortality and worsening clinical outcomes in vaccinates in trials, with only limited success reported in others (Perez-Casal et al., 2017; Kaplan et al., 2024b). Recent advances in modified-live attenuated *Manheimia haemolytica*-vectored *M. bovis* vaccines have improved clinical outcomes for vaccinated domestic calves in laboratory trials, but more work is needed to evaluate their efficacy in field settings in bison and cattle (Briggs et al., 2021).

A recent bison-specific vaccination study evaluated a novel injectable subunit vaccine containing recombinant *M. bovis* Elongation Factor Tu (EFTu) and Heat Shock Protein 70 (Hsp70) – two highly conserved, membrane-associated bacterial proteins (Kaplan et al., 2024b). Intramuscular administration of two doses resulted in antigen specific serum IgG and T cell responses. Thirty days after experimental inoculation, *M. bovis* was detected via PCR in the nasal cavity, trachea, and middle ear in all bison, with several bison also PCR positive for *M. bovis* in joints and one of the unvaccinated animals also having evidence of necrotizing pharyngitis. A reduction in *M. bovis* counts was observed in the lungs of vaccinated bison compared to unvaccinated controls, and vaccinated bison had lower lung pathology scores and smaller lung lesions compared to unvaccinated controls. Although all bison had histologic abnormalities in the lungs, unvaccinated animals had

more than 30% of lungs affected compared to just over 10% of the lung affected in vaccinated animals, demonstrating that this vaccine may provide partial protection from *M. bovis* disease (Kaplan et al., 2024b). Several herds with prior histories of *M. bovis* mortality report decreased mortality in bison that were alive during prior outbreaks compared to animals born since the prior *M. bovis* outbreak, supporting the hypothesis that acquired immunity can develop and may offer some protection (T. Bragg, D. Buttke, pers comm.). Research supporting further vaccine development efforts is needed.

The status of *M. bovis* as a primary pathogen in bison, strong correlation between herd-level detection and history of previous disease, combined with the overall apparent rarity of *M. bovis* in individual bison suggests that elimination of subclinical carriers may be a feasible management strategy to reduce recurrent disease in affected herds (Schwartz et al., 2024). Similar test-and-remove approaches have been applied to wild bighorn sheep populations in an attempt to manage *Mycoplasma ovipneumoniae*, a devastating wild bighorn sheep pathogen that routinely spills over from domestic sheep where it rarely causes significant disease (Cassirer and Besser, 2025). The intermittent and variable shedding of *Mycoplasma* species in chronically infected animals makes correctly identifying carriers challenging and highlights the urgent need for accurate antemortem diagnostic approaches. Furthermore, work to differentiate newly infected animals that may successfully recover from infection from those that are likely to continue to harbor the bacteria and risk of transmission is also needed.

In the absence of a sufficiently sensitive antemortem diagnostic test at the individual animal level, testing and de-population of *M. bovis*-affected cattle herds is being attempted to eliminate *M. bovis* from New Zealand, where *M. bovis* was introduced in 2017 and had not yet reached a high prevalence in cattle populations (Shadbolt et al., 2021). Test-and-depopulate eradication programs have been successfully used to eliminate other diseases in domestic cattle populations even with imperfect antemortem diagnostic testing (Zhang et al., 2018). However, de-population-based eradication programs require significant financial resources and thus are only practical with low-prevalence diseases of significant national economic impact, and even then, have only been implemented in captive populations. The unique status of bison as both wildlife and ranched animals coupled with the small size of the industry presents challenges for disease eradication and control programs, and optimization of antemortem test performance is essential to support evaluation of a test-and-removal approach for managing *M. bovis* in bison.

7 Knowledge gaps and future needs

As wild bison restoration efforts and bison production industries both continue to expand, the need to better understand *M. bovis* in bison is critical. Despite considerable advances in the past decade, significant gaps in our knowledge and understanding of *M. bovis* in bison persist. Calcutt et al. (2018) provides a comprehensive overview of the gaps and needs in improving our understanding of *M. bovis* virulence, pathogenicity, detection, and control that will benefit cattle and bison health alike, but there are unique and urgent needs specific to bison health that are addressed here.

7.1 Epidemiology

Surveys such as Bras et al. (2016) and Martin et al. (2025) shed light on the significance of *M. bovis* in affected herds, but data on the true incidence of the disease is lacking. These studies also highlighted the risk of introducing subclinical animals and fenceline contact with cattle as potential disease sources, while recent reports of disease in pronghorn and mule deer emphasize the need to understand the impact to and influence of free-ranging wildlife in *M. bovis* ecology (Malmberg et al., 2020; Johnson et al., 2022; Malmberg et al., 2025). Comprehensive whole-genome sequencing comparison of a larger suite of cattle, bison, and other wild ungulate species isolates is currently underway and will likely help elucidate the relative importance of these risk factors and their role in the epidemiology and ecology of *M. bovis*.

Environmental transmission has not been documented to play a role in *M. bovis* transmission in any system to date, but knowledge gaps on *M. bovis* transmission, incubation period, and disease kinetics in bison have limited the ability to fully evaluate environmental contamination or fomites in disease transmission. While studies suggest that environmental transmission may not play a significant role in *M. bovis* disease in bison (Buttke et al., 2025; Johnson et al., 2022), most studies evaluating the potential for environmental transmission of *M. bovis* have been conducted in confinement cattle operations. *Mycoplasma* species were successfully cultured from moist, recycled dairy bedding sourced from herds known to be infected with *M. bovis* and other *Mycoplasmas*, but *M. bovis* could not be experimentally transmitted to naïve dairy calves through contaminated bedding, even when high levels of inoculant were used (Wilson et al., 2011). Because temperature and moisture are known to be important factors predicting environmental persistence of *Mycoplasma* species, environmental transmission may be less important in outdoor and arid environments in the western United States, where bison and pronghorn *M. bovis* outbreaks have been most commonly reported. Several of the authors have attempted to isolate *M. bovis* from biofilms in water sources used by known-infected animals but none have been successful to date (B. Kaplan, D. Buttke, T. Bragg, J. Malmberg, pers comm.).

7.2 Disease course and outcomes

The two experimental infection studies conducted in captive bison to date have produced valuable information about the role of *M. bovis* as a primary pathogen in bison (Register et al., 2018). However, laboratory space constraints in both of these studies limited the timeline available to study the disease course, and the presence of *M. bovis* in the respiratory tract of all but 1 bison prior to inoculation in the Register et al. (2018) study and the use of

bovine herpes virus-1 as a co-infection in an attempt to enhance disease presentation in hinder our ability to fully evaluate *M. bovis* as a single pathogen in naïve animals, as commonly occurs in the field. No studies to date have been able to describe the disease course of natural infection due to the lack of validated antemortem diagnostic tests as described above, the cryptic nature of *M. bovis* infections, and the difficulty in repeated handling and sampling required of bison. Captive rearing of bison in a dedicated bison research facility would improve our ability to understand *M. bovis* disease course, clinical presentation and the bison immune response and health more broadly. The paradoxical disparity in age-related disease presentation between bison and cattle, coupled with the lack of significant differences detected to date between bison and cattle *M. bovis* virulence genes, suggests significant species-specific immunologic differences likely exist (Buttke et al., 2025; Kinnear et al., 2021). The ability to study both the disease course and bison immune response in a controlled setting would greatly enhance our understanding of *M. bovis* and bison health more broadly.

More work is also needed to understand factors that may drive the variable disease presentations reported in bison (Dyer et al., 2008). Some *M. bovis* outbreaks in bison present almost exclusively with pneumonia, while others present almost exclusively with necrotizing pharyngitis (T. Bragg, pers comm.). *Mycoplasma bovis* has been noted to be one of the most prolific biofilm-forming *Mycoplasma* species, capable of surviving environmental laboratory conditions for over 30 hours (Mcauliffe et al., 2006). Biofilm formation was reported to be enhanced when co-occurring with *Trueperella pyogenes*, an opportunistic pathogen previously noted to be associated with necrotizing pharyngitis lesions in bison (Nishi et al., 2025; Dyer et al., 2008). Whether co-infection or biofilm formation influences site selection, disease presentation (i.e., pneumonic versus pharyngetic), or persistence is unknown and warrants further study. Cattle studies have identified disease-enhancing co-infections such as bovine herpes virus-1 and influenza D virus that may also influence clinical presentation and disease outcomes in bison (Prysliak et al., 2011; Lion et al., 2021).

In addition to studying acute infections in naïve bison, close study of chronically infected bison throughout the prolonged course of disease is also needed to understand both how and why some animals develop subclinical infections and what factors stimulate disease recrudescence or shedding. Buttke et al. documented the apparent prolonged carriage of *M. bovis* by sub clinically infected bison with no transmission to naïve conspecifics (under review), and seasonal differences in disease occurrence have also been noted (Martin et al., 2025). Whether environmental, physiologic, or epidemiologic factors influence this apparent seasonal disease presentation typically associated with periodic recurring outbreaks is unknown, and further study of how seasonally-influenced variables or co-infections may stimulate disease recrudescence or shedding is needed.

8 Conclusions

Climate change and human activities that alter environments and global species distributions have led to increases in disease emergence and spillover, resulting in more threats to bison health (Carlson et al., 2022). As a keystone species integral to native cultures and national identities, preserving and restoring healthy, wild bison is a critical conservation priority (Jones et al., 2020; Oyler-McCance et al., 2024). As a more resilient, sustainable food source compared to many conventional animal proteins (see Martin et al., 2025), enhancing bison health and markets also has a multitude of benefits. Both species restoration and food industry efforts require live animal translocations in order to accomplish their respective goals. As live-animal translocations carry the risk of disease translocation and likely contributed to the dissemination of *M. bovis* in the private bison industry, coordinated efforts are urgently needed on multiple fronts to increase awareness and education of the risk of *M. bovis*, develop accurate antemortem testing to identify and prevent the movement of infected animals, and identify science-based management strategies to reduce both initial and recurrent mortality events. Preventing *M. bovis* introduction into wild bison herds is critical to the survival and restoration of this native North American wildlife species, with benefits extending to other wild ungulate hosts such as pronghorn and deer.

Author contributions

DB: Conceptualization, Data curation, Investigation, Writing – original draft, Writing – review & editing. BK: Writing – original draft, Writing – review & editing. TB: Writing – original draft, Writing – review & editing. LJ: Writing – original draft, Writing – review & editing. JM: Writing – original draft, Writing – review & editing.

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