DISSERTATION

ADVANCING DISEASE SURVEILLANCE AND BIOSECURITY IN NORTH AMERICAN BISON (BISON BISON): MULTIDISCIPLINARY APPROACHES TO BISON HEALTH MONITORING

Submitted by

Catherine Bridget Krus

Department of Clinical Sciences

In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Spring 2025

Doctoral Committee:

Advisor: Christie Mayo
Co-Advisor: Sarah Raabis

Danielle Buttke
Georgia Titcomb

ABSTRACT

ADVANCING DISEASE SURVEILLANCE AND BIOSECURITY IN NORTH AMERICAN BISON (*BISON BISON*): MULTIDISCIPLINARY APPROACHES TO BISON HEALTH MONITORING

North American bison (*Bison bison*) play a critical ecological, cultural, and economic role, yet their long-term conservation and management face challenges from disease threats, anthropologic changes, and translocation-associated risks. This dissertation integrates a One Health approach to assess key aspects of bison health, disease surveillance, and biosecurity policy through a multidisciplinary framework. Specifically, this research (1) establishes a stakeholder-driven definition of bison health, (2) evaluates the epidemiology of Bluetongue Virus (BTV) and Epizootic Hemorrhagic Disease Virus (EHDV) in bison, and (3) assesses the diagnostic performance of serological assays for *Mycoplasma bovis*, a pathogen of increasing concern.

To define bison health from a multisectoral perspective, a two-round Delphi survey was conducted with experts from public, tribal, nonprofit, and private sectors. Experts defined bison health as the ability of populations to express natural behaviors, demonstrate resilience to external stressors, and sustain high reproductive output with minimal intervention. *Mycoplasma bovis* was identified as a high-priority pathogen, with participants highlighting the urgent need for improved diagnostics, biosecurity measures, and cross-sectoral disease management strategies.

In response to the research and biosecurity gaps in bison health identified in the Delphi manuscript, as well as the expanding geographical range of *Orbiviruses* in the

United States, we conducted the first cross-sectional serosurvey of BTV and EHDV in North American bison. This study analyzed samples from 287 animals across nine herds in seven U.S. states. Competitive ELISA assays revealed a seroprevalence of 56.5% for BTV and 57.5% for EHDV, with logistic regression identifying age as a significant predictor of seropositivity ($p < 0.01$). PCR-based detection of circulating BTV serotypes (6, 11, 13, 17) was noted, indicating exposure to endemic serotypes. Additionally, a significant decline in viremia with increasing age suggesting age-related immune dynamics. These findings provide foundational data for incorporating bison into vector-borne disease surveillance and highlight the need for further investigation into their role as incidental hosts.

Given the increasing impact of *M. bovis* on bison health along with the economic impacts highlighted in the Delphi manuscript, this dissertation also evaluates the diagnostic performance of a newly designed P48 ELISA compared to a commercially available ELISA for *M. bovis* detection in bison. This chapter assesses sensitivity, specificity, and cross-reactivity, identifying key limitations of current assays and providing recommendations for improving serological surveillance in bison populations. Findings from this study emphasize the need for species-specific diagnostic validation to enhance disease monitoring and outbreak prevention.

By integrating Delphi consensus-building, epidemiological surveillance, and diagnostic evaluation, this dissertation provides critical insights into bison disease ecology, biosecurity needs, and translocation risks. The findings support evidence-based policies for disease mitigation and contribute to the sustainable management of bison populations under a One Health framework.

ACKNOWLEGMENTS

First and foremost, I'd like to acknowledge my parents, Ted and Donna, and my siblings, Robert, Monica, and Ted for their constant support, genuine interest, and belief in me as I went through veterinary school…and then a veterinary internship…and then a veterinary residency…and then a PhD. I don't think we ever thought I would finish school. Without you, the hard times would have felt a lot harder. I love you all.

To my friends who lent a listening ear, who advised me, who showed me empathy and compassion throughout this journey, thank you so much. You are the bricks that helped build this home and I will be forever grateful for the beautiful friendships and community we built together.

To the veterinarians, biologists, producers, tribes, conservationists that helped contribute to this work, collaboration is key. I appreciate all of the behind the scenes work that you put in to help me succeed. I couldn't have done it without you.

My PhD committee members. Thank you for teaching me how to be strong by being kind. You break the vicious cycle of academic abuse that is so engrained into this culture and instead foster knowledge with thoughtful deliberation and collaboration. If there is one "transferable skill" that I take away from this PhD, it is that.

And of course, to my incredible partner, Giovanni. Wow, what a ride. You have been my best friend, shoulder to lean on, my cheerleader, and most of all, my hope throughout this PhD. I am deeply grateful for your ability to see beyond the 9 to 5 work week and for offering me a perspective on life that I had long forgotten. Your kindness and compassion are infectious. Thank you for all that you have done for me.

DEDICATION

To the buffalo- whose presence, resilience, and sacrifice has made this research possible. I honor their contribution to science, to the land, and to the people who have lived alongside them for generations.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1 – INTRODUCTION AND LITERATURE REVIEW

Bison Health in a One Health Context

North American bison (*Bison bison*) embody the principles of One Health and Traditional Ecological Knowledge, serving as a powerful example of the interconnectedness of environmental, animal, and human health. As a keystone species in grassland ecosystems, bison shape their environment through grazing behaviors that enhance plant diversity, improve soil health, and support biodiversity across multiple taxa, including birds and small mammals (1,2). Additionally, their reintroduction has been linked to increased native plant richness and greater ecosystem resilience to climate extremes such as drought (1). This ecological influence directly impacts the health of the environment, which in turn influences the health of the animals and the human populations that depend on them.

Beyond their role as ecosystem engineers, bison are deeply valued by diverse stakeholders, including conservationists, producers, and Indigenous communities. Approximately 80% of U.S. bison now reside on private farms and ranches, reflecting the expansion of bison production as an industry (3). Bison meat is recognized for its nutritional benefits, containing only 2.8 grams of fat per 100-gram serving compared to 6.4 grams in beef (4). For Indigenous communities, bison restoration is not just an ecological or economic matter but also a fundamental aspect of food sovereignty, sustainable land management, and the reinforcement of cultural ties to the land (5). A One Health approach considers the collective need for healthy food and water, linking bison to broader aspects of human well-being and sustainable development (6).

Challenges to Bison Health and Sustainability

Despite their ecological, economic, and cultural significance, the long-term sustainability of bison populations remains under threat due to historical large-scale slaughter and ongoing challenges such as introduction of disease and habitat loss. The historical slaughter of bison was not just an ecological disaster but also a calculated effort to undermine Native American nations, as the U.S. government and military recognized that the destruction of bison, who are central to Indigenous survival, economy, and culture, would force Plains nations into dependence on reservations (7,8). By the late 19th century, due to these factors, plains bison populations were decimated, reduced from tens of millions to fewer than 1,200 individuals (7,9,10). Due to the small initial herd sizes, nearly all present-day bison in North America descend from fewer than 100 individuals (11,12). This historic bottleneck, along with restricted herd sizes and geographic ranges, has resulted in limited genetic diversity within many herds (13,14).

Given these perspectives, maintaining bison health is of utmost importance. Conservation efforts are complicated by genetic constraints, disease risks, and environmental pressures. To enhance genetic diversity and improve production, contemporary bison conservation and production often rely on translocating animals across various public, tribal, NGO, and private operations. However, translocations have presented challenges due to these disease risks and exposure to novel environments. The movement of bison across large geographic and environmental gradients can result in increased morbidity and mortality, reduced reproductive output, and the introduction of diseases to new locations, affecting all stakeholders (15–17). Similar examples can

be seen with zebra's translocating African horse sickness to Spain, warble and nostril flies transmitted between reindeer and caribou in Greenland, and Brucellosis translocated from hare to domestic animals transported from Hungary to Italy (17).

Environmental conditions also drastically influence disease transmission dynamics, particularly for parasitic and vector-borne diseases (18,19). Translocations over long distances expose bison to novel environments that can alter infection dynamics in unexpected ways. Certain helminths with free-living stages, for example, have broad geographic ranges but cause production losses only under specific environmental conditions (20–22). Additionally, rising temperatures, shifting precipitation patterns, and increased weather variability can expand the range of arthropod vectors such as *Culicoides* midges, which transmit Bluetongue Virus (BTV) and Epizootic Hemorrhagic Disease Virus (EHDV)(23–25). As environmental conditions continue to evolve for bison, whether through translocations or climate change itself, understanding the intersection of climate, vector ecology, and bison health will be crucial for disease management and conservation strategies.

The Need for a Holistic Approach: Harnessing the Delphi Method for Bison Health Management

The challenges bison face highlight the importance of a holistic health management approach that accounts for the complex interplay of factors influencing their well-being. This approach should focus on defining metrics of health in the context of bison, surveillance and biosecurity needs, along with developing tools to help managers mitigate the effects of translocation and climate change on bison health. For a holistic approach to be applied, the scope must move beyond a narrow focus of health

as simply "the absence of disease" and embrace a broader view that incorporates biological, social, and environmental determinants (26). While disease can lead to large-scale wildlife die-offs, most population declines stem from habitat degradation, fragmentation, and reduced resilience (27,28). Furthermore, external factors influencing health are not evenly distributed across populations, leading to variations in individual outcomes (29,30). How population health is assessed, interpreted, and valued depends on both the context in which a population exists and the perspectives of those conducting the evaluation (26,31,32). Therefore, establishing a shared definition of health provides a crucial reference point for stakeholders, aligning them toward unified goals and serving as a critical first step in disease risk evaluation (33).

The Delphi method has proven to be a valuable tool in wildlife health for unifying diverse expert opinions into clear, actionable consensus. Delphi studies have consistently reinforced that wildlife health is multifaceted, encompassing ecological, biological, and social dimensions, with a strong emphasis on resilience and sustainability (28,34,35). These studies have yielded tangible outcomes, such as prioritizing disease risks (e.g., identifying critical pathogens or stressors), establishing standardized monitoring metrics, and highlighting knowledge gaps, all of which inform conservation policy and research agendas (28,34).

In the context of bison health management, the Delphi method offers a structured approach to gather and synthesize expert insights, facilitating a systematic convergence of opinion through iterative rounds of surveys. This process minimizes bias, fosters thoughtful deliberation, and enables consensus-building among diverse stakeholders. By engaging a broad panel, Delphi surveys can help identify knowledge gaps, prioritize

research needs, and develop consensus-driven strategies for disease surveillance and biosecurity. Moreover, this participatory process builds a sense of shared ownership over wildlife health goals, which is crucial for implementing disease management and conservation interventions that require cross-sectoral collaboration.

To effectively translate One Health from theory into practice, communication, coordination, collaboration, and capacity building (the “4 Cs”) are of utmost importance (6). The Delphi method aligns with these principles by serving as a platform for structured dialogue and knowledge exchange among diverse stakeholders. By fostering consensus across institutional and cultural boundaries, it facilitates collaborative decision-making and ensures that conservation and health management strategies for bison are based on broad stakeholder agreement. This alignment can also help reduce conflicts related to controversial interventions, such as culling or translocation, since all parties contribute to defining problems and solutions.

Ultimately, using the Delphi method in wildlife health management not only produces well-defined priorities and strategies but also cultivates a collaborative network of experts and stakeholders who share a common vision for wildlife health. This synergy is invaluable for addressing emerging disease challenges and conservation dilemmas, ensuring that responses are proactive, science-driven, and widely supported across agencies, jurisdictions, and disciplines.

Disease Threats and Surveillance Gaps: *Orbiviruses*

Vector-borne diseases represent a significant challenge for wildlife health management, particularly in species that occupy the interface between domestic

livestock and free-ranging ecosystems. Bluetongue Virus (BTV) and Epizootic Hemorrhagic Disease Virus (EHDV) are two closely related *Orbiviruses* that have historically been associated with severe morbidity and mortality in domestic and wild ruminants. While extensively studied in cattle, sheep, and white-tailed deer, the role of bison in the epidemiology of these viruses remains poorly understood. As climate change alters vector distributions and serotype diversity continues to expand in North America, the potential impact of BTV and EHDV on bison health and conservation warrants further investigation. This section reviews the existing literature on BTV and EHDV, including their transmission ecology, host susceptibility, and global emergence, while highlighting knowledge gaps that need to be addressed to inform bison health management and surveillance strategies.

Introduction to Bluetongue Virus and Epizootic Hemorrhagic Disease Virus

Bluetongue (BT) and Epizootic Hemorrhagic Disease (EHD) are two arthropod-borne viral diseases that cause significant health impacts in domestic and wild ruminants, ranging from reproductive losses to vascular injury, hemorrhage and death. The causative agents, bluetongue virus and epizootic hemorrhagic disease virus, respectively, belong to the *Orbivirus* genus (family *Reoviridae*) and are transmitted by biting midges of the genus *Culicoides*.

Bluetongue was first described in Merino sheep in South Africa, where it was initially referred to as "Malarial Catarrhal Fever" due to its association with lameness and mouth lesions (36). In North America, periodic outbreaks of epizootic hemorrhagic disease in white-tailed deer (*Odocoileus virginianus*) were reported by hunters as early

as the late 1800s, when the condition was commonly referred to as "black tongue" (37,38). The first confirmed EHD outbreak, however, was documented in New Jersey in 1955, where EHDV-1 was identified as the causative agent (39). While the role of insects in *Orbivirus* transmission was suspected for decades, it was not until 1944 that *Culicoides* biting midges were experimentally confirmed as vectors of BTV (40). To date, both viruses remain economically significant, causing devastating outbreaks in domestic livestock and wildlife globally, including major epizootics in the Netherlands. The net costs of the 2006 Bluetongue epidemic were estimated at €32.4 million, while the 2007 epidemic resulted in losses ranging from €117 to €128 million, showing the substantial economic burden associated with these outbreaks (41).

There are 29 described BTV serotypes, 27–29 of which are putative and capable of segment reassortment, and seven EHDV serotypes (42–44). The BTV and EHDV genome consists of 10 linear segments of double-stranded RNA and is enclosed in a bi-layered, icosahedral capsid (45). The 10 segments code seven structural (VP1-VP7) and four nonstructural proteins (NS1-NS4), which are responsible for viral replication, host immune evasion, and transmission dynamics (45,46).

BTV and EHDV preferentially replicate in endothelial cells, monocytes/macrophages, and lymphocytes, leading to vascular injury (47,48). After the initial replication phase, the viruses are highly assoicated with red blood cells, which aids in persistence and transmission (47–49). Due to these mechanisms, the clinical evidence of *Orbivirus* infections reflect systemic vasculitis and include coronitis and laminitis, mucosal erosions, pulmonary and subcutaneous edema, pericardial effusion,

hemorrhage and coagulopathy, among others (47,50–53). The severity of illness is influenced by factors such as host species, age, immune status, vector competence, and circulating serotypes (38).

Host and Vector Dynamics

The transmission of vector-borne diseases such as BTV and EHDV is shaped by complex interactions between vertebrate hosts, vector species, and environmental conditions. *Orbiviruses* are no exception with host susceptibility and vector competence contributing to the virus's ability to establish and persist in different ecological landscapes.

Although there are regional variations in the prevalence of *Orbiviruses* in the US, overall, BTV and EHDV transmission in the United States is highly seasonal, correlating with the abundance of competent *Culicoides* vectors, which fluctuate with temperature and precipitation patterns (25). Warmer temperatures accelerate virus replication within vectors, shortening the extrinsic incubation period and increasing transmission efficiency (54). Additionally, habitat features, including water sources and vegetation cover, influence midge breeding sites and host-vector interactions. In temperate regions, vector populations typically peak in late summer and early autumn, aligning with increased BTV transmission (25).

Because *Culicoides* midges rely on temporary aquatic habitats such as manure pools, small puddles, and organic-rich water sources, and their eggs are susceptible to desiccation, they face significant ecological dispersal challenges. However, wind-borne dispersal of *Culicoides* has been documented, with midges traveling distances of 130–

200 km from their original emergence sites (54). This long-distance dispersal can facilitate the spread of BTV and EHDV into new regions, particularly when combined with anthropogenic factors such as livestock movement.

Human activities significantly impact *Orbivirus* transmission by modifying vector habitats and host distribution. For example, intensive livestock farming creates ideal breeding conditions for *Culicoides* midges, increasing host-vector contact rates. Additionally, the global movement of livestock can introduce novel virus strains into new areas, facilitating reassortment events and increasing disease emergence risks (25).

Transmission of Orbiviruses: Vectors

There are over 1,400 known species of *Culicodies*, but only approximately 30 have been demonstrated to be vectors for BTV (55). In North America, the primary *Orbivirus* vector is *Culicodies sonorensis*, however, as climate changes occur, other ranges of competent vectors such *C. insignis* are expected to expand, altering transmission dynamics (38,55). In the genus *Culicodies*, females require a blood meal for ovarian maturation and egg production (54). Once a *Culicoides* midge ingests a blood meal from an *Orbivirus*-infected host, the virus undergoes an extrinsic incubation period within the insect, typically lasting between 7 and 14 days, depending on environmental factors such as temperature (56). During this time, the virus must overcome multiple physiological barriers within the midge, including the mesenteron infection barrier, which limits initial viral replication in the midgut, and the mesenteron escape barrier, which prevents viral dissemination beyond the gut (56,57). If the virus successfully bypasses these defenses, it disseminates throughout the midge's body and reaches the salivary glands, often within five days post-infection, where no known

barrier exists to prevent further replication and transmission (57). Once established in the salivary glands, the virus can be efficiently transmitted to a new susceptible ruminant host during subsequent blood meals, completing the transmission cycle (57). Importantly, once a female midge becomes infected, it remains infected for the rest of its life, continuously transmitting the virus as long as it continues to take blood meals. Under optimal laboratory conditions (25°C and 70% relative humidity), *Culicoides sonorensis* females can complete three to four gonotrophic cycles within their three- to six-week lifespan (58). However, environmental factors such as rising temperatures can accelerate egg development, increasing blood-feeding frequency while potentially shortening lifespan (58). Consequently, the total number of blood meals a female midge takes in her lifetime can vary significantly based on environmental conditions and impact *Orbivirus* transmission dynamics. Although there has been documentation of horizontal transmission routes for BTV, these are likely negligible in the overall epidemiology of BT as compared to vector-mediated spread (56,59).

Transmission of Orbiviruses: Hosts

Bluetongue tends to occur in ruminants with a seeming predilection for certain sheep breeds (60). Among domestic animals, cattle and goats often experience subclinical infections with prolonged periods of viremia, making them efficient reservoirs often without overt clinical disease (61,62). In contrast, sheep typically develop more severe clinical signs, thus serving as both indicator and reservoir hosts in many outbreaks (38). In addition to domestic ruminants, several wild ruminants have been known to be infected with BT including bison, elk, deer, antelope, among others with clinical signs ranging from subclinical infection to death (63–67).

For epizootic hemorrhagic disease virus, the virus predominantly affects wild ruminants, with white-tailed deer in North America being the primary reservoir. These deer are highly susceptible to infection and have been historically linked to severe epizootics, such as the outbreak described in New Jersey (39). While other ruminants, including cattle and additional deer species, can be infected and act as incidental hosts, they generally do not maintain the virus in nature to the same extent as the white-tailed deer reservoir host (68).

BTV and EHDV in Bison

While there is extensive research on BTV and EHDV in domestic livestock and wild deer, the epidemiology of these viruses in bison remains poorly understood. Few studies have specifically investigated BTV and EHDV in North American or European bison, with most data coming from isolated outbreak reports or serological surveys (23,65,69–71).

Seroprevalence rates for BTV in European bison (*Bison bonasus*) have ranged from 12.83% to 22.1% (69,70). While European bison seroprevalence data provide useful context, direct comparisons must account for fundamental differences in BTV and EHDV transmission dynamics between North America and Europe. In the United States, these viruses circulate in endemic cycles, driven by sustained vector activity and environmental conditions conducive to year-round transmission (72). In contrast, European outbreaks are typically sporadic, with stricter disease control measures such as vaccination and pre-movement testing, limiting disease persistence (73,74). Additionally, North American and European bison are distinct species with potentially different susceptibilities, immune responses, and interactions with *Culicoides* vectors,

further complicating direct epidemiological comparisons. Understanding these regional and species-specific differences is essential for accurately assessing disease risk and developing targeted surveillance strategies. Experimental infections with BTV-11 in North American bison demonstrated the ability of bison to develop viremia and mount an immune response without showing clinical signs (65). While EHD outbreaks in North America are commonly associated with high mortality in white-tailed deer, bison are generally considered incidental hosts. A 2007 EHD outbreak in a captive facility in Colorado confirmed seroconversion in bison without clinical disease, although the role of bison in EHDV transmission remains unclear (67). Contrastingly, during the 2012 EHDV outbreak in the United States, morbidity in bison reached 7%, highlighting the potential for disease under specific conditions (75).

Challenges in Orbivirus Control

In 1963, the World Organisation for Animal Health (WOAH), then known as the Office International des Epizooties (OIE), designated bluetongue as a notifiable disease due to severe health impact on animals as well as its detection beyond Africa (76). Initially thought to be restricted between 35°S and 40°N, the virus has now been detected up to 58°N and on every continent except Antarctica, driven by vector range expansion, viral evolution, and anthropogenic factors (55,59,77,78).

Since 1989, ten previously exotic bluetongue virus serotypes (BTV-1, BTV-3, BTV-5, BTV-6, BTV-9, BTV-12, BTV-14, BTV-19, BTV-22, and BTV-24) have been detected in enzootic regions of the southeastern United States, highlighting the ongoing expansion of BTV beyond its historical range (25,59). Prior to this, only five BTV serotypes (BTV-2, BTV-10, BTV-11, BTV-13, and BTV-17) and two EHDV serotypes

(EHDV-1 and EHDV-2) were considered endemic to the country. The increasing diversity of BTV serotypes in North America emphasizes the role of environmental changes, vector range shifts, and global livestock movement in facilitating the spread of *Orbiviruses* into new geographic regions.

Since the turn of the century, several economically devastating BTV and EHDV epizootic events have occurred, affecting both domestic and wild ruminants. The unprecedented outbreak of BTV-8 in Europe in 2006 marked a turning point, causing widespread morbidity and mortality among livestock, particularly sheep, and resulting in significant financial losses due to trade restrictions, vaccination costs, and decreased production (59). The outbreak, which initially began in the Netherlands, rapidly spread across Northern and Western Europe, with remarkable expansion to 58°N (73). Additionally, over 60,000 cases were recorded at its peak in 2007 and 27,000 cases in the following year (59). Bison were among the affected species in this outbreak, where BTV-8 led to up to 40% morbidity and 20% mortality in captive populations (53,70). Clinical signs observed in infected bison included lethargy, fever, mouth ulcers, drooling, difficulty eating, conjunctivitis, corneal edema, respiratory difficulty, lameness, and coronary band inflammation, with some cases resulting in sudden death (53). The severity of disease in bison during this outbreak highlights their susceptibility to novel BTV serotypes, particularly in populations without prior immunity.

Beyond individual outbreaks, the epidemiology of *Orbiviruses* is shaped by the co-circulation of multiple BTV and EHDV serotypes and the potential for viral reassortment, further complicating disease dynamics. Immunity acquired against one serotype often fails to confer cross-protection against others, increasing the likelihood of

repeated infections (79–81). Additionally, variations in virulence among strains within the same serotype can result in a wide spectrum of clinical outcomes, ranging from mild to severe disease (82,83). The complexity of *Orbivirus* epidemiology shows challenges in predicting disease impact and developing effective management strategies.

Vaccination for bluetongue virus (BTV) has significant limitations despite its role in controlling the disease. Live attenuated vaccines, while effective in some cases, carry risks such as teratogenic effects, incomplete attenuation, and potential transmission to non-vaccinated animals (84). These vaccines can also recombine with circulating strains, potentially reverting to virulence or creating reassorted viruses (84–86). Furthermore, they may lead to viremia sufficient for vector uptake and onward spread, exacerbating outbreaks (87,88). Inactivated vaccines are safer but require multiple doses, increasing costs and logistical challenges (86). Additionally, the diversity of BTV serotypes means that vaccination against one serotype often does not offer cross-protection against others, limiting the efficacy of current vaccines (84,86). As a result, control measures for BTV and EHDV must extend beyond vaccination alone, incorporating vector management strategies, movement restrictions, and targeted surveillance to mitigate disease spread. Given the increasing geographic expansion of BTV and EHDV, a comprehensive approach that integrates host, vector, and environmental factors is necessary to improve disease forecasting and response efforts.

Lack of Epidemiological Orbivirus Data in Bison: Summary

As a species of ecological, economic, and cultural importance, bison occupy a unique niche at the interface between domestic livestock and wildlife. Many states classify bison under dual designations, which has implications for disease monitoring

and movement regulations. Despite their increasing commercial production and translocation needs, surveillance efforts for infectious diseases in bison remain limited especially when compared to those in domestic livestock. Current *Orbivirus* surveillance efforts largely focus on species known to exhibit severe clinical disease, often overlooking bison due to their presumed incidental host status. However, environmental changes, shifting vector distributions, and bison-specific ecological behaviors may influence disease dynamics in ways that differ from other ruminants.

Geographic variability plays a critical role in *Orbivirus* transmission dynamics with cattle studies demonstrating regional clustering in the United States (89). While similar patterns are likely to exist in bison, systematic surveillance data are lacking. Additionally, environmental factors such as *Culicoides* breeding habitats, including bison wallows, may create localized hotspots for virus transmission, further complicating exposure risk assessments (71). Genetic factors may also contribute to species-specific differences in disease susceptibility and immune response, as the historical bottleneck in North American bison has resulted in reduced genetic diversity, potentially affecting pathogen resilience.

Overall, climate change is expected to increase the frequency, intensity, and geographic range of outbreaks for both viruses due to rising global temperatures and shifting environmental conditions. Collaborative efforts between wildlife and livestock health sectors will be essential for integrating bison into broader *Orbivirus* surveillance under a One Health framework.

Disease Threats and Surveillance Gaps: *Mycoplasma bovis*

Effective health surveillance is critical for managing disease threats in bison populations, yet significant gaps remain in our ability to detect, monitor, and mitigate emerging pathogens. Among these, *Mycoplasma bovis* has become a major concern due to its severe impact on herd health, high mortality rates, and the challenges associated with diagnosis and management. Unlike in cattle, where *M. bovis* is often a secondary pathogen associated with bovine respiratory disease complex, bison appear to be uniquely susceptible to *M. bovis* as a primary pathogen. However, much *M. bovis* research as well as diagnostic tools have to this date been extrapolated from cattle research.

Cross-Species Transmission

Mycoplasma species demonstrate remarkable capacities for cross-species transmission through integrated biological adaptations with consequences ranging from asymptomatic colonization to population level mortality events (90–95). Despite lacking a cell wall, which would typically reduce their ability to persist in the environment, mycoplasmas exhibit remarkable environmental durability likely in part due to the formation of biofilms (96). *Mycoplasma bovirhinis* has been shown to survive for 154 days and *Mycoplasma bovis* for 126 days on dry substrates at 4°C, while *Mycoplasma arginini* remains viable for 185 days in liquid media(97). This extended survival makes mycoplasmas durable in the environment, increasing the potential for indirect transmission across species at shared resource sites. Due to this, critical risk factors can emerge at anthropogenic interfaces such as *M. bovis* transmission events from cattle to pronghorn via contaminated water troughs (98). A documented outbreak in Austria further illustrates this risk, where *M. bovis* spilled over from cattle to pigs via

whey consumption and later re-emerged in cattle, emphasizing how shared resources can facilitate host-jumping events and prolonged pathogen circulation (94). Additional *Mycoplasma* species, such as *Mycoplasma ovipneumoniae,* can cause catastrophic pneumonia in bighorn sheep populations following spillover events from domestic counterparts (99). The bacterium has also been identified in diseased mule and white-tailed deer, as well as in apparently healthy Dall sheep, moose, caribou, and mule deer. This variation in disease expression emphasizes how host-specific immune responses and coevolutionary histories can also influence infection outcomes (100).

Given the growing evidence of *Mycoplasma* species crossing host barriers, bison, especially those with exposure to infected cattle or wildlife, may face an increased risk of spillover events. Indirect and direct transmission through respiratory aerosols, physical contact with infected animals, environmental reservoirs, shared water sources, or contaminated feed may present an ongoing threat to herd health. Therefore, there is a need for enhanced biosecurity measures and continued surveillance to prevent disease introduction and mitigate potential outbreaks.

M. bovis in Cattle: A Comparative Baseline

Mycoplasma bovis was first isolated in the United States in 1961 from the milk of a cow with mastitis (101). Initially described as *Mycoplasma agalactia* var *bovis*, it was later confirmed as a distinct species through serological responses and DNA homology studies (102). *M. bovis* spread globally through international trade in cattle and cattle products, reaching the UK and the rest of Europe in the mid-1970s (103). It continued to spread to all continents where cattle are kept, with New Zealand becoming the last major cattle-rearing country to be infected in 2017 (103).

M. bovis causes a variety of diseases in cattle, including mastitis, pneumonia, arthritis, otitis media, among others with presentation of disease often affected by management and age (103–106). In cattle, *M. bovis* primarily affects three target groups: feedlot cattle, where it is frequently associated with chronic pneumonia and polyarthritis syndrome (CPPS); dairy cattle, where it tends to manifest as mastitis; and calves, where it often presents as otitis media (104). The pathogen is frequently implicated in bovine respiratory disease complex (BRD) alongside *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni*, and respiratory viruses such as bovine herpesvirus-1 and bovine respiratory syncytial virus (104,107). Unlike some bacterial pathogens, *M. bovis* in cattle is rarely an isolated cause of BRD (107). Transmission primarily occurs through direct contact, respiratory secretions, and potentially through fomites, with evidence suggesting that asymptomatic carriers may serve as reservoirs for future outbreaks (104,108). Regardless of the production system, *M. bovis* infections are widely associated with economic losses due to decreased productivity and treatment challenges (103). In the United States, losses due to reduced weight gain and carcass value have been estimated at $32 million per year, while losses from bovine mastitis amount to approximately $108 million per year (109). The pathogen's ability to evade host immune responses, persist in infected cattle, and develop increasing antimicrobial resistance has made it a formidable challenge for the cattle industry, necessitating ongoing research into effective prevention and control strategies.

M. bovis in Bison: Disease Emergence and Severity

In bison, *M. bovis* was first noted as a major threat to feedlot herds in the late 2000s and noted as an emerging disease by the USDA APHIS in 2013 (110–112). Since then, the pathogen has continued to expand, with a notable surge in 2021 for both the number of affected herds and the geographic extent of outbreaks, threatening both conservation and production herds (113). The economic and conservation consequences of *M. bovis* in bison are severe (103,112). In production settings, high mortality and reduced reproductive success can result in substantial financial losses (114). Conservation herds face additional threats, as disease outbreaks can decimate limited populations, disrupt long-term restoration efforts, and hinder genetic diversity.

The disease course of *M. bovis* is divergent from cattle in many ways. *M. bovis* in bison appears more severe than in cattle, suggesting host-specific vulnerabilities that may contribute to increased morbidity and mortality. Whereas cattle typically experience *M. bovis* as a part of a polymicrobial disease complex, bison appear to have a unique susceptibility that allows the pathogen to act as a primary cause of severe respiratory disease (111,114,115). Comparative genomic analysis reveals that while *M. bovis* strains from cattle and bison share core virulence factors, key genetic differences may influence host adaptation and disease severity (116). Expanding sequencing efforts across bison herds could clarify whether strain-specific differences contribute to increased disease impact in this species.

Presentation of *M. bovis* in bison includes severe caseonecrotic pneumonia, lameness, chronic weight loss, and necrotizing pharyngitis, with affected herds experiencing mortality rates ranging from 10–45% (111,117). These outbreaks are often recurrent, indicating that *M. bovis* may establish persistent, latent infections in some

individuals, similar to *Mycobacterium bovis* (112,114). The most common clinical signs include lameness, reluctance to move, swollen joints, difficulty breathing, coughing, sluggishness and loss of body condition (114). Additionally, *M. bovis* has been reported to cause reproductive failure including dystocia, abortion, and reduced calving rates (114,118).

Pathologically, pulmonary lesions show necrotizing and granulomatous pneumonia with or without fibrinous pleuritis. Systemic dissemination can lead to abscess formation in the liver, spleen, mesentery, uterus and intestines (111,119). Histologically, infected joints show evidence of severe necropurulent synovitis and arthritis, which can be correlated with observed lameness and reluctance to move (111,119).

Another distinction from cattle is the apparent predilection of *M. bovis* for adult females in bison herds. This pattern is believed to be influenced by the matriarchal social structure of bison, where females lead herd movements and have more frequent close-contact interactions (120). Additionally, female bison may experience seasonal immunosuppression during late summer and fall due to the physiological demands of lactation and nutritional stress, further predisposing them to infection (112).

Due to the high mortality, reduced reproductive success, and loss of animals from translocation and breeding, the economic consequences of *M. bovis* outbreaks in bison herds are substantial. Conservation herds, which depend on genetic diversity and successful recruitment, are particularly vulnerable, as *M. bovis* outbreaks can decimate limited populations and disrupt long-term restoration efforts. Additionally, the inability to safely translocate bison due to uncertain *M. bovis* infection status threatens

conservation initiatives aimed at restoring herds across their historic range. Therefore, understanding the dynamics of this pathogen in bison is of utmost importance.

Pathogenesis and Immune Evasion of M. bovis

Mycoplasmas are small, with a genome mass between 500 to 1,000 kD and belong to the class *Mollicutes* (121). The bacterium is unique in its ability to evade host immune defenses, persist within tissues, and establish chronic infections. Unlike many bacterial pathogens, *M. bovis* lacks a cell wall, making it naturally resistant to antibiotics that target cell wall synthesis, such as beta-lactams (121,122). It primarily colonizes the respiratory tract but can disseminate to other organ systems, including the mammary glands, joints, and middle ear (123).

A key feature of *M. bovis* pathogenesis is its ability to evade immune clearance, which facilitates chronic infections and prolonged bacterial shedding. The bacterium expresses at least thirteen variable surface proteins (Vsps) that facilitate attachment to bronchial epithelial cells, a process that is crucial for infection establishment (124). *M. bovis* employs high frequency DNA rearrangements in its vsp gene locus to drive phase variation which enables antigenic switching through site specific chromosomal inversions and recombination events (125). This adaptive mechanism allows immune evasion by continuously altering surface epitopes, helping M. bovis persist in host environments despite antibody pressure (126,127). Additionally, *M. bovis* can move between different epithelial cell types, enabling systemic spread and persistent colonization (121). Isolates from animals exhibiting clinical disease demonstrate greater adherence capabilities than those from asymptomatic carriers, suggesting that adhesion

plays a role in virulence (124). Within biofilms, *M. bovis* can alter its antigenic profile, further complicating immune recognition and clearance (128).

In addition to these mechanisms, invasion studies have demonstrated that bison isolates can survive within bronchoalveolar lavage macrophages for extended periods, with some isolates displaying higher intracellular survival rates than cattle strains (129). This intracellular persistence may contribute to recurrent outbreaks in herds, as infected bison serve as long-term carriers. The ability of *M. bovis* to persist intracellularly and evade immune detection highlights the critical challenge of identifying and managing chronic carriers, particularly in conservation herds where translocation and genetic preservation efforts are at risk.

Despite its ability to evade early immune recognition, *M. bovis* eventually triggers a dysregulated inflammatory response, with high levels of pro- and anti-inflammatory cytokines, including IL-12, IL-10, IFN-γ, and TNF-α (130,131). This inflammatory imbalance may contribute to excessive lung tissue damage and caseonecrotic pneumonia, which is a common occurrence in severe *M. bovis* outbreaks in bison (130,131). However, much of the current understanding of the immune response to *M. bovis* in bison is based on in vitro studies, many of which have been conducted using cattle peripheral blood mononuclear cells (PBMCs), and these findings have yet to be fully validated in vivo. Preliminary evidence suggests that *M. bovis* isolates from bison differ from those in cattle, potentially contributing to the severe disease outcomes observed in bison. Bison derived strains are less effective at suppressing immune cell proliferation, leading to a stronger inflammatory response and increased tissue damage (129). In cattle, this intensified immune activation is further compounded by the

production of nitric oxide and TNF-α by alveolar macrophages, both of which are known to exacerbate lung pathology (132). Additionally, cytokine dysregulation can weaken lung epithelial junctions, further compromising respiratory integrity and may worsen disease severity (133,134).

Infected cattle often show a Th2-skewed immune response, characterized by increased IgG1 and IL-4 production, which is less effective at clearing infection and contributes to chronic disease (108). In bison, there is growing evidence that IgG2 levels play a role in bacterial clearance. A study on naturally infected bison found that higher serum IgG2 levels were associated with reduced PCR detection of *M. bovis*, suggesting that a stronger Th1-associated immune response may enhance bacterial clearance (135). Bison with low IgG2 responses had persistent *M. bovis* detection, highlighting the importance of Th-1 responses in bacterial clearance.

The dysregulated immune response in *M. bovis* infection is further compounded by immune exhaustion mechanisms. Chronic infections in cattle have been associated with upregulation of inhibitory receptors, such as programmed cell death-1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), which suppress T-cell responses and further impairs the host's ability to clear infection (136). This may suggest that bison with persistent infections could experience a combination of immune exhaustion and inadequate Th1-mediated bacterial clearance, reinforcing *M. bovis*'s ability to establish long-term, subclinical infections in some individuals. Bison that develop chronic infections might be more likely to experience long-term immune suppression, which not only facilitates bacterial persistence but may also increase susceptibility to secondary infections.

The differences in *M. bovis* pathogenesis between cattle and bison suggest that bison-specific isolates may have evolved unique adaptations, or that bison immune responses differ significantly from cattle, leading to greater susceptibility and severe disease outcomes. The ability of *M. bovis* to persist within host tissues, evade immune responses, and induce excessive inflammation expresses the need for bison-specific diagnostic tools and targeted biosecurity strategies. Further genomic and proteomic studies on bison isolates may provide insight into the molecular mechanisms underlying these host-pathogen differences, which could inform vaccine development and disease management practices in bison populations.

Diagnostic Limitations of M. bovis in Bison

The accurate detection of *M. bovis* in bison presents several challenges due to the pathogen's biological characteristics, host interactions, and the limitations of available antemortem diagnostic assays. One of the major diagnostic challenges is the presence of subclinical infections and chronic carriers within bison herds that can silently promote persistence (137,138). Additionally, chronic carriers in cattle are known to shed *M. bovis* intermittently and this is speculated to be true in bison (104,139,140). This further complicates disease detection, as a negative test result does not necessarily indicate the absence of infection.

The current diagnostic tools for *M. bovis* are primarily developed for cattle and lack validation for bison. The most reliable method for detecting *M. bovis* is bacterial culture; however, this process is challenging and time-consuming. *Mycoplasma bovis* is notoriously difficult to grow in laboratory conditions due to its specific growth requirements, and its colonies can easily be overwhelmed by other bacteria making

isolation and identification difficult (106). PCR testing of deep nasopharyngeal swabs is currently considered the most sensitive method for detecting active infections in bison (82.8%), however there is poor agreement between superficial nasal and deep nasopharyngeal swabs suggesting that stage of infection and site of colonization may influence test outcome (140).

While ELISA-based serological tests are widely used in cattle, they have demonstrated low sensitivity and specificity in bison due to cross-reactivity with non-pathogenic *Mycoplasma* species such as *M. bovirhinis* and *M. bovoculi* (138). Additionally, it has been reported that *M. bovis* can persist in the respiratory tract of health bison and have a variable antibody response ranging from no to high responders (135,138). It has been suggested that bison that mount a strong humoral immune response initially, may be more efficient at clearing infection (135). Additional reports demonstrate that commercial ELISA tests developed for cattle sera are not optimal for bison, particularly when antibody levels are low to moderate (141). The antigenic variability of *M. bovis* strains found in bison may also reduce assay sensitivity, as these strains may differ from those used in cattle-based diagnostic tests (142–144). The lack of an optimized serological assay for bison complicates biosecurity efforts and limits the ability to make informed management decisions regarding herd health and animal translocation.

In summary, the diagnosis of *M. bovis* in bison is challenged by subclinical and chronic carriers, immunogenic variation in the host, variable surface proteins expressed by *M. bovis,* and challenges associated with sample collection. Developing a reliable serological test would provide critical tools for herd health monitoring, facilitating early

detection of *M. bovis* outbreaks and enabling safer translocation of disease-free bison for conservation efforts. Moreover, improved diagnostics would support research into chronic infections and transmission dynamics, informing targeted management strategies to reduce disease burden in both production and conservation herds.

Research Objectives and Hypotheses

The overarching objective of this dissertation is to advance the understanding of disease surveillance, biosecurity, and health management in North American bison through a multidisciplinary One Health framework. By integrating epidemiological studies, expert consensus methodologies, and ecological assessments, this research seeks to bridge gaps in bison health monitoring and inform more effective management strategies across public, private, tribal, and conservation sectors.

The specific objectives of this research are:

1. To define bison health and identify gaps in bison health surveillance, biosecurity, and diagnostic tools from a multidisciplinary perspective using the Delphi method
    - Hypothesis 1: Agreement on a definition of bison health, key disease concerns and management priorities can be reached across stakeholder groups, despite differences in objectives.
    - Hypothesis 2: The current state of bison disease surveillance lacks standardization across sectors highlighted by lack of bison-specific diagnostic tools and research.

2. To characterize the prevalence, serotype diversity, and epidemiology of Bluetongue Virus (BTV) and Epizootic Hemorrhagic Disease Virus (EHDV) in North American bison
    - Hypothesis 1: BTV and EHDV seroprevalence in bison is high and varies geographically, influenced by climate, vector distributions, and habitat characteristics.
    - Hypothesis 2: Age-related differences exist in seroprevalence and active infection rates, leading to differences in disease management strategies.
3. To evaluate the accuracy and reliability of ELISA-based serological assays for detecting *Mycoplasma bovis* antibodies in bison
    - Hypothesis 1: Commercially available ELISA assays designed for cattle sera will demonstrate reduced sensitivity and specificity when applied to bison due to antigenic variation and cross-reactivity with non-pathogenic *Mycoplasma* species.
    - Hypothesis 2: The newly designed P48 ELISA will demonstrate improved sensitivity and specificity compared to commercially available ELISAs when detecting *M. bovis* antibodies in bison.
    - Hypothesis 3: The P48 ELISA will reduce cross-reactivity with non-pathogenic *Mycoplasma* species commonly found in bison, leading to more accurate serological results.

Structure of the Dissertation

Chapter 1: Introduction and Literature Review

This chapter provides background on bison health in a One Health context and reviews translocation-related health risks, the need for a multidisciplinary approach for bison health, and major pathogen threats including bluetongue virus, epizootic hemorrhagic disease virus, and *Mycoplasma bovis*.

Chapter 2: Delphi Survey Leads to Multi-Stakeholder Consensus on the Definition of Health in North American Bison (Bison Bison)

This chapter presents a Delphi survey that engaged experts from diverse sectors to develop a working definition of bison health. It identifies key health determinants, common disease concerns, and management/research priorities from conservation, production, and tribal perspectives.

Chapter 3: High Seroprevalence and Age-Associated Dynamics of Bluetongue and Epizootic Hemorrhagic Disease in North American Bison (Bison Bison)

This chapter details the epidemiological study on BTV and EHDV in bison herds, highlighting seroprevalence trends, regional variations, and the influence of environmental and herd factors on disease transmission.

Chapter 4: Evaluation of a P48 ELISA for Mycoplasma bovis in North American Bison (Bison bison): Inferior Performance Compared to a Commercially Available ELISA

This chapter assesses the diagnostic accuracy of a newly designed P48 ELISA against an existing commercial ELISA assay. It examines differences in sensitivity,

specificity, and cross-reactivity, revealing key limitations and opportunities for assay refinement.

Chapter 5: Discussion and Conclusions

This final chapter synthesizes findings across all studies, emphasizing their implications for bison health management and future research directions. It provides recommendations for enhancing disease surveillance, improving diagnostic accuracy, and integrating One Health principles into bison conservation and production systems.

REFERENCES

1. Ratajczak Z, Collins Sl, Blair Jm, Koerner Se, Louthan Am, Smith Md, et al. Reintroducing Bison Results in Long-Running and Resilient Increases in Grassland Diversity. *Proceedings of the National Academy of Sciences.* 2022 Sep 6;119(36).
2. Truett Jc, Phillips M, Kunkel K, Miller R. Managing Bison to Restore Biodiversity [Internet]. Vol. 11, *Plains Research.* 2001. Available from: https://about.jstor.org/terms
3. National Bison Association. Bison by the Numbers.
4. Mcdaniel J, Askew W, Bennett D, Mihalopoulos J, Anantharaman S, Fjeldstad As, et al. Bison Meat Has a Lower Atherogenic Risk Than Beef in Healthy Men. *Nutrition Research.* 2013 Apr;33(4):293–302.
5. Shamon H, Cosby Og, Andersen Cl, Augare H, Bearcub Stiffarm J, Bresnan Ce, et al. The Potential of Bison Restoration as an Ecological Approach to Future Tribal Food Sovereignty on the Northern Great Plains. *Front Ecol Evol.* 2022 Jan 28;10.
6. Adisasmito Wb, Almuhairi S, Behravesh Cb, Bilivogui P, Bukachi Sa, Casas N, et al. One Health: A New Definition for a Sustainable and Healthy Future. *PLoS Pathog.* 2022 Jun 1;18(6).
7. Moloney Cj, Chambliss Wj. Slaughtering the Bison, Controlling Native Americans: A State Crime and Green Criminology Synthesis. *Crit Criminol.* 2014;22(3):319–38.
8. Feir Rob Gillezeau Maggie Ec Jones Dl, Ferrara A, Gregg M, Jaremski M, Leonard B, Parker D, et al. The Slaughter of the Bison and Reversal of Fortunes on the Great Plains [Internet]. 2022. Available from: http://www.nber.org/data-appendix/w30368
9. The Extermination of the American Bison From the Report of the National Museum. 1886.
10. Shaw Jh. How Many Bison Originally Populated Western Rangelands? Vol. 17, *148 Rangelands.* 1995.
11. Taylor Ms. Buffalo Hunt: International Trade and the Virtual Extinction of the North American Bison. *American Economic Review.* 2011 Dec;101(7):3162–95.
12. Hedrick Pw. Conservation Genetics and North American Bison (Bison Bison). *Journal of Heredity.* 2009 Jul 1;100(4):411–20.
13. Sanderson Ew, Redford Kh, Weber B, Aune K, Baldes D, Berger J, et al. The Ecological Future of the North American Bison: Conceiving Long-Term, Large-Scale Conservation of Wildlife. Vol. 22, *Conservation Biology.* 2008. p. 252–66.
14. Gross Je, Wang G, Halbert Nd, Gogan Pa, Derr Jn, Templeton Jw. Effects of Population Control Strategies on Retention of Genetic Diversity in National Park Service Bison (Bison Bison) Herds. 2006.
15. Hedrick Pw, Kalinowski St. Inbreeding Depression in Conservation Biology. *Annu Rev Ecol Syst.* 2000 Nov;31(1):139–62.

16. Keller L, Waller D. Inbreeding Effects in Wild Populations. *Trends Ecol Evol.* 2002 May 1;17(5):230–41.
17. Kock Ra, Woodford Mh, Rossiter Pb. Disease Risks Associated With the Translocation of Wildlife. *Revue Scientifique et Technique de l'OIE.* 2010 Aug 1;29(2):329–50.
18. Lafferty Kd, Kuris Am. How Environmental Stress Affects the Impacts of Parasites. *Limnol Oceanogr.* 1999 May 15;44(3part2):925–31.
19. Johnson Wl, Reynolds S, Adkins Cl, Wehus-Tow B, Brennan J, Krus Cb, et al. A Comparison of Mini-FLOTAC and McMaster Techniques, Overdispersion and Prevalence of Parasites in Naturally Infected North American Bison (Bison Bison) in the USA. *Current Research in Parasitology & Vector-Borne Diseases.* 2022;2:100103.
20. Penzhorn Bl, Knapp Se, Speer Ca. Enteric Coccidia in Free-Ranging American Bison (Bison Bison) in Montana. *J Wildl Dis.* 1994 Apr;30(2):267–9.
21. Woodbury Mr, Wagner B, Ben-Ezra E, Douma D, Wilkins W. A Survey to Detect Toxocara Vitulorum and Other Gastrointestinal Parasites in Bison (Bison Bison) Herds From Manitoba and Saskatchewan. *Can Vet J.* 2014 Sep;55(9):870–4.
22. Wiese Jd, Caven Aj, Zarlenga Ds, Topliff Cl, Kelling Cl, Salter J. Gastrointestinal Parasites of a Reintroduced Semi-Wild Plains Bison (Bison Bison Bison) Herd: Examining Effects of Demographic Variation, Deworming Treatments, and Management Strategy. *Int J Parasitol Parasites Wildl.* 2021 Apr;14:216–27.
23. Krzysiak Mk, Iwaniak W, Kęsik-Maliszewska J, Olech W, Larska M. Serological Study of Exposure to Selected Arthropod-Borne Pathogens in European Bison (Bison Bonasus) in Poland. *Transbound Emerg Dis.* 2017 Oct 1;64(5):1411–23.
24. Gould Ea, Higgs S. Impact of Climate Change and Other Factors on Emerging Arbovirus Diseases. Vol. 103, *Transactions of the Royal Society of Tropical Medicine and Hygiene.* 2009. p. 109–21.
25. Mayo C, Mcdermott E, Kopanke J, Stenglein M, Lee J, Mathiason C, et al. Ecological Dynamics Impacting Bluetongue Virus Transmission in North America. Vol. 7, *Frontiers in Veterinary Science.* Frontiers Media S.A.; 2020.
26. Stephen C. Toward a Modernized Definition of Wildlife Health. *J Wildl Dis.* 2014;50(3):427–30.
27. Deem Sl, Karesh Wb, Weisman W. Putting Theory Into Practice: Wildlife Health in Conservation. *Conservation Biology.* 2001 Oct 20;15(5):1224–33.
28. Hanisch Sl, Riley Sj, Nelson Mp. Promoting Wildlife Health or Fighting Wildlife Disease: Insights From History, Philosophy, and Science. *Wildl Soc Bull.* 2012 Sep;36(3):477–82.
29. Kindig D, Stoddart G. What Is Population Health? [Internet]. Vol. 93, *American Journal of Public Health.* 2003. Available from: http://www.cihr-irsc.gc.ca/

30. Pampalon R. Developing a Healthy Communities Index: A Collection of Papers. 2005.
31. Arah Oa. On the Relationship Between Individual and Population Health. *Med Health Care Philos.* 2009;12(3):235–44.
32. Gunnarsson S. The Conceptualisation of Health and Disease in Veterinary Medicine. *Acta Vet Scand.* 2006;48(1).
33. OIE, IUCN. World Organisation for Animal Health (OIE) & International Union for Conservation of Nature (IUCN) (2014) – Guidelines for Wildlife Disease Risk Analysis. 2014.
34. Patyk Ka, Duncan C, Nol P, Sonne C, Laidre K, Obbard M, et al. Establishing a Definition of Polar Bear (Ursus Maritimus) Health: A Guide to Research and Management Activities. *Science of the Total Environment.* 2015 May 1;514:371–8.
35. Wittrock J, Duncan C, Stephen C. A Determinants of Health Conceptual Model for Fish and Wildlife Health. *J Wildl Dis.* 2019 Apr 1;55(2):285–97.
36. Spreull J. Malarial Catarrhal Fever (Bluetongue) of Sheep in South Africa. *Journal of Comparative Pathology and Therapeutics.* 1905 Jan;18:321–37.
37. Savini G, Afonso A, Mellor P, Aradaib I, Yadin H, Sanaa M, et al. Epizootic Heamorragic Disease. Vol. 91, *Research in Veterinary Science.* 2011. p. 1–17.
38. Rivera Na, Varga C, Ruder Mg, Dorak Sj, Roca Al, Novakofski Je, et al. Bluetongue and Epizootic Hemorrhagic Disease in the United States of America at the Wildlife-Livestock Interface. *Pathogens.* 2021 Aug 1;10(8).
39. Shope Re, Macnamara Lg, Mangold R. A Virus-Induced Epizootic Hemorrhagic Disease of the Virginia White-Tailed Deer (Odocoileus Virginianus).
40. Du Toit Rm. The Transmission of Blue-Tongue and Horse-Sickness by Culicoides. *Ondersrepoort Journal of Veterinary Science and Animal Industry.* 1994;7–15.
41. Velthuis Agj, Saatkamp Hw, Mourits Mcm, De Aa, Elbers Arw. 72 Economic Consequences of the Dutch Bluetongue Serotype 8 Epidemic in 2006 and 2007.
42. Anthony Sj, Maan S, Maan N, Kgosana L, Bachanek-Bankowska K, Batten C, et al. Genetic and Phylogenetic Analysis of the Outer-Coat Proteins VP2 and VP5 of Epizootic Haemorrhagic Disease Virus (EHDV): Comparison of Genetic and Serological Data to Characterise the EHDV Serogroup. *Virus Res.* 2009 Nov;145(2):200–10.
43. Yang H, Gu W, Li Z, Zhang L, Liao D, Song J, et al. Novel Putative Bluetongue Virus Serotype 29 Isolated From Inapparently Infected Goat in Xinjiang of China. *Transbound Emerg Dis.* 2021 Jul 6;68(4):2543–55.
44. Bumbarov V, Golender N, Jenckel M, Wernike K, Beer M, Khinich E, et al. Characterization of Bluetongue Virus Serotype 28. *Transbound Emerg Dis.* 2020 Jan 10;67(1):171–82.
45. Roy P. Bluetongue Virus Structure and Assembly. *Curr Opin Virol.* 2017 Jun;24:115–23.
46. Ratinier M, Caporale M, Golder M, Franzoni G, Allan K, Nunes Sf, et al. Identification and Characterization of a Novel Non-Structural Protein of Bluetongue Virus. *PLoS Pathog.* 2011 Dec;7(12).

47. Maclachlan Nj, Drew Cp, Darpel Ke, Worwa G. The Pathology and Pathogenesis of Bluetongue. Vol. 141, *Journal of Comparative Pathology.* 2009. p. 1–16.
48. Barratt-Boyes Sm, Rossitto P V., Stott Jl, Maclachlan Nj. Flow Cytometric Analysis of In Vitro Bluetongue Virus Infection of Bovine Blood Mononuclear Cells. *Journal of General Virology.* 1992 Aug 1;73(8):1953–60.
49. Bonneau Kr, Demaula Cd, Mullens Ba, Maclachlan Nj. Duration of Viraemia Infectious to Culicoides Sonorensis in Bluetongue Virus-Infected Cattle and Sheep.
50. Darpel Ke, Batten Ca, Veronesi E, Shaw Ae, Anthony S, Bachanek-Bankowska K, et al. Clinical Signs and Pathology Shown by British Sheep and Cattle Infected With Bluetongue Virus Serotype 8 Derived From the 2006 Outbreak in Northern Europe. *Veterinary Record.* 2007 Aug 25;161(8):253–61.
51. Maclachlan Nj, Mayo Ce, Daniels Pw, Savini G, Zientara S, Gibbs Epj. Bluetongue. Vol. 34, *Rev. Sci. Tech. Off. Int. Epiz.* 2015.
52. Schwartz-Cornil I, Mertens Ppc, Contreras V, Hemati B, Pascale F, Bréard E, et al. Bluetongue Virus: Virology, Pathogenesis and Immunity. Vol. 39, *Veterinary Research.* 2008.
53. Sanderson S, Zoo C. Bluetongue in Non-Domestic Ruminants: Experiences Gained in EAZA Zoos During the 2007 & 2008 BTV8 and BTV1 Epizootics.
54. Marquardt Wc, Kondratieff Bc, Borkent A. The Biting Midges, the Ceratopogonidae (Diptera). In: Marquardt Wh, editor. *Biology of Disease Vectors* [Internet]. Elsevier Science & Technology; 2004. p. 113–26. Available from: http://ebookcentral.proquest.com/lib/csu/detail.action?docID=297059.
55. Mellor P, Boorman J, Baylis M. Culicoides Biting Midges: Their Role as Arbovirus Vectors [Internet]. Vol. 45, *Annu. Rev. Entomol.* 2000. Available from: www.annualreviews.org
56. Mellor Ps, Carpenter S, White Dm. Bluetongue Virus in the Insect Host 14. 2009.
57. Mills Mk, Michel K, Pfannenstiel Rs, Ruder Mg, Veronesi E, Nayduch D. Culicoides–Virus Interactions: Infection Barriers and Possible Factors Underlying Vector Competence. Vol. 22, *Current Opinion in Insect Science.* Elsevier Inc.; 2017. p. 7–15.
58. Rozo-Lopez P, Park Y, Drolet Bs. Effect of Constant Temperatures on Culicoides Sonorensis Midge Physiology and Vesicular Stomatitis Virus Infection. *Insects.* 2022 Apr 1;13(4).
59. Maclachlan Nj. Global Implications of the Recent Emergence of Bluetongue Virus in Europe. Vol. 26, *Veterinary Clinics of North America - Food Animal Practice.* 2010. p. 163–71.
60. Coetzee P, Van Vuuren M, Venter Eh, Stokstad M. A Review of Experimental Infections With Bluetongue Virus in the Mammalian Host. *Virus Res.* 2014 Mar 28;182:21–34.
61. Singer Rs, Maclachlan Nj, Carpenter Te. Maximal Predicted Duration of Viremia in Bluetongue Virus-Infected Cattle. Vol. 13, *J Vet Diagn Invest.* 2001.
62. Koumbati M, Mangana O, Nomikou K, Mellor Ps, Papadopoulos O. Duration of Bluetongue Viraemia and Serological Responses in Experimentally Infected European Breeds of Sheep and Goats.
63. Work Tm, Jessup Da, Sawyer Mm. Experimental Bluetongue and Epizootic Hemorrhagic Disease Virus Infection in California Black-Tailed Deer [Internet]. Vol.

28, *Journal of Wildlife Diseases.* 1992. Available from: http://meridian.allenpress.com/jwd/article-pdf/28/4/623/2234218/0090-3558-28_4_623.pdf
64. Murray J 0, Trainer D 0. Bluetongue Virus in North American Elk [Internet]. Vol. 6, *Journal of Wildlife Diseases.* 1970. Available from: http://meridian.allenpress.com/jwd/article-pdf/6/3/144/2243580/0090-3558-6_3_144.pdf
65. Tessaro S V, Clavijo A. Duration of Bluetongue Viremia in Experimentally Infected American Bison [Internet]. Vol. 37, *Journal of Wildlife Diseases.* 2001. Available from: http://meridian.allenpress.com/jwd/article-pdf/37/4/722/2235624/0090-3558-37_4_722.pdf
66. Drolet Bs, Mills Kw, Belden El, Mecham34 J 0. Enzyme-Linked Immunosorbent Assay for Efficient Detection of Antibody to Bluetongue Virus in Pronghorn (Antilocapra Americana) [Internet]. Vol. 26, *Journal of Wildlife Diseases.* 1990. Available from: http://meridian.allenpress.com/jwd/article-pdf/26/1/34/2232902/0090-3558-26_1_34.pdf
67. Nol P, Kato C, Reeves Wk, Rhyan J, Spraker T, Gidlewski T, et al. Epizootic Hemorrhagic Disease Outbreak in a Captive Facility Housing White-Tailed Deer (Odocoileus Virginianus), Bison (Bison Bison), Elk (Cervus Elaphus), Cattle (Bos Taurus), and Goats (Capra Hircus) in Colorado, USA. *Journal of Zoo and Wildlife Medicine.* 2010 Sep;41(3):510–5.
68. Maclachlan Nj, Zientara S, Wilson Wc, Richt Ja, Savini G. Bluetongue and Epizootic Hemorrhagic Disease Viruses: Recent Developments With These Globally Re-Emerging Arboviral Infections of Ruminants [Internet]. 2018. Available from: https://www.elsevier.com/open-access/userlicense/1.0/
69. Larska M, Tomana J, Socha W, Rola J, Kubiś P, Olech W, et al. Learn the Past and Present to Teach the Future—Role of Active Surveillance of Exposure to Endemic and Emerging Viruses in the Approach of European Bison Health Protection. *Diversity (Basel).* 2023 Apr 1;15(4).
70. Didkowska A, Klich D, Nowak M, Wojciechowska M, Prolejko K, Kwiecień E, et al. A Serological Survey of Pathogens Associated With the Respiratory and Digestive System in the Polish European Bison (Bison Bonasus) Population in 2017–2022. *BMC Vet Res.* 2023 Dec 1;19(1).
71. Pfannenstiel Rs, Ruder Mg. Colonization of Bison (Bison Bison) Wallows in a Tallgrass Prairie by Culicoides Spp (Diptera: Ceratopogonidae). *Journal of Vector Ecology.* 2015 Jun 1;40(1):187–90.
72. Committee on Foreign and Emerging Animal Diseases. Re-Evaluation of Endemic Bluetongue Virus Serotypes in the United States. 2020 Oct.
73. Wilson Aj, Mellor Ps. Bluetongue in Europe: Past, Present and Future. Vol. 364, *Philosophical Transactions of the Royal Society B: Biological Sciences.* Royal Society; 2009. p. 2669–81.
74. Gondard M, Postic L, Garin E, Turpaud M, Vorimore F, Ngwa-Mbot D, et al. Exceptional Bluetongue Virus (BTV) and Epizootic Hemorrhagic Disease Virus (EHDV) Circulation in France in 2023. *Virus Res* [Internet]. 2024 Oct 27;199489. Available from: http://www.ncbi.nlm.nih.gov/pubmed/39471970

75. Stevens G, Mccluskey B, King A, O'hearn E, Mayr G. Review of the 2012 Epizootic Hemorrhagic Disease Outbreak in Domestic Ruminants in the United States. *PLoS One.* 2015 Aug 5;10(8).
76. Coetzee P, Stokstad M, Venter Eh, Myrmel M, Van Vuuren M. Bluetongue: A Historical and Epidemiological Perspective With the Emphasis on South Africa. Vol. 9, *Virology Journal.* 2012.
77. Nomikou K, Hughes J, Wash R, Kellam P, Breard E, Zientara S, et al. Widespread Reassortment Shapes the Evolution and Epidemiology of Bluetongue Virus Following European Invasion. *PLoS Pathog.* 2015 Aug 1;11(8).
78. Lysyk Tj, Dergousoff Sj. Distribution of Culicoides Sonorensis (Diptera: Ceratopogonidae) in Alberta, Canada. *J Med Entomol.* 2014;51(3):560–71.
79. Fay Pc, Jaafar Fm, Batten C, Attoui H, Saunders K, Lomonossoff Gp, et al. Serological Cross-Reactions Between Expressed VP2 Proteins From Different Bluetongue Virus Serotypes. *Viruses.* 2021 Aug 1;13(8).
80. Martinelle L, Dal Pozzo F, Thys C, De Leeuw I, Van Campe W, De Clercq K, et al. Assessment of Cross-Protection Induced by a Bluetongue Virus (BTV) Serotype 8 Vaccine Towards Other BTV Serotypes in Experimental Conditions. *Vet Res.* 2018 Jul 16;49(1).
81. Bissett Sl, Roy P. Impact of VP2 Structure on Antigenicity: Comparison of BTV1 and the Highly Virulent BTV8 Serotype. Wobus Ce, editor. *J Virol* [Internet]. 2024 Oct 22;98(10). Available from: https://journals.asm.org/doi/10.1128/jvi.00953-24
82. Jiménez-Cabello L, Utrilla-Trigo S, Lorenzo G, Ortego J, Calvo-Pinilla E. Epizootic Hemorrhagic Disease Virus: Current Knowledge and Emerging Perspectives. Vol. 11, *Microorganisms.* MDPI; 2023.
83. Janowicz A, Caporale M, Shaw A, Gulletta S, Di Gialleonardo L, Ratinier M, et al. Multiple Genome Segments Determine Virulence of Bluetongue Virus Serotype 8. *J Virol.* 2015 May 15;89(10):5238–49.
84. Celma Cc, Stewart M, Wernike K, Eschbaumer M, Gonzalez-Molleda L, Breard E, et al. Replication-Deficient Particles: New Insights Into the Next Generation of Bluetongue Virus Vaccines. *J Virol.* 2017 Jan;91(1).
85. Roy P, Boyce M, Noad R. Prospects for Improved Bluetongue Vaccines. Vol. 7, *Nature Reviews Microbiology.* 2009. p. 120–8.
86. Chapter 3.1.3 Bluetongue (Infection With Bluetongue Virus). 2021.
87. Venter Gj, Gerdes Gh, Mellor Ps, Paweska Jt. Transmission Potential of South African Culicoides Species for Live-Attenuated Bluetongue Virus. *Vet Ital.* 2004;40(3):198–202.
88. Listeš E, Monaco F, Labrović A, Paladini C, Leone A, Di Gialleonardo L, et al. First Evidence of Bluetongue Virus Serotype 16 in Croatia. *Vet Microbiol.* 2009 Jul 2;138(1–2):92–7.
89. Hoar Br, Carpenter Te, Singer Rs, Gardner Ia. Regional Risk of Exporting Cattle Seropositive for Bluetongue Virus From the United States. Vol. 64, *AJVR.* 2003.

90. Hartup Bk, Dhondt Aa, Sydenstricker K V., Hochachka Wm, Kollias G V. Host Range and Dynamics of Mycoplasmal Conjunctivitis Among Birds in North America. *J Wildl Dis.* 2001 Jan;37(1):72–81.
91. Butler Cj, Edwards Wh, Paterson Jt, Proffitt Km, Jennings-Gaines Je, Killion Hj, et al. Respiratory Pathogens and Their Association With Population Performance in Montana and Wyoming Bighorn Sheep Populations. *PLoS One.* 2018 Nov 26;13(11):e0207780.
92. Besser Te, Frances Cassirer E, Highland Ma, Wolff P, Justice-Allen A, Mansfield K, et al. Bighorn Sheep Pneumonia: Sorting Out the Cause of a Polymicrobial Disease. *Prev Vet Med.* 2013 Feb;108(2–3):85–93.
93. Dhondt Aa, Tessaglia Dl, Slothower Rl. Epidemic Mycoplasmal Conjunctivitis in House Finches From Eastern North America. *J Wildl Dis.* 1998 Apr;34(2):265–80.
94. Spergser J, Macher K, Kargl M, Lysnyansky I, Rosengarten R. Emergence, Re-Emergence, Spread and Host Species Crossing of Mycoplasma Bovis in the Austrian Alps Caused by a Single Endemic Strain. *Vet Microbiol.* 2013;164(3–4):299–306.
95. Malmberg Jl, O'toole D, Creekmore T, Peckham E, Killion H, Vance M, et al. Mycoplasma Bovis Infections in Free-Ranging Pronghorn, Wyoming, USA. In: *Emerging Infectious Diseases.* Centers for Disease Control and Prevention (CDC); 2020. p. 2807–14.
96. Mcauliffe L, Ellis Rj, Miles K, Ayling Rd, Nicholas Raj. Biofilm Formation by Mycoplasma Species and Its Role in Environmental Persistence and Survival. *Microbiology (N Y).* 2006 Apr 1;152(4):913–22.
97. Nagatomo H, Takegahara Y, Sonoda T, Yamaguchi A, Uemura R, Hagiwara S, et al. Comparative Studies of the Persistence of Animal Mycoplasmas Under Different Environmental Conditions.
98. Johnson M, Macglover C, Peckham E, Killion H, Allen Se, Creekmore T, et al. Source and Seasonality of Epizootic Mycoplasmosis in Free-Ranging Pronghorn (Antilocapra Americana). *J Wildl Dis.* 2022 Jul 1;58(3):524–36.
99. Besser Te, Highland Ma, Baker K, Cassirer Ef, Anderson Nj, Ramsey Jm, et al. Causes of Pneumonia Epizootics Among Bighorn Sheep, Western United States, 2008–2010. *Emerg Infect Dis.* 2012 Mar;18(3):406–14.
100. Highland Ma, Herndon Dr, Bender Sc, Hansen L, Gerlach Rf, Beckmen Kb. Mycoplasma Ovipneumoniae in Wildlife Species Beyond Subfamily Caprinae. *Emerg Infect Dis.* 2018 Dec;24(12):2384–6.
101. Hale Hh, Helmboldt Cf, Plastridge Wn, Stula Ef. Bovine Mastitis Caused by a Mycoplasma Species. *Cornell Vet.* 1962 Oct;52:582–91.
102. Askaa G, Erno H. Elevation of Mycoplasma Agalactiae Subsp. Bovis to Species Rank: Mycoplasma Bovis (Hale et al.) Comb. Nov. *Int J Syst Bacteriol.* 1976 Jul 1;26(3):323–5.

103. Dudek K, Nicholas Raj, Szacawa E, Bednarek D. Mycoplasma Bovis Infections—Occurrence, Diagnosis and Control. Vol. 9, *Pathogens.* MDPI AG; 2020. p. 1–21.
104. Maunsell Fp, Woolums Ar, Francoz D, Rosenbusch Rf, Step Dl, Wilson Dj, et al. Mycoplasma Bovis Infections in Cattle. *J Vet Intern Med.* 2011 Jul;25(4):772–83.
105. Hermeyer K, Buchenau I, Thomasmeyer A, Baum B, Spergser J, Rosengarten R, et al. Chronic Pneumonia in Calves After Experimental Infection With Mycoplasma Bovis Strain 1067: Characterization of Lung Pathology, Persistence of Variable Surface Protein Antigens and Local Immune Response. *Acta Vet Scand.* 2012 Feb 4;54(1).
106. Gelgie Ae, Desai Se, Gelalcha Bd, Kerro Dego O. Mycoplasma Bovis Mastitis in Dairy Cattle. Vol. 11, *Frontiers in Veterinary Science.* Frontiers Media SA; 2024.
107. Oliveira Tes, Pelaquim If, Flores Ef, Massi Rp, Valdiviezo Mjj, Pretto-Giordano Lg, et al. Mycoplasma Bovis and Viral Agents Associated With the Development of Bovine Respiratory Disease in Adult Dairy Cows. *Transbound Emerg Dis.* 2020 Jul 1;67(S2):82–93.
108. Nicholas Raj, Ayling Rd, Stipkovits Lp. An Experimental Vaccine for Calf Pneumonia Caused by Mycoplasma Bovis: Clinical, Cultural, Serological and Pathological Findings. *Vaccine.* 2002 Oct;20(29–30):3569–75.
109. Stipkovits L, Rosengarten R, Frey J. Mycoplasmas of Ruminants: Pathogenicity, Diagnostics, Epidemiology and Molecular Genetics. *European Cooperation on Scientific and Technical Research.* 1999;3.
110. Dyer N, Register Kb, Miskimins D, Newell T. Necrotic Pharyngitis Associated With Mycoplasma Bovis Infections in American Bison (Bison Bison). *Journal of Veterinary Diagnostic Investigation.* 2013 Mar 1;25(2):301–3.
111. Janardhan Ks, Hays M, Dyer N, Oberst Rd, Debey Bm. Mycoplasma Bovis Outbreak in a Herd of North American Bison (Bison Bison). *Journal of Veterinary Diagnostic Investigation.* 2010 Sep 1;22(5):797–801.
112. Sweeney S, Jones R, Patyk K, Losapio C. Mycoplasma Bovis – An Emerging Pathogen in Ranched Bison. *USDA APHIS Emerging Disease Notice.* 2013.
113. NBA Mycoplasma Bovis Fact Sheet – May 2022. 2022.
114. Bras Al, Barkema Hw, Woodbury M, Ribble C, Perez-Casal J, Windeyer Mc. Risk Factors for Mycoplasma Bovis–Associated Disease in Farmed Bison (Bison Bison) Herds in Western Canada: A Case-Control Study. *Prev Vet Med.* 2016 Jul 1;129:67–73.
115. Register Kb, Olsen Sc, Sacco Re, Ridpath J, Falkenberg S, Briggs R, et al. Relative Virulence in Bison and Cattle of Bison-Associated Genotypes of Mycoplasma Bovis. *Vet Microbiol.* 2018 Aug 1;222:55–63.
116. Menghwar H, Perez-Casal J. Comparative Genomic Analysis of Canadian Mycoplasma Bovis Strains Isolated From Bison and Cattle. *Comp Immunol Microbiol Infect Dis.* 2022 Aug 1;87.
117. Bras Al, Barkema Hw, Woodbury Mr, Ribble Cs, Perez-Casal J, Windeyer Mc. Clinical Presentation, Prevalence, and Risk Factors Associated With Mycoplasma Bovis–Associated Disease in Farmed Bison (Bison Bison) Herds in Western Canada. *J Am Vet Med Assoc.* 2017 May 15;250(10):1167–75.
118. Register Kb, Woodbury Mr, Davies Jl, Trujillo Jd, Perez-Casal J, Burrage Ph, et al. Systemic Mycoplasmosis With Dystocia and Abortion in a North American Bison

(Bison Bison) Herd. *Journal of Veterinary Diagnostic Investigation.* 2013 Jul;25(4):541–5.
119. Dyer N, Hansen-Lardy L, Krogh D, Schaan L, Schamber E. An Outbreak of Chronic Pneumonia and Polyarthritis Syndrome Caused by Mycoplasma Bovis in Feedlot Bison (Bison Bison). 2008.
120. King Kc, Caven Aj, Leung Kg, Ranglack Dh, Arcilla N. High Society: Behavioral Patterns as a Feedback Loop to Social Structure in Plains Bison (Bison Bison Bison). *Mamm Res.* 2019 Jul 1;64(3):365–76.
121. Smith B. Large Animal Internal Medicine – 5th Edition. Vol. 5. 2015. p. 599–601.
122. Chernov Vm, Chernova Oa, Mouzykantov Aa, Medvedeva Es, Baranova Nb, Malygina Ty, et al. Antimicrobial Resistance in Mollicutes: Known and Newly Emerging Mechanisms. *FEMS Microbiol Lett.* 2018 Sep 1;365(18).
123. Thomas Lh, Howard Cj, Stott Ej, Parsons Kr. Mycoplasma Bovis Infection in Gnotobiotic Calves and Combined Infection With Respiratory Syncytial Virus. *Vet Pathol.* 1986 Sep 26;23(5):571–8.
124. Thomas A, Sachse K, Farnir F, Dizier I, Mainil J, Linden A. Adherence of Mycoplasma Bovis to Bovine Bronchial Epithelial Cells. *Microb Pathog.* 2003 Mar 1;34(3):141–8.
125. Lysnyansky I, Rosengarten R, Yogev D. Phenotypic Switching of Variable Surface Lipoproteins in Mycoplasma Bovis Involves High-Frequency Chromosomal Rearrangements [Internet]. Vol. 178, *Journal of Bacteriology.* 1996. Available from: https://journals.asm.org/journal/jb
126. Grand D Le, Solsona M, Rosengarten R, Poumarat F. Adaptive Surface Antigen Variation in Mycoplasma Bovis to the Host Immune Response. *FEMS Microbiol Lett.* 1996 Nov;144(2–3):267–75.
127. Wynn El, Browne As, Clawson Ml. Diversity and Antigenic Potentials of Mycoplasmopsis Bovis Secreted and Outer Membrane Proteins Within a Core Genome of Strains Isolated From North American Bison and Cattle. *Genome.* 2024 Jun 1;67(6):204–9.
128. Razin S, Yogev D, Naot Y. Molecular Biology and Pathogenicity of Mycoplasmas. *Microbiology and Molecular Biology Reviews.* 1998 Dec;62(4):1094–156.
129. Suleman M, Prysliak T, Clarke K, Burrage P, Windeyer C, Perez-Casal J. Mycoplasma Bovis Isolates Recovered From Cattle and Bison (Bison Bison) Show Differential In Vitro Effects on PBMC Proliferation, Alveolar Macrophage Apoptosis and Invasion of Epithelial and Immune Cells. *Vet Microbiol.* 2016 Apr 15;186:28–36.
130. Jungi Tw, Krampe M, Sileghem M, Griot Ch, Nicolet J. Differential and Strain-Specific Triggering of Bovine Alveolar Macrophage Effector Functions by Mycoplasmas. *Microb Pathog.* 1996 Dec;21(6):487–98.

131. Jimbo S, Suleman M, Maina T, Prysliak T, Mulongo M, Perez-Casal J. Effect of Mycoplasma Bovis on Bovine Neutrophils. *Vet Immunol Immunopathol.* 2017 Jun 1;188:27–33.
132. Hermeyer K, Jacobsen B, Spergser J, Rosengarten R, Hewicker-Trautwein M. Detection of Mycoplasma Bovis by In-Situ Hybridization and Expression of Inducible Nitric Oxide Synthase, Nitrotyrosine and Manganese Superoxide Dismutase in the Lungs of Experimentally-Infected Calves. *J Comp Pathol.* 2011 Aug;145(2–3):240–50.
133. Ahdieh M, Vandenbos T, Youakim A. Lung Epithelial Barrier Function and Wound Healing Are Decreased by IL-4 and IL-13 and Enhanced by IFN-γ. *American Journal of Physiology-Cell Physiology.* 2001 Dec 1;281(6):C2029–38.
134. Temann Ua, Ray P, Flavell Ra. Pulmonary Overexpression of IL-9 Induces Th2 Cytokine Expression, Leading to Immune Pathology. *Journal of Clinical Investigation.* 2002 Jan 1;109(1):29–39.
135. Kaplan Bs, Malmberg Jl, Sondgeroth Ks, Davila Ks, Dassanayake Rp, Sacco Re, et al. Serum IgG Immunoglobulin Levels Are Associated With Reduced PCR Detection of Mycoplasma Bovis in Naturally Infected American Bison (Bison Bison). *J Wildl Dis.* 2024 Jul 1;60(3):594–604.
136. Gondaira S, Nishi K, Tanaka T, Yamamoto T, Nebu T, Watanabe R, et al. Immunosuppression in Cows Following Intramammary Infusion of Mycoplasma Bovis. *Infect Immun.* 2020 Feb 20;88(3).
137. Bras Al, Suleman M, Woodbury M, Register K, Barkema Hw, Perez-Casal J, et al. A Serologic Survey of Mycoplasma spp. in Farmed Bison (Bison Bison) Herds in Western Canada. *Journal of Veterinary Diagnostic Investigation.* 2017 Jul 1;29(4):513–21.
138. Register Kb, Jones Lc, Boatwright Wd, Shury Tk, Woodbury M, Hamilton Rg, et al. Prevalence of Mycoplasma spp. in the Respiratory Tract of Healthy North American Bison (Bison Bison) and Comparison With Serum Antibody Status. *J Wildl Dis.* 2021 Jul 1;57(3):683–8.
139. Biddle Mk, Fox Lk, Hancock Dd. Patterns of Mycoplasma Shedding in the Milk of Dairy Cows With Intramammary Mycoplasma Infection. *J Am Vet Med Assoc.* 2003 Oct 15;223(8):1163–6.
140. Schwartz K, Schwalbe E, Buttke D, Bragg T, Killion H, Sondgeroth Ks, et al. Evaluating Two Sampling Methods for Mycoplasma Bovis Diagnosis in American Bison (Bison Bison). *J Wildl Dis.* 2024 Jul 1;60(3):584–93.
141. Register Kb, Sacco Re, Olsen Sc. Evaluation of Enzyme-Linked Immunosorbent Assays for Detection of Mycoplasma Bovis-Specific Antibody in Bison Sera. *Clinical and Vaccine Immunology.* 2013 Sep;20(9):1405–9.
142. Beier T, Hotzel H, Lysnyansky I, Grajetzki C, Heller M, Rabeling B, et al. Intraspecies Polymorphism of vsp Genes and Expression Profiles of Variable

Surface Protein Antigens (Vsps) in Field Isolates of Mycoplasma Bovis. *Vet Microbiol.* 1998 Oct;63(2–4):189–203.
143. Nussbaum S, Lysnyansky I, Sachse K, Levisohn S, Yogev D. Extended Repertoire of Genes Encoding Variable Surface Lipoproteins in Mycoplasma Bovis Strains. *Infect Immun.* 2002;70(4):2220–5.
144. Suleman M, Cyprian Fs, Jimbo S, Maina T, Prysliak T, Windeyer C, et al. Mycoplasma Bovis-Induced Inhibition of Bovine Peripheral Blood Mononuclear Cell Proliferation Is Ameliorated After Blocking the Immune-Inhibitory Programmed Death 1 Receptor. *Infect Immun.* 2018 Mar 1;86(3).
145. Boyce Aj, Shamon H, Mcshea Wj. Bison Reintroduction to Mixed-Grass Prairie Is Associated With Increases in Bird Diversity and Cervid Occupancy in Riparian Areas. *Front Ecol Evol.* 2022 Mar 18;10.
146. Register Kb, Parker M, Patyk Ka, Sweeney Sj, Boatwright Wd, Jones Lc, et al. Serological Evidence for Historical and Present-Day Exposure of North American Bison to Mycoplasma Bovis. *BMC Vet Res.* 2021 Dec 1;17(1).
147. Robino P, Alberti A, Pittau M, Chessa B, Miciletta M, Nebbia P, et al. Genetic and Antigenic Characterization of the Surface Lipoprotein P48 of Mycoplasma Bovis. *Vet Microbiol.* 2005 Aug 30;109(3–4):201–9.
148. Fu P, Sun Z, Zhang Y, Yu Z, Zhang H, Su D, et al. Development of a Direct Competitive ELISA for the Detection of Mycoplasma Bovis Infection Based on a Monoclonal Antibody of P48 Protein. *BMC Vet Res.* 2014 Feb 18;10.
149. Schwartz Pm, Shipman C, Carlson Rh, Drach Jc, Tritsch L, Cheda Gb, et al. Thermal Inactivation as a Means of Inhibiting the Serum-Associated Deamination of 9-F3-D-Arabinofuranosyladenine in Tissue Culture Media [Internet]. Vol. 5, *Antimicrobial Agents and Chemotherapy.* 1974. Available from: https://journals.asm.org/journal/aac
150. Moskaluk A, Nehring M, Vande Woude S. Serum Samples From Co-Infected and Domestic Cat Field Isolates Nonspecifically Bind FIV and Other Antigens in Enzyme-Linked Immunosorbent Assays. *Pathogens.* 2021 Jun 1;10(6).
151. Wawegama Nk, Browning Gf, Kanci A, Marenda Ms, Markham Pf. Development of a Recombinant Protein-Based Enzyme-Linked Immunosorbent Assay for Diagnosis of Mycoplasma Bovis Infection in Cattle. *Clinical and Vaccine Immunology.* 2014 Feb;21(2):196–202.
152. Perez-Casal J, Prysliak T. Detection of Antibodies Against the Mycoplasma Bovis Glyceraldehyde-3-Phosphate Dehydrogenase Protein in Beef Cattle. *Microb Pathog.* 2007 Nov;43(5–6):189–97.
153. Sun Z, Fu P, Wei K, Zhang H, Zhang Y, Xu J, et al. Identification of Novel Immunogenic Proteins From Mycoplasma Bovis and Establishment of an Indirect ELISA Based on Recombinant E1 Beta Subunit of the Pyruvate Dehydrogenase Complex. *PLoS One.* 2014 Feb 10;9(2).

CHAPTER 2- DELPHI SURVEY LEADS TO MULTI-STAKEHOLDER INSIGHTS ON DEFINITION OF HEALTH IN NORTH AMERICAN BISON (*BISON BISON*)[1]

Introduction

Bison are uniquely susceptible to genetic inbreeding due to their very small founder population as well as the restricted movement between herds that prevents genetic exchange (1). Loss of genetic diversity and inbreeding depression can negatively impact the viability of a species due to reproductive failure, poor recruitment, reduced disease resistance, and inability to adapt to changing climate or environmental conditions (2,3). Smaller, fragmented populations also increase the potential negative impacts of disease introduction. One such pathogen, *Mycoplasma bovis*, was listed as an emerging disease by the United States Department of Agriculture in 2013 (4) *Mycoplasma bovis* is an important primary bacterial pathogen of bison that expanded rapidly in both numbers of affected herds and geographic range in 2021 (5,6) The

---

[1] **Journal of Wildlife Diseases**

Catherine B. Krus[1], Jameson R. Brennan[3,5], Jeff M. Martin[4,5], Danielle Buttke[2,6]

1. Department of Clinical Sciences, College of Veterinary Medicine, Colorado State University, Ft. Collins, CO 80523, USA
2. National Park Service Biological Resources Division and Office of Public Health, 1201 Oakridge Drive, Suite 200, Fort Collins, CO 80524, USA
3. Department of Animal Sciences, South Dakota State University, West River Research and Extension Center, 711 N. Creek Dr., Rapid City, South Dakota 57703, USA
4. Department of Natural Resource Management, South Dakota State University, West River Research and Extension Center, 711 N. Creek Dr., Rapid City, South Dakota 57703, USA
5. Center of Excellence for Bison Studies, South Dakota State University, Rapid City, SD 57701, USA
6. Corresponding author (Danielle Buttke, Danielle_buttke@nps.gov)

emergence of *M. bovis* heightened awareness of the need for a comprehensive and collaboratively developed definition of bison health that could unify differing stakeholder sectors. Without a clear and collective understanding of what constitutes bison health, efforts to manage disease, translocate animals, and support long-term population resilience remain fragmented and inconsistent. A consensus definition serves as a foundational framework for aligning conservation and production strategies, guiding health monitoring, informing policy, and enhancing the effectiveness of coordinated responses to current and emerging health threats. Furthermore, bison industry mortality rates are much higher than that of the cattle industry (2.3% vs. 1.5%, respectively) and, of those bison deaths, 60% are health related problems (7,8) Bison health is a common value shared across sectors that can help bridge differences in these goals and ultimately help bison to become more resilient to health threats.

Bison translocation, essential for improving genetics and conservation, presents significant challenges due to disease risks and exposure to novel environments. Especially of concern is bison movement across large geographic areas and environmental gradients which can result in high morbidity and mortality, reduced animal performance and reproductive output, and disease introductions into new locations, with far-reaching implications for multiple livestock stakeholders and rightsholders (2,3,9) Stress from translocation may suppress immune function, reactivate dormant pathogens, and heighten susceptibility to infections, while environmental changes can alter disease dynamics unpredictably (10–14). For example, certain helminths with free-living stages have broad geographic ranges but cause production losses only under specific environmental conditions (15–17) In these

ways, animal translocations can increase disease risks for both previously infected hosts as well as promote infectious agent introductions and shedding to new individuals and herds (18) These risks underscore the critical need for a One Health approach and practical tools to mitigate the effects of translocation on bison health.

Geospatial tools such as remote sensing and GIS play a key role in a One Health approach, which emphasizes the interconnected health of humans, animals, and the environment. These technologies help identify habitat overlap, environmental stressors, and climate shifts that influence disease dynamics, as demonstrated in studies of infectious keratoconjunctivitis in Italy (19). Additional advancements, such as the Sen2WQ application developed within Google Earth Engine, have been utilized to enable indirect assessment of water quality indicators using satellite data (20). By supporting early detection and surveillance, these tools provide timely, actionable data to inform disease prevention and control policies.

Defining wildlife and livestock health is the first step in determining disease risk (21). However, this definition can vary greatly between stakeholders and across management goals (22,23). By having a population-level health definition for a species, one can better identify species and sector needs to find mutually beneficial goals between stakeholders. The Delphi method is a tool for achieving expert consensus through iterative feedback, particularly useful when time or data are insufficient for quantitative hazard assessment. It is well-suited for fields where insights are based largely on professional experience (24–26). Experts are surveyed on topics such as species health definitions, key health determinants, common diseases, and medical interventions. Survey results are compiled, shared anonymously, and refined through

subsequent rounds to develop consensus and mitigation guidelines. This survey aimed to establish a definition of bison health, identify common health hazards, and prioritize treatment practices, providing a foundation for multidisciplinary collaboration. The findings will guide efforts to address bison health needs and infectious disease risks associated with translocation.

Materials and Methods

We identified and invited top bison experts (n=22) representing each of four separate sectors (public, private, tribal, nonprofit organization) to participate in a two-round Delphi survey (24,27)(Figure 2.1). We use the terms bison and buffalo interchangeably to be inclusive of preferred nouns for *Bison bison* by myriad stakeholders in North America. This study was approved by the South Dakota State University Institutional Review Board: IRB-2112004-EXM. These participants were identified by their peers as having extensive knowledge of and experience with bison health, research, and management and were responsible for bison health decisions within their sector as evidenced by job titles and years of experience in the bison sector. We sent the identified experts an electronic survey link via email with a description of the study objectives. Experts were given 6 weeks to respond, with three follow-up emails.

In the first round of the survey, 11 open-ended, 4 Likert, and 3 multiple choice questions were presented to the participants. Completed questionnaires were compiled anonymously via Qualtrics. Three members of the research team reviewed the survey responses blinded to one another to identify common themes within the survey answers. Once individual reviews were completed, results were evaluated as a group.

From this discussion, results were gathered into qualitative or quantitative data and served as the premise for the second round of the survey.

The second round of the survey gave the respondents the opportunity to review and comment on aggregated answers from the first-round survey. New question styles included ranking (n=3), Likert (1), check box (1), and open ended (1). Each question was followed by an area to leave comments. The results from the round 2 questionnaire were evaluated quantitatively or qualitatively, as applicable. Descriptive statistics, including means, standard deviations, modes, and ranges, were calculated to summarize the data using Microsoft Excel (Version 2403 [Build 17425.20146 c2r]). Qualitative data was evaluated using thematic analysis.

In order to identify the determinants of bison health, the research team categorized the first-round responses into six themes identified by Wittrock et al. as being associated with fish and wildlife health when determining a health model (28) Wittrock et al. provided a conceptual model that adapted the determinants of health framework from human public health to wildlife, validated through both literature review and expert consensus across two ecologically and culturally significant species, caribou and salmon. This model offered a flexible yet structured foundation for categorizing multifactorial drivers of health, making it well-suited for applying to bison health in a similarly complex ecological and management context. In the second round of this question, survey participants were provided with information to obtain the Wittrock et al. manuscript for clarification, and Figure 1 from the paper was included directly within the survey. The six determinants of health from the Wittrock et al. manuscript are listed below.

1) The biologic endowment of the individual and population
2) The animal's social environment
3) The quality and abundance of the animal's needs for daily living
4) The abiotic environment in which the animal lives
5) Sources of direct mortality
6) Changing human expectations

Results

We compiled both the first and second round questionnaires in Qualtrics. Round one included 12 survey responses resulting in a response rate of 55% (12/22). One survey response was excluded due to lack of consent. First round respondents included in the survey analysis comprised of 3 public, 3 private, 3 nonprofit organization, and 2 tribal sector experts. Ten of the included experts resided in the United States and 1 resided in Canada at the time of the survey. Nine survey respondents were recorded for the second round of the Delphi survey (41% response rate).

3.1 Bison Health

3.1.a. Definition

A consensus definition of bison health was developed. Stakeholders consistently identified reproductive capacity (n=7), also referred to as fitness, environmental factors (5), and the ability to display natural behaviors (4) as the most important aspects defining bison health. This led to a final consensus on the definition of bison health as follows: "Bison health can be assessed by a population's ability to express natural behaviors, be resilient to external stressors that include disease, and display high levels

of lifetime reproductive output within its environment with minimal intervention" (Mean and SD, respectively: 8.33 ±1.33). Participants were also invited to provide open-ended feedback and suggest modifications. One participant commented that the concept of health is a human construct, therefore, as human health expectations evolve so will this definition. Another added that bison health is intimately related to the health of the environment in which the animals live and is dependent on the ability of that environment to sustain and support the bison population.

3.1.b. Determinants of Health

Key determinants of bison health were ranked in importance from 1 (utmost importance) to 6 (little or no importance) with biological endowment (mean 1.5), and need for daily living (mean 1.88) rated as the highest priorities. Social (mean 3) and abiotic environments (mean 4.25) were also significant but secondary considerations. Metrics for monitoring bison health included reproductive performance (n=6), body condition (5), forage and habitat use (4), parasite load (2), and mineral status (2).

3.1.c. Monitoring and diagnostics

There was broad agreement on the need for improved resources for bison health monitoring, prevention, and treatment. Respondents identified that needs exist for research and development (89%), disease prevention (78%), and biosecurity protocols (44%). Respondents highlighted gaps in diagnostic capabilities, including the need for bison-specific tests for *Mycoplasma bovis* (n=5), fecal parasitology (2), Johne's disease (2), tuberculosis (2), and brucellosis (1). Specific concerns included the lack of antemortem diagnostic tools and standardized mineral reference ranges as well as the

lack of knowledgeable bison veterinarians. As one respondent noted “There has been very little done in the way of research and development of bison health and treatment from drug companies, research institutions, and government entities to support the producer”.

3.2 Bison Health Concerns

3.2.a. Current threats

Respondents were asked to list the top 5 most significant threats to bison. *Mycoplasma bovis* was identified as the most significant health threat to bison (mean=2.88 ±1.96) with severe economic and health impacts due to high mortality rates and production losses (n=8). Other prominent threats included habitat loss/modification (3.00 ±2.07), general diseases (3.75 ±2.12) such as brucellosis, tuberculosis, and parasitism, policy (4.6 ±1.07), and climate (4.8 ±1.55). One participant stated, “*Mycoplasma bovis* is not only lethal, but it severely impacts herd productivity by reducing pregnancy rates and restricting animal movement between herds.”

3.2.b. Disease management of adult bison

Respondents were then asked to identify the top 5 most common diseases seen in bison adults. The most common to least common included *Mycoplasma bovis* (mean=2.1), parasites (3.5), pink eye (4.3), stress (4.3), and injury (4.6). A commenter stated, “Good pasture management is often overlooked and the basis of overall herd health including in calves”.

A combination of open-ended and Likert style questions were asked regarding the medical interventions, consequences, and economic consequences of the most

common diseases listed by the respondents. Management strategies for common diseases included mineral supplementation (n=5), vaccination (4), test-and-cull approaches (3), and anthelmintic use (2), though efficacy varied. Respondents highlighted the need for research on biosecurity measures, disease surveillance, and non-invasive health monitoring methods. Additionally, commenters mentioned the need to maintain genetic diversity as a way to mitigate disease risk and another commenter stated that some of the consequences of these diseases include the inability to move animals due to disease status.

3.2.c. Disease management of bison calves

The most common health issues in calves included parasitism (n=5), respiratory diseases (4), diarrhea (3), and bovine viral diarrhea virus (2). Management approaches focused on improving pasture conditions (n=3) and targeted interventions (3), though consensus on effective strategies was limited. Respondents reported most calf diseases were of low to moderate health consequence and of low to moderate economic consequence. One producer shared, “We lost 41 weaned bison calves in fall 2021 due to Pasteurella pneumonia, and yet these losses did not qualify for USDA disaster relief.” Others emphasized that “calf diseases are generally related to management practices and hygiene,” especially in more intensively managed herds.

3.3 Shared Needs and Priorities

The results emphasized the need for multidisciplinary research, improved diagnostics, and dedicated funding for bison health. Stakeholders stressed the importance of collaboration to address health challenges and translocation-associated

disease risks. Commenters noted that bison health threats vary by geography and herd use (e.g., conservation or production) and highlighted the importance of including Native American perspectives. They also called for more research, public dissemination of findings, and expanded government compensation for losses.

Discussion

Bison health is a complex and multifaceted concept, particularly for wild populations, as highlighted by stakeholders in this study. Given that bison are recognized as having multiple statuses as livestock, wildlife, cultural resources, ecosystem engineers, ecological keystone species, significant management differences exist. For example, public and tribal sectors often mentioned the importance of bison thriving within their landscape as well as their ability to cope with environmental stress (i.e., resiliency). NGO and private sectors frequently brought up aspects such as productive capabilities, body condition, and veterinary care as important aspects of bison health. Despite these differences, stakeholders aligned on the interconnectedness of animal health and environmental health, supporting the utility of the Delphi method in fostering consensus across disciplines.

This study achieved a consensus definition of bison health and identified key challenges and priorities for improving bison health management. The definition—emphasizing natural behaviors, resilience to stressors, and reproductive success with minimal human intervention—provides a foundation for collaborative efforts. The results call attention to the opportunity to address shared challenges, including infectious disease risks and gaps in diagnostic and biosecurity resources.

The emergence of *Mycoplasma bovis* as an infectious disease risk was consistently identified as the most pressing health threat to bison, with significant economic and biological consequences. High mortality rates and limited diagnostic options highlight the urgent need for antemortem testing strategies, the ability to quickly on-board new bison specific diagnostic tests for emerging diseases, and interpretations of test results (18,29). Harm reduction may be a good way to approach all aspects of bison health so that bison can thrive, knowing that the threats will vary across time and space and diagnostic limitations remain (30). Additionally, the emergence of *M. bovis* revealed critical gaps in bison health monitoring, prevention, and treatment (6,31). Stakeholders emphasized the need for dedicated funding to support research and develop bison-specific diagnostic tools. A lack of bison health veterinary expertise compared to other livestock species further complicates management, stressing the need for specialized training and collaborative research efforts.

Stakeholders also identified pasture management as a fundamental but often overlooked aspect of herd health, highlighting the needs of a One Health approach involving experts in rangeland and grazing sciences, habitat restoration specialists, and monitoring indicator species for grassland health (32) Additionally, these approaches can lead to economic advancements along with additional benefits to ranchers including lower invasive plant and pest pressures, increased biodiversity that could foster recreation and ecotourism opportunities, nutritional diversity for grazing bison, improved soil health and resulting sustained productivity, and more resilience against extreme weather and climate change (33–35). Agricultural and natural resource conservation agencies must tailor program resources specifically to bison for the role they play as a

keystone species. Through One Health initiatives such as these, bison restoration can support the goals of multiple human sectors.

The integration of Traditional Ecological Knowledge (TEK) and One Health principles enriches the understanding of bison health. Several commenters noted that bison well-being contributes to and reflects the health of their environment, emphasizing the interconnection between wildlife, ecosystems, and human communities (33,36). TEK, rooted in indigenous observations and cultural knowledge, emphasizes the reciprocal relationship between people and nature (37). This perspective highlights the importance of ecosystem balance in sustaining bison health and suggests that the Bison Management System implicitly incorporates these principles (32). Collaboration across sectors and the inclusion of Native American perspectives are essential for advancing holistic, culturally informed bison health strategies.

This study has several limitations that should be considered when interpreting the findings. Although a recent review suggests that Delphi studies aiming for statistical confidence may require larger samples (60–80 participants) and response rates of at least 70% per round for methodological rigor, this study reflects the reality of conducting expert elicitation within a small and highly specialized field (38,39). Experts (n = 22) were recruited through peer nomination and represented leading authorities across tribal, public, nonprofit, and private bison sectors. While only 12 individuals completed the first round, and a subset continued to the second round, this response rate is consistent with similar Delphi studies involving niche or hard-to-reach populations (26,40,41). Second, while qualitative responses added critical nuance to the interpretation of health and management challenges, further thematic analysis and

respondent validation could strengthen the integration of these perspectives. Finally, as with many Delphi studies, participant dropout between rounds may have affected the consistency of feedback and the robustness of the consensus. These limitations are acknowledged; however, the diversity of perspectives and the high level of expertise still allowed for meaningful synthesis and consensus development. Future efforts may benefit from additional incentives or institutional partnerships to improve response rates and panel continuity.

Ultimately, this study provides a consensus definition of bison health and highlights the need for expanded research into both specific disease threats, such as *M. bovis*, and broader factors affecting bison health, including environmental influences on disease dynamics. Addressing gaps in diagnostics, biosecurity, and resource availability will be critical for improving bison health outcomes and fostering resilience. By integrating One Health and TEK principles into management strategies, stakeholders can create a comprehensive framework to support the ecological, cultural, and economic roles of bison in a changing world.

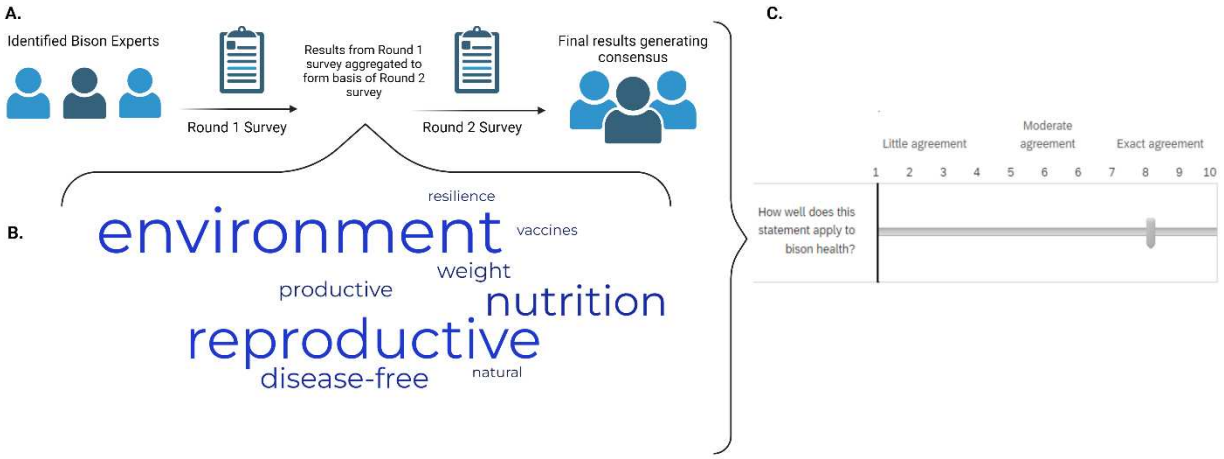

Figure 2.1. Reproductive capacity (fitness) and environment are identified as the most important aspects defining bison health in a two-round Delphi survey.

A Delphi survey is an iterative process used to summarize knowledge and opinions of a group of experts by using a series of questionnaires (Fig 1A). Larger words represent a larger frequency than smaller words identified in expert responses in the first round of the Delphi survey (Fig 1B).

The results from Round 1 were used to create a definition of bison health that was relayed back to the experts in the Round 2 questionnaire. (Fig 1C). The results from the Round 2 questionnaire led to a final consensus on the definition of bison health as follows: “Bison health can be assessed by a population’s ability to express natural behaviors, be resilient to external stressors that include disease, and display high levels of lifetime reproductive output within its environment with minimal intervention”. This, along with other survey questions will be used to identify needs pertaining to bison health as well as serve as a premise for creating a disease risk analysis for bison translocation.

REFERENCES

1. Hedrick PW. Conservation Genetics and North American Bison (Bison bison). Journal of Heredity. 2009 Jul 1;100(4):411–20.
2. Keller L, Waller D. Inbreeding Effects in Wild Populations. Trends Ecol Evol. 2002 May 1;17(5):230–41.
3. Hedrick PW, Kalinowski ST. Inbreeding Depression in Conservation Biology. Annu Rev Ecol Syst. 2000 Nov;31(1):139–62.
4. Sweeney S, Jones R, Patyk K, LoSapio C. Mycoplasma Bovis- An Emerging Pathogen in Ranched Bison. USDA APHIS Emerging Disease Notice. 2013;
5. Janardhan KS, Hays M, Dyer N, Oberst RD, DeBey BM. *Mycoplasma Bovis* Outbreak in a Herd of North American Bison (*Bison Bison*). Journal of Veterinary Diagnostic Investigation. 2010 Sep 1;22(5):797–801.
6. Register KB, Sacco RE, Olsen SC. Evaluation of Enzyme-Linked Immunosorbent Assays for Detection of Mycoplasma bovis-Specific Antibody in Bison Sera. Clinical and Vaccine Immunology. 2013 Sep;20(9):1405–9.
7. USDA–APHIS–NAHMS. Health and Management Practices on U.S. Ranched-Bison Operations, 2014. Fort Collins, Colorado; 2016.
8. Martin JM. Facing into the Blizzard: Resiliency and Mortality of Native and Domestic North American Ungulates to Extreme Weather Events. Diversity (Basel). 2022 Dec 21;15(1):11.
9. Kock RA, Woodford MH, Rossiter PB. Disease Risks Associated with the Translocation of Wildlife. Revue Scientifique et Technique de l'OIE. 2010 Aug 1;29(2):329–50.
10. Maunsell FP, Woolums AR, Francoz D, Rosenbusch RF, Step DL, Wilson DJ, et al. Mycoplasma bovis Infections in Cattle. J Vet Intern Med. 2011;25(4):772–83.
11. Callaway TR, Morrow JL, Edrington TS, Genovese KJ, Dowd S, Carroll J, et al. Social Stress Increases Fecal Shedding of Salmonella typhimurium by Early Weaned Piglets. Curr Issues Intest Microbiol. 2006 Sep;7(2):65–71.
12. Rostagno MH. Can Stress in Farm Animals Increase Food Safety Risk? Foodborne Pathog Dis. 2009 Sep;6(7):767–76.
13. Reed R, Raison C. Environmental Influences on the Immune System. Vienna: Springer Vienna; 2016. 97–126 p.
14. Maunsell FP, Chase C. Mycoplasma bovis. Veterinary Clinics of North America: Food Animal Practice. 2019 Nov;35(3):471–83.

15. Wiese JD, Caven AJ, Zarlenga DS, Topliff CL, Kelling CL, Salter J. Gastrointestinal Parasites of a Reintroduced Semi-Wild Plains Bison (Bison bison bison) herd: Examining Effects of Demographic Variation, Deworming Treatments, and Management Strategy. Int J Parasitol Parasites Wildl. 2021 Apr;14:216–27.

16. Penzhorn BL, Knapp SE, Speer CA. Enteric Coccidia in Free-Ranging American Bison (Bison bison) in Montana. J Wildl Dis. 1994 Apr;30(2):267–9.

17. Woodbury MR, Wagner B, Ben-Ezra E, Douma D, Wilkins W. A Survey to Detect Toxocara vitulorum and other Gastrointestinal Parasites in Bison (Bison bison) Herds from Manitoba and Saskatchewan. Can Vet J. 2014 Sep;55(9):870–4.

18. Bras AL, Barkema HW, Woodbury M, Ribble C, Perez-Casal J, Windeyer MC. Risk Factors for Mycoplasma bovis-Associated Disease in Farmed Bison (Bison bison) Herds in Western Canada: A Case-Control Study. Prev Vet Med. 2016 Jul 1;129:67–73.

19. Orusa T, Orusa R, Viani A, Carella E, Mondino EB. Geomatics and EO Data to Support Wildlife Diseases Assessment at Landscape Level: A Pilot Experience to Map Infectious Keratoconjunctivitis in Chamois and Phenological Trends in Aosta Valley (NW Italy). Remote Sens (Basel). 2020 Nov 1;12(21):1–22.

20. Viani A, Orusa T, Borgogno-Mondino E, Orusa R. A One Health Google Earth Engine Web-GIS Application to Evaluate and Monitor Water Quality Worldwide. EuroMediterr J Environ Integr. 2024 Dec 14;9(4):1873–86.

21. OIE, IUCN. World Organisation for Animal Health (OIE) & International Union for Conservation of Nature (IUCN) (2014). – Guidelines for Wildlife Disease Risk Analysis. 2014.

22. Stephen C. Toward a Modernized Definition of Wildlife Health. J Wildl Dis. 2014 Jul;50(3):427–30.

23. Gunnarsson S. The Conceptualisation of Health and Disease in Veterinary Medicine. Acta Vet Scand. 2006 Dec 7;48(1):20.

24. Dalkey N, Helmer O. An Experimental Application of the Delphi Method to the Use of Experts. Manage Sci. 1963 Apr;9(3):458–67.

25. Duncan C, Patyk K, Wild MA, Shury T, Leong KM, Stephen C. Perspectives on Wildlife Health in National Parks: Concurrence with Recent Definitions of Health. Human Dimensions of Wildlife. 2019 Nov 2;24(6):579–87.

26. Patyk KA, Duncan C, Nol P, Sonne C, Laidre K, Obbard M, et al. Establishing a Definition of Polar Bear (Ursus maritimus) Health: A Guide to Research and Management Activities. Science of the Total Environment. 2015 May 1;514:371–8.

27. Mukherjee N, Hugé J, Sutherland WJ, McNeill J, Van Opstal M, Dahdouh-Guebas F, et al. The Delphi Technique in Ecology and Biological Conservation: Applications and Guidelines. Methods Ecol Evol. 2015 Sep 28;6(9):1097–109.

28. Wittrock J, Duncan C, Stephen C. A Determinants of Health Conceptual Model for Fish and Wildlife Health. J Wildl Dis. 2019 Apr 9;55(2):285.

29. Dyer N, Register KB, Miskimins D, Newell T. Necrotic Pharyngitis Associated with Mycoplasma bovis Infections in American bison (Bison bison). Journal of Veterinary Diagnostic Investigation. 2013 Mar 1;25(2):301–3.

30. Gallagher CA, Keehner JR, Hervé-Claude LP, Stephen C. Health Promotion and Harm Reduction Attributes in One Health Literature: A Scoping Review. Vol. 13, One Health. Elsevier B.V.; 2021.

31. Schwartz K, Schwalbe E, Buttke D, Bragg T, Killion H, Sondgeroth KS, et al. Evaluating Two Sampling Methods for Mycoplasma Bovis Diagnosis in American Bison (Bison bison). J Wildl Dis. 2024 Jul 1;60(3):584–93.

32. Martin JM, Zarestky J, Briske DD, Barboza PS. Vulnerability Assessment of the Multi-sector North American Bison Bison bison Management System to Climate Change. People and Nature. 2021 Jun 25;3(3):711–22.

33. Shamon H, Cosby OG, Andersen CL, Augare H, BearCub Stiffarm J, Bresnan CE, et al. The Potential of Bison Restoration as an Ecological Approach to Future Tribal Food Sovereignty on the Northern Great Plains. Front Ecol Evol. 2022 Jan 28;10.

34. Ratajczak Z, Collins SL, Blair JM, Koerner SE, Louthan AM, Smith MD, et al. Reintroducing Bison Results in Long-Running and Resilient Increases in Grassland Diversity. Proceedings of the National Academy of Sciences. 2022 Sep 6;119(36).

35. Boyce AJ, Shamon H, McShea WJ. Bison Reintroduction to Mixed-Grass Prairie Is Associated With Increases in Bird Diversity and Cervid Occupancy in Riparian Areas. Front Ecol Evol. 2022 Mar 18;10.

36. Bartlett C, Marshall M, Marshall A. Two-Eyed Seeing and Other Lessons Learned Within a Co-learning Journey of Bringing Together Indigenous and Mainstream Knowledges and Ways of Knowing. J Environ Stud Sci. 2012 Nov 16;2(4):331–40.

37. Isaac G, Finn S, Joe JR, Hoover E, Gone JP, Lefthand-Begay C, et al. Native American Perspectives on Health and Traditional Ecological Knowledge. Environ Health Perspect. 2018 Dec;126(12).

38. Chuenjitwongsa S. Determination of Required Anatomical Knowledge for Clinical Practice in Emergency Medicine: National Curriculum Planning Using a Modified Delphi Technique [Internet]. Vol. 27, Emergency Medicine Journal. 2005. 693 p. Available from: https://meded.walesdeanery.org/how-to-guides

39. Manyara AM, Purvis A, Ciani O, Collins GS, Taylor RS. Sample Size in Multistakeholder Delphi Surveys: at What Minimum Sample Size do Replicability of Results Stabilize? J Clin Epidemiol. 2024 Oct 1;174.

40. Hess GR, King TJ. Planning Open Spaces for Wildlife. Selecting Focal Species Using a Delphi Survey Approach.

41. Hanisch SL, Riley SJ, Nelson MP. Promoting Wildlife Health or Fighting Wildlife Disease: Insights from History, Philosophy, and Science. Wildl Soc Bull. 2012 Sep;36(3):477–82.

CHAPTER 3- HIGH SEROPREVALENCE AND AGE-ASSOCIATED DYNAMICS OF BLUETONGUE AND EPIZOOTIC HEMORRHAGIC DISEASE IN NORTH AMERICAN BISON (*BISON BISON*)[2]

Introduction

Shaped by climate change, globalization, and anthropogenic activities such as farming, land-use modifications, and trade, vector-borne viruses of ruminants (often referred to as arboviruses despite the term having no taxonomic significance) have undergone significant shifts in their epidemiology over the past several decades (1–3) These shifts have contributed to the re-emergence and geographic spread of many arboviruses, including the economically significant bluetongue (BT) and epizootic hemorrhagic disease (EHD).

---

[2] Frontiers in Veterinary Science, section Veterinary Infectious Diseases

Catherine Krus[1], Ian Zander[2], Tyler J. Sherman[2], Courtney Maichak[3], Danielle E. Buttke[4], Lee Jones[5], Christie Mayo[2*]

[1] Department of Clinical Sciences, Colorado State University, Fort Collins, CO, United States

[2]Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO, United States

[3]Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO, United States

[4]Biological Resources Division, National Park Service, Fort Collins, CO, United States

[5]Natural Resource Program Center – Wildlife Health, United States Fish and Wildlife Service, Bozeman, MT, United States

Bluetongue Virus (BTV) and Epizootic Hemorrhagic Disease Virus (EHDV) are classified in the genus *Orbivirus* and are transmitted by various species of *Culicoides* midges (4,5). In the genus *Culicodies*, females require a blood meal for ovarian maturation and egg production (6). When a *Culicodies* midge feeds on an *Orbivirus*-infected host, the virus undergoes an extrinsic incubation period of 7 to 14 days, depending on environmental factors like temperature (5). During this time, the virus must overcome physiological barriers in the midgut before disseminating to the salivary glands, where it replicates unchecked and becomes transmissible to a new host during subsequent blood meals (5,7). Historically, these viruses were confined to tropical and subtropical regions of Africa, but have since expanded into more temperate zones, including Europe, the Americas, and Asia, with projections indicating continued geographic expansion (8–11).

Bluetongue Virus consists of 29 described serotypes, 27–29 of which are putative and capable of segment reassortment (12–15). This contributes to its genetic diversity and complicates vaccine development (16). The clinical manifestations of BTV and EHDV infections vary depending on the host species and virus serotype but can include fever, hemorrhage, abortion, oral ulceration, and edema, with outcomes ranging from subclinical to severe (17–20). These diseases impose significant economic costs through decreased productivity, direct veterinary expenses, and the implementation of control measures (21–23).

While extensive research on BT and EHD has focused on domestic livestock, the epidemiology of these diseases in bison remain poorly understood, with only a handful of studies specifically exploring these diseases in North American or European bison

(18,24–27). As an iconic species of ecological, economic, and cultural importance, bison are uniquely positioned at the interface between domestic livestock and wildlife, with many states listing them under dual classifications, highlighting the critical importance of their health status in understanding disease dynamics (28–31).

This gap is particularly concerning given the unknown potential for bison to facilitate the maintenance and vector-mediated transmission of these viruses to co-grazing domestic livestock and other wildlife. To address this, our study investigated the seroprevalence of BTV and EHDV antibodies in range-limited, minimally managed (handled yearly or biennially) North American bison, identified circulating BTV serotypes via RT-qPCR; and examined age-associated dynamics in correlation to current and past exposure. These findings offer valuable insight into the epidemiology of BTV and EHDV in bison and emphasize the importance of integrated surveillance and control measures to mitigate the broader impact of vector-borne diseases on diverse ruminant populations and ecosystems.

Materials and Methods

Sample Collection

Samples from a total of 287 North American bison (285 serum, 216 whole blood, with 214 paired) were collected from nine herds in seven states (Table 3.1, Figure 3.1). The states included in this study (CO, IA, KS, MT, NE, OK, SD) encompass regions within the historical range of North American plains bison and include areas where significant bison populations persist today. While comprehensive data on private bison herds remain limited, these states are home to several Department of the Interior (DOI)

conservation herds that serve as an essential resource for bison conservation and management, along with a few conservation herds managed similarly by other agencies and organizations (32). Sampling was conducted opportunistically during routine management-assoicated capture and handling events scheduled between August 31st and November 13th, 2023, with individuals sampled based on accessibility and availability. All sampling procedures were performed in coordination with herd managers, veterinary personnel, and wildlife biologists to minimize stress and ensure activities met currently accepted professional standards of animal welfare as identified by each agency or managing organization. Serum samples were extracted through centrifugation in the field prior to storage alongside K3EDTA preserved whole blood samples in coolers with ice packs during transport to the Colorado State University Veterinary Diagnostic Laboratory for further diagnostic processing.

ELISA Testing for BTV and EHDV Antibodies

Competitive enzyme-linked immunosorbent assays (cELISAs) were conducted to detect antibodies against Bluetongue Virus and Epizootic Hemorrhagic Disease Virus using the Bluetongue Virus Antibody Test Kit (VMRD, Washington, US) and EHD Virus Antibody Test Kit (ID Screen, France) respectively. Plates with antigen-coated wells were incubated with serum samples and controls that were supplied by the kit, washed, and sequentially treated with substrate and stop solutions following the manufacturers' protocols. Optical densities were read on a plate reader (BioTek Instruments Inc., Vermont, US) at 630 nm for BTV and 450 nm for EHDV. Final classifications were based on kit validation criteria provided by the manufacturer.

For BTV, samples with a mean percent inhibition (%I) ≥60% were classified as positive. For EHDV, samples with a percent sample-to-negative (%S/N) ratio ≤30% were classified as positive, while values of 30% < %S/N < 40% were retested to confirm results.

RNA Extraction and Duplex RT-qPCR

RNA Extraction

RNA was extracted from whole blood samples using the MagMAX™ Pathogen RNA/DNA Kit (ThermoFisher, Massachusetts, USA) on a KingFisher 96 automated system according to the manufacturer's protocol. Extracted RNA was stored at −80°C until analysis.

Duplex RT-qPCR

Bluetongue Virus and Epizootic Hemorrhagic Disease Virus RNA were simultaneously detected using the SuperScript™ III Platinum™ One-Step qRT-PCR kit on an Applied Biosystems™ 7500 Real-Time PCR System (ThermoFisher, Massachusetts, USA). Reactions were conducted in a single tube with 25 µL reaction volumes containing primers and probes targeting conserved regions of BTV and EHDV genomes (33–35). 5 uL of extracted nucleic acids were first denatured at 95°C for 5 mins prior to the addition of the reaction master mix. Thermal cycling conditions then included a reverse transcription step (48°C for 30 min), initial denaturation (95°C for 2 min), and 40 amplification cycles (95°C for 15 sec, 56°C for 30 sec, 72°C for 30 sec). Negative and positive controls were included in each run to validate results. Cycle

thresholds (Ct) were determined at 10% of the amplification plateau of the positive amplification control (PAC).

RT-qPCR BTV Serotyping

Samples previously confirmed positive for BTV via RT-qPCR were tested for specific serotypes using RT-qPCR with serotype-specific primers for BTV-2w, BTV-3, BTV-6, BTV-10, BTV-11, BTV-13, and BTV-17 (36). Reactions were performed using the SuperScript™ III Platinum™ One-Step qRT-PCR System, as described above. Thermal cycling conditions then included a reverse transcription step (48°C for 30 min), initial denaturation (95°C for 2 min), and 40 amplification cycles (95°C for 15 sec, 56°C for 30 sec, 72°C for 30 sec). Positive and negative controls were included for quality assurance. Amplification was performed using the Applied Biosystems 7500 Real-Time PCR System. Ct thresholds were determined at 10% of the amplification plateau of the positive amplification control (PAC).

Data Analysis

Data analysis was conducted in R (v4.3.0) using the tidyverse package for data preprocessing and visualization. We obtained weather data from Open-Meteo, which provides meteorological data through numerical weather prediction models. Logistic regression models were used to evaluate associations between demographics and meteorological data (e.g., age, sex, herd demographics, land area, and weather conditions) and outcomes (EHDV/BTV serostatus and RT-qPCR status). Predictors were selected based on biological relevance and exploratory analysis.

Models were optimized using the Akaike Information Criterion (AIC) to balance fit and complexity. To assess multicollinearity among predictor variables, Variance Inflation Factors (VIFs) were calculated using the car package in R. Odds ratios (OR) with 95% confidence intervals (CI) were calculated to assess the strength of associations. Visualizations, including probability curves with confidence intervals, were generated using ggplot2. Effect sizes for significant predictors were highlighted to enhance interpretability.

Results

Antibodies against BTV were detected in 56.5% (161/285) of bison sera using cELISA, with EHDV seroprevalence at 57.5% (164/285), and 45.3% (129/285) testing positive for antibodies to both viruses. Among the 9 tested herds, BTV seroprevalence ranged from 0% (MT and NE herds) to 83.3% (KS herd), while EHDV seroprevalence ranged from 3.2% (MT herd) to 86.7% (KS herd) (Table 3.1).

RT-qPCR-based serotyping revealed the presence of multiple circulating BTV serotypes, including BTV-6, BTV-11, BTV-13, and BTV-17. These serotypes were detected in herds from South Dakota, Kansas, and Oklahoma (Table 3.1). BTV and EHDV RT-qPCR positivity were lower than antibody detection, with BTV RT-qPCR positivity ranging from 0% to 7% and EHDV RT-qPCR positivity between 0% and 20%.

Variation in seroprevalence and RT-qPCR positivity among herds highlighted potential influences of herd size, geography, and local environmental factors. However, broader herd-level logistic regression models incorporating factors such as herd

composition, land area, and climatic variables (temperature, precipitation, windspeed) did not identify statistically significant predictors of seropositivity or RT-qPCR positivity.

Variables with high multicollinearity (VIF > 10) were excluded from the final analysis to improve model stability and interpretation. Logistic regression analysis identified significant relationships between age and seropositivity for both viruses (Figure 3.2). For BTV, each additional year of age increased the odds of seropositivity by 15% (OR: 1.15, CI: 1.05–1.26, p-value: 0.006). Similarly, for EHDV, each additional year of age was associated with a 16% increase in the odds of seropositivity (OR: 1.16, CI: 1.06–1.28, p-value: 0.0014). Age was also significantly negatively associated with RT-qPCR positivity for both viruses. For BTV, animals were less likely to be RT-qPCR positive (OR: 0.70, CI: 0.53–0.93, p-value: 0.014) as they aged, and the same pattern was observed for EHDV (OR: 0.56, CI: 0.33–0.93, p-value: 0.024). No significant associations with sex were observed for either seropositivity or RT-qPCR positivity (Table 3.2). No significance was associated with daily precipitation or 5-day average temperature and RT-qPCR positivity (Table 3.3).

Discussion

This study identified notably higher seroprevalence rates for BTV and EHDV in North American bison compared to previously reported values of 12.83% and 22.1% for BTV in European bison (*Bison bonasus*) populations (24,25). While comparisons with European bison seroprevalence are valuable, it is crucial to recognize fundamental differences in BTV and EHDV transmission dynamics between North America and Europe, including differences in habitat and climate. In the United States, these viruses

persist in endemic cycles, sustained by continuous vector activity in some geographic regions, whereas in Europe, outbreaks tend to be sporadic and are more tightly controlled through mitigation efforts (37–39). Additionally, European bison and North American bison are distinct species with potentially differing susceptibilities, immune responses, and ecological interactions with vectors. While EHD outbreaks in North America are commonly associated with high mortality in white-tailed deer, bison are generally considered incidental hosts and often remain asymptomatic, as noted during a natural outbreak in a captive facility (40). However, the 2012 EHDV epidemic in the United States revealed that morbidity in bison could reach as high as 7%, highlighting the potential risk to this species under certain conditions (20). Combined with the observed high seroprevalence in the present study, these findings suggest that bison could potentially contribute to *Orbivirus* transmission as incidental hosts, particularly during the first two years of life when they are more likely to be infective. As with European red deer, which do not appear to maintain BTV in France, it remains uncertain whether bison can sustain transmission cycles of if they are spillover hosts (41). Bison's role in transmission ecology is likely limited compared to species with prolonged periods of viremia, such as noted with EHDV in white-tailed deer (42). Further research into their role in *Orbivirus* ecology and implications for disease transmission dynamics is warranted.

There are many aspects that further complicate disease dynamics in bison, including BTV/EHDV serotype co-circulation and reassortment. Immunity to one serotype does not often provide effective protection against another (43–45). Additionally, BTV and EHDV exhibit strain-dependent variations in virulence, with

different strains of the same serotype causing varying levels of clinical disease (46,47). The detection of multiple BTV serotypes in the present study highlights the complexities associated with serotype-specific immunity and the potential for novel serotype introductions to precipitate outbreaks. During the BTV-8 novel outbreak in Europe (2006-2008), BTV-8 caused the deaths of 10 of 33 bison in a German breeding center along with up to a 40% morbidity and 20% mortality in European zoos (19,25). Reported clinical signs included lethargy, fever, mouth ulcers, drooling, difficulty eating, conjunctivitis, corneal edema, respiratory difficulty, lameness, inflammation of the coronary band, and sudden death (19). Notably, North American bison experimentally infected with BTV-11 developed detectable antibodies without exhibiting clinical signs (18). This finding highlights the ability of bison to mount an immune response to a specific serotype, potentially reducing clinical disease severity in subsequent exposures to the same serotype. The age-dependent dynamics observed in the current study may also reflect the accumulation of partial immunity in older animals due to prior exposures, which could mitigate the impact of subsequent infections. However, the introduction of a new serotype, to which the population has no prior exposure, can still result in high morbidity and mortality, as evidenced by the recent European outbreaks (39,48). These events emphasize how exposure to novel serotypes can lead to significant disease outbreaks, particularly in populations lacking prior immunity.

Geographic variability also plays a critical role in *Orbivirus* transmission (42,49). The wide range of seroprevalence observed between sites in the current study highlights the role of localized environmental factors and vector habitats in shaping *Orbivirus* transmission dynamics. Bison wallows, for example, serve as temporary

breeding sites for *Culicoides* spp., with species like *Culicoides sonorensis* favoring active wallows enriched by bison activity (27). These findings suggest that landscape features and host behaviors can create focal points for vector-host interactions, influencing disease transmission at a local level. Additionally, the 2012 EHD outbreak in the U.S. demonstrated geographic clustering, with most cases in cattle and bison occurring in Nebraska, South Dakota, and Iowa (20). Similarly, cattle studies have reported region-specific seroprevalence rates, emphasizing the impact of geographic and ecological factors on disease exposure risk (22).

In a broader context, climate change is expected to further influence the distribution and epidemiology of orbiviruses. Rising temperatures and altered precipitation patterns are predicted to expand the geographic range of *Culicoides* vectors, extend their active seasons, and increase the availability of suitable breeding habitats (3,8). For example, higher mean annual temperatures and age were correlated with increased BTV seroprevalence in water buffalo and cattle in southern Italy (50). These environmental shifts may promote viral transmission dynamics, particularly in landscapes with high host densities and modified habitats like wastewater lagoons and fragmented agricultural areas (1).

Although logistic regression modeling in the present study did not find significant associations in weather or herd demographics and BTV/EHDV status, the small sample size likely limited the power of the analyses. Additionally, the cross-sectional design of the study provides a snapshot of vector-host interactions but does not allow us to determine causality. Future research should include longitudinal studies to track seroconversion and PCR positivity over time, coupled with ecological assessments of

vector populations and habitats. Collaborations between wildlife and livestock health sectors are essential for integrating surveillance within a One Health framework.

Overall, our findings reveal significant age-associated dynamics in the epidemiology of BTV and EHDV in North American bison, with older animals showing higher seroprevalence but reduced PCR positivity. This suggests that immunity to orbiviruses accumulates over time due to repeated exposures, while risk of detectable viremia declines with age. Long term persistence of antibodies to both BTV and EHDV serotypes have been noted, supporting the concept that immunity following infection is generally long lasting for that specific serotype (51,52). Additionally, the timing of sampling within the vector season may influence observed seroprevalence and viremia rates, as animals sampled at the end of the season may have had more opportunities for exposure and antibody development, while those sampled earlier may be more likely to exhibit active infection. Nonetheless, these dynamics highlight the importance of considering life stage in disease surveillance and management strategies. Younger bison, which were more likely to exhibit active infections in our study, may contribute disproportionately to virus transmission during peak vector activity, while older individuals may serve as immunological sentinels, reflecting historical exposure to these viruses. To better understand these patterns, longitudinal sampling across multiple seasons to capture variations in exposure, immune response, and viremia over time is advised.

Understanding these age-related patterns is critical for designing targeted surveillance programs and control measures. Future research should explore how these dynamics interact with environmental and vector-related factors to influence disease

transmission at the wildlife-livestock interface. Enhanced surveillance efforts that incorporate bison as a model for understanding *Orbivirus* ecology can provide valuable insights into mitigating risks to livestock, wildlife, and ecosystem health. These results contribute to the growing body of evidence supporting the importance of wildlife in *Orbivirus* dynamics, particularly under the influence of climate change.

TABLES AND FIGURES

Table 3.1. Geographic distribution, seroprevalence, PCR-positivity, and serotype identification of *orbiviruses* in a 2023 cross-sectional survey of North American bison herds.

State	Serum*	Whole Blood*	Total Animals Sampled	BTV ELISA + (%)	EHDV ELISA + (%)	EHDV PCR + (n)	BTV PCR + (n)	BTV Serotypes
CO	10	10	10	70.0	70.0	0	3	N/A
IA	5	5	5	0.0	20.0	1	1	N/A
KS	30	29	30	83.3	86.7	2	5	6,13
MT	31	31	31	0.0	3.2	0	0	-
NE	30	30	30	0.0	6.7	1	0	-
OK	30	30	30	60.0	53.3	2	7	11,13,13,13
SD	30	31	32	80.0	76.7	0	0	-
SD	101	50	101	82.2	80.2	4	5	6,11,17,17
SD	18	0	18	22.2	33.3	-	-	-

* Total serum and whole blood samples collected per herd.

**BTV serotypes identified using PCR serotyping. Some positive samples could not be successfully serotyped.

N/A was placed in locations where serotypes could not be identified via RT-qPCR.

Table 3.2. Logistic regression analysis of sex as a function of BTV and EHDV test results from a BTV/EHDV cross-sectional survey in bison. No significance was noted.

Dependent Variable	Independent Variable	Estimate (β)	Std. Error	p-Value
BTV PCR	Sex	0.8245	0.4825	0.08747
EHDV PCR	Sex	0.9327	0.6814	0.171021
BTV ELISA	Sex	0.1089	0.4082	0.789565
EHDV ELISA	Sex	-0.3441	0.4075	0.398326

Table 3.3. Weather variables and BTV/EHDV PCR results from logistic regression modeling for a BTV/EHDV cross-sectional survey in bison. No significance was noted.

	EHDV PCR (β)	EHDV 95% CI	BTV PCR (β)	BTV 95% CI
Daily Precipitation	0.090	-0.361, 0.541	0.105	-0.230, 0.439
5-Day Avg Temp.	-0.228	-1.286, 0.830	-0.060	-0.697, 0.576
				* p<0.1; ** p<0.05; *** p<0.01

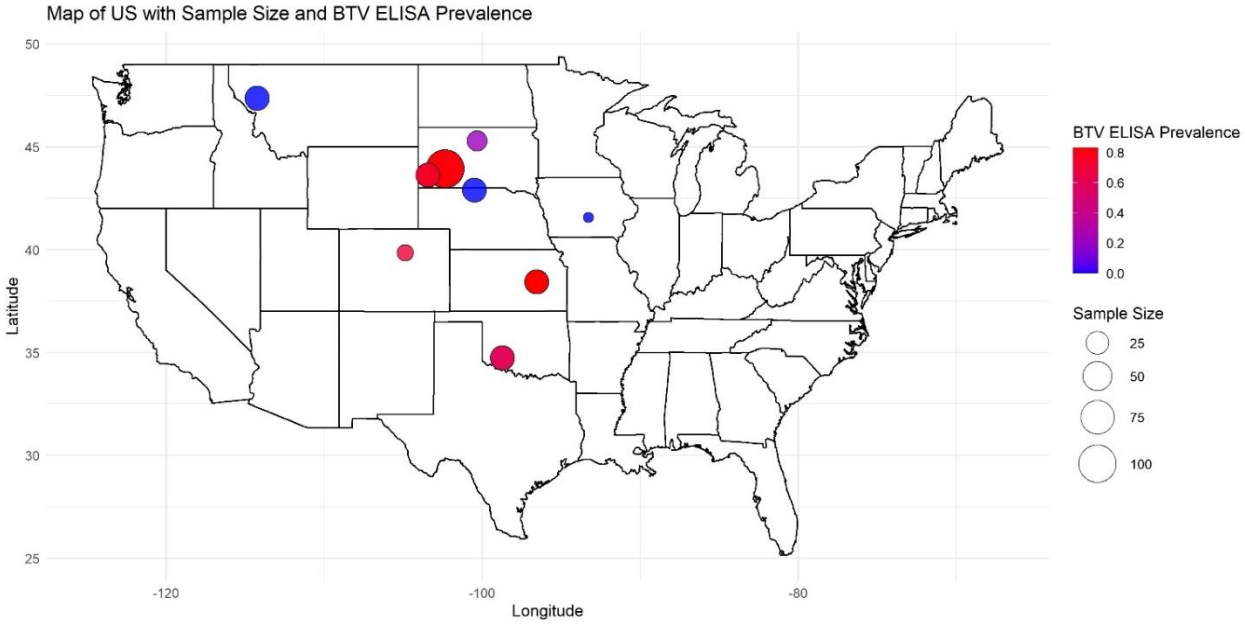

Figure 3.1. Geographic distribution and herd size of North American bison with associated BTV seroprevalence in a 2023 cross-sectional study on Bluetongue Virus and Epizootic Hemorrhagic Disease.

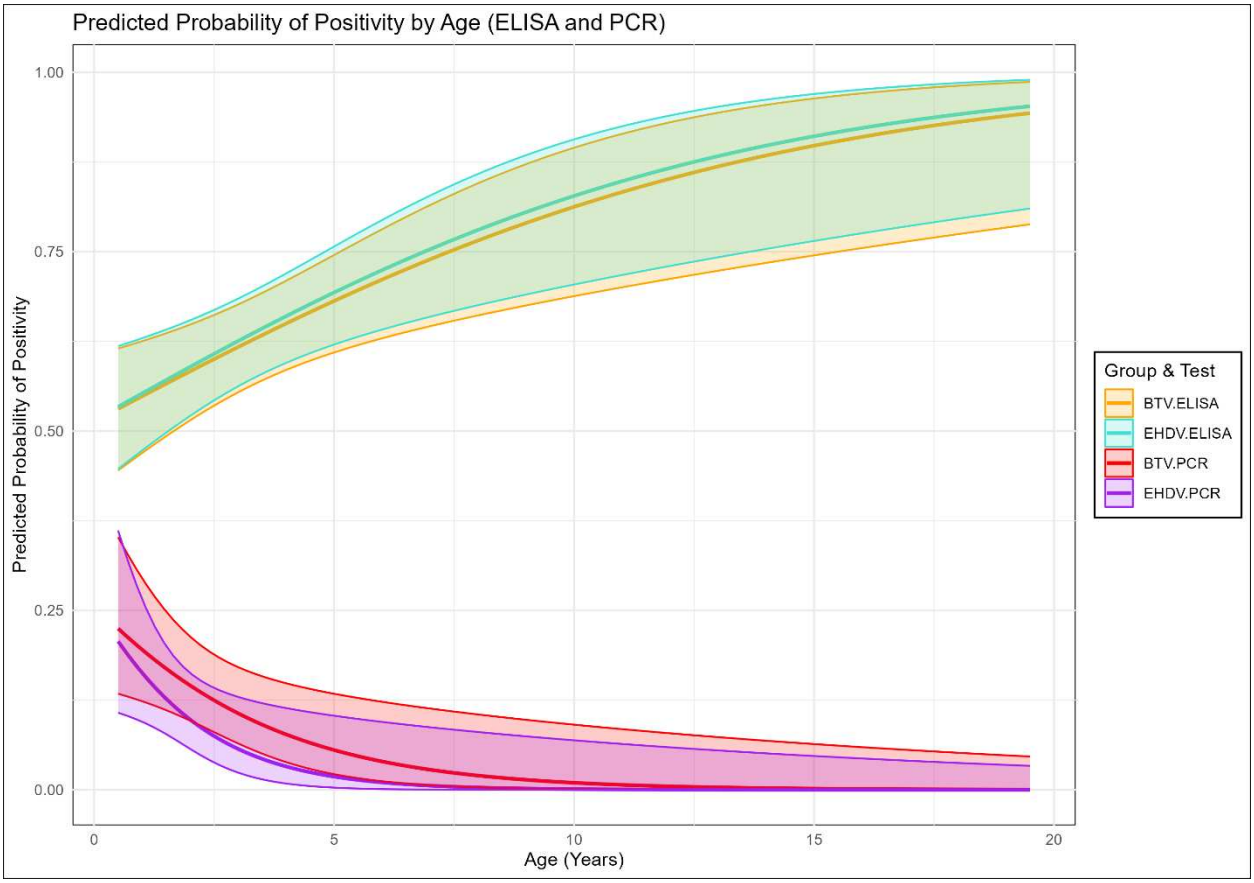

Figure 3.2. Predicted probability of positivity (ELISA and PCR) for Bluetongue Virus and Epizootic Hemorrhagic Disease Virus by age in bison from a 2023 cross-sectional study, including 95% confidence intervals.

REFERENCES

1. Mayo C, McDermott E, Kopanke J, Stenglein M, Lee J, Mathiason C, Carpenter M, Reed K, Perkins TA. Ecological Dynamics Impacting Bluetongue Virus Transmission in North America. *Front Vet Sci* (2020) 7: doi: 10.3389/fvets.2020.00186

2. Gould EA, Higgs S. Impact of Climate Change and Other Factors on Emerging Arbovirus Diseases. *Trans R Soc Trop Med Hyg* (2009) 103:109–121. doi: 10.1016/j.trstmh.2008.07.025

3. Navarro Mamani DA, Ramos Huere H, Vera Buendia R, Rojas M, Chunga WA, Valdez Gutierrez E, Vergara Abarca W, Rivera Gerónimo H, Altamiranda-Saavedra M. Would Climate Change Influence the Potential Distribution and Ecological Niche of Bluetongue Virus and Its Main Vector in Peru? *Viruses* (2023) 15: doi: 10.3390/v15040892

4. Mellor P, Boorman J, Baylis M. Culicoides Biting Midges: Their Role as Arbovirus Vectors. (2000). 307–340 p. www.annualreviews.org

5. Mellor PS, Carpenter S, White DM. *Bluetongue Virus in the Insect Host 14*. (2009). doi: 10.1016/B978-0-12-369368-6.50018-6

6. Marquardt WC, Kondratieff BC, Borkent A. “The Biting Midges, the Ceratopogonidae (Diptera).,” In: Marquardt WH, editor. *Biology of Disease Vectors*. Elsevier Science & Technology (2004). p. 113–126 http://ebookcentral.proquest.com/lib/csu/detail.action?docID=297059.

7. Mills MK, Michel K, Pfannenstiel RS, Ruder MG, Veronesi E, Nayduch D. Culicoides–Virus Interactions: Infection Barriers and Possible Factors Underlying Vector Competence. *Curr Opin Insect Sci* (2017) 22:7–15. doi: 10.1016/j.cois.2017.05.003

8. Samy AM, Peterson AT. Climate Change Influences on the Global Potential Distribution of Bluetongue Virus. *PLoS One* (2016) 11: doi: 10.1371/journal.pone.0150489

9. Maclachlan NJ. Global Implications of the Recent Emergence of Bluetongue Virus in Europe. *Veterinary Clinics of North America - Food Animal Practice* (2010) 26:163–171. doi: 10.1016/j.cvfa.2009.10.012

10. Coetzee P, Stokstad M, Venter EH, Myrmel M, Van Vuuren M. Bluetongue: A Historical and Epidemiological Perspective with the Emphasis on South Africa. *Virol J* (2012) 9: doi: 10.1186/1743-422X-9-198

11. Walton TE. The History of Bluetongue and a Current Global Overview. (2004).

12. Schulz C, Bréard E, Sailleau C, Jenckel M, Viarouge C, Vitour D, Palmarini M, Gallois M, Höper D, Hoffmann B, et al. Bluetongue Virus Serotype 27: Detection

and Characterization of Two Novel Variants in Corsica, France. *Journal of General Virology* (2016) 97:2073–2083. doi: 10.1099/jgv.0.000557

13. Yang H, Gu W, Li Z, Zhang L, Liao D, Song J, Shi B, Hasimu J, Li Z, Yang Z, et al. Novel Putative Bluetongue Virus Serotype 29 Isolated from Inapparently Infected Goat in Xinjiang of China. *Transbound Emerg Dis* (2021) 68:2543–2555. doi: 10.1111/tbed.13927

14. Bumbarov V, Golender N, Jenckel M, Wernike K, Beer M, Khinich E, Zalesky O, Erster O. Characterization of Bluetongue Virus Serotype 28. *Transbound Emerg Dis* (2020) 67:171–182. doi: 10.1111/tbed.13338

15. Jiménez-Cabello L, Utrilla-Trigo S, Calvo-Pinilla E, Moreno S, Nogales A, Ortego J, Marín-López A. Viral Vector Vaccines Against Bluetongue Virus. *Microorganisms* (2020) 9:42. doi: 10.3390/microorganisms9010042

16. Maan NS, Maan S, Belaganahalli MN, Ostlund EN, Johnson DJ, Nomikou K, Mertens PPC. Identification and Differentiation of the Twenty Six Bluetongue Virus Serotypes by RT-PCR Amplification of the Serotype-Specific Genome Segment 2. *PLoS One* (2012) 7: doi: 10.1371/journal.pone.0032601

17. Rao PP, Hegde NR, Singh KP, Putty K, Hemadri D, Maan NS, Reddy YN, Maan S, Mertens PPC. “Bluetongue: Aetiology, Epidemiology, Pathogenesis, Diagnosis and Control.,” *Emerging and Re-emerging Infectious Diseases of Livestock*. Springer International Publishing (2017). p. 3–54 doi: 10.1007/978-3-319-47426-7_1

18. Tessaro S V, Clavijo A. Duration of Bluetongue Viremia in Experimentally Infected American Bison. (2001). 722–729 p. http://meridian.allenpress.com/jwd/article-pdf/37/4/722/2235624/0090-3558-37_4_722.pdf

19. Sanderson S, Zoo C. Bluetongue in Non-Domestic Ruminants: Experiences Gained in EAZA Zoos During the 2007 & 2008 BTV8 and BTV1 Epizootics.

20. Stevens G, McCluskey B, King A, O’Hearn E, Mayr G. Review of the 2012 Epizootic Hemorrhagic Disease Outbreak in Domestic Ruminants in the United States. *PLoS One* (2015) 10: doi: 10.1371/journal.pone.0133359

21. Kedmi M, Van Straten M, Ezra E, Galon N, Klement E. Assessment of the Productivity Effects Associated with Epizootic Hemorrhagic Disease in Dairy Herds. *J Dairy Sci* (2010) 93:2486–2495. doi: 10.3168/jds.2009-2850

22. Hoar BR, Carpenter TE, Singer RS, Gardner IA. Regional Risk of Exporting Cattle Seropositive for Bluetongue Virus from the United States. (2003).

23. Velthuis AGJ, Saatkamp HW, Mourits MCM, de Koeijer AA, Elbers ARW. Financial Consequences of the Dutch Bluetongue Serotype 8 Epidemics of 2006 and 2007. *Prev Vet Med* (2010) 93:294–304. doi: 10.1016/j.prevetmed.2009.11.007

24. Larska M, Tomana J, Socha W, Rola J, Kubiś P, Olech W, Krzysiak MK. Learn the Past and Present to Teach the Future—Role of Active Surveillance of Exposure to

Endemic and Emerging Viruses in the Approach of European Bison Health Protection. *Diversity (Basel)* (2023) 15:535. doi: 10.3390/d15040535

25. Didkowska A, Klich D, Nowak M, Wojciechowska M, Prolejko K, Kwiecień E, Rzewuska M, Olech W, Anusz K. A Serological Survey of Pathogens Associated with the Respiratory and Digestive System in the Polish European Bison (Bison bonasus) Population in 2017–2022. *BMC Vet Res* (2023) 19:74. doi: 10.1186/s12917-023-03627-y

26. Krzysiak MK, Iwaniak W, Kęsik-Maliszewska J, Olech W, Larska M. Serological Study of Exposure to Selected Arthropod-Borne Pathogens in European Bison (Bison bonasus) in Poland. *Transbound Emerg Dis* (2017) 64:1411–1423. doi: 10.1111/tbed.12524

27. Pfannenstiel RS, Ruder MG. Colonization of Bison (Bison bison) Wallows in a Tallgrass Prairie by Culicoides spp (Diptera: Ceratopogonidae). *Journal of Vector Ecology* (2015) 40:187–190. doi: 10.1111/jvec.12150

28. Shamon H, Cosby OG, Andersen CL, Augare H, BearCub Stiffarm J, Bresnan CE, Brock BL, Carlson E, Deichmann JL, Epps A, et al. The Potential of Bison Restoration as an Ecological Approach to Future Tribal Food Sovereignty on the Northern Great Plains. *Front Ecol Evol* (2022) 10: doi: 10.3389/fevo.2022.826282

29. Heuer K, Farr J, Littlebear L, Hebblewhite M. Reintroducing Bison to Banff National Park – an Ecocultural Case Study. *Frontiers in Conservation Science* (2023) 4: doi: 10.3389/fcosc.2023.1305932

30. Boyce AJ, Shamon H, McShea WJ. Bison Reintroduction to Mixed-Grass Prairie Is Associated With Increases in Bird Diversity and Cervid Occupancy in Riparian Areas. *Front Ecol Evol* (2022) 10: doi: 10.3389/fevo.2022.821822

31. Miller RS, Farnsworth ML, Malmberg JL. Diseases at the Livestock–Wildlife Interface: Status, Challenges, and Opportunities in the United States. *Prev Vet Med* (2013) 110:119–132. doi: 10.1016/j.prevetmed.2012.11.021

32. National Park Service. Department of the Interior Bison Conservation Initiative. *Bison Bellows* (2016)

33. Wilson WC, Hindson BJ, O'hearn ES, Hall S, Tellgren-Roth C, Torres C, Naraghi-Arani P, Mecham JO, Lenhoff RJ. A Multiplex Real-Time Reverse Transcription Polymerase Chain Reaction Assay for Detection and Differentiation of Bluetongue Virus and Epizootic Hemorrhagic Disease Virus Serogroups.

34. Maan NS, Maan S, Potgieter AC, Wright IM, Belaganahalli M, Mertens PPC. Development of Real-Time RT-PCR Assays for Detection and Typing of Epizootic Haemorrhagic Disease Virus. *Transbound Emerg Dis* (2017) 64:1120–1132. doi: 10.1111/tbed.12477

35. Hofmann M, Griot C, Chaignat V, Perler L, Thür B. [Bluetongue Disease Reaches Switzerland]. *Schweiz Arch Tierheilkd* (2008) 150:49–56. doi: 10.1024/0036-7281.150.2.49

36. Maan S, Maan NS, Belaganahalli MN, Potgieter AC, Kumar V, Batra K, Wright IM, Kirkland PD, Mertens PPC. Development and Evaluation of Real Time RT-PCR Assays for Detection and Typing of Bluetongue Virus. *PLoS One* (2016) 11: doi: 10.1371/journal.pone.0163014

37. Committee on Foreign and Emerging Animal Diseases. Re-evaluation of Endemic Bluetongue Virus Serotypes in the United States. (2020).

38. Wilson AJ, Mellor PS. Bluetongue in Europe: Past, present and Future. *Philosophical Transactions of the Royal Society B: Biological Sciences* (2009) 364:2669–2681. doi: 10.1098/rstb.2009.0091

39. Gondard M, Postic L, Garin E, Turpaud M, Vorimore F, Ngwa-Mbot D, Tran M-L, Hoffmann B, Warembourg C, Savini G, et al. Exceptional Bluetongue Virus (BTV) and Epizootic Hemorrhagic Disease Virus (EHDV) Circulation in France in 2023. *Virus Res* (2024)199489. doi: 10.1016/j.virusres.2024.199489

40. Nol P, Kato C, Reeves WK, Rhyan J, Spraker T, Gidlewski T, Vercauteren K, Salman M. Epizootic Hemorrhagic Disease Outbreak in a Captive Facility Housing White-Tailed Deer (Odocoileus virginianus), Bison (Bison bison), Elk (Cervus elaphus), Cattle (Bos taurus), and Goats (Capra hircus) in Colorado, USA. *Journal of Zoo and Wildlife Medicine* (2010) 41:510–515. doi: 10.1638/2009-0216.1

41. Rossi S, Balenghien T, Viarouge C, Faure E, Zanella G, Sailleau C, Mathieu B, Delécolle JC, Ninio C, Garros C, et al. Red Deer (Cervus elaphus) did not Play the Role of Maintenance Host for Bluetongue Virus in France: The burden of Proof by Long-Term Wildlife Monitoring and Culicoides Snapshots. *Viruses* (2019) 11: doi: 10.3390/v11100903

42. Rivera NA, Varga C, Ruder MG, Dorak SJ, Roca AL, Novakofski JE, Mateus-Pinilla NE. Bluetongue and Epizootic Hemorrhagic Disease in the United States of America at the Wildlife-Livestock Interface. *Pathogens* (2021) 10: doi: 10.3390/pathogens10080915

43. Martinelle L, Dal Pozzo F, Thys C, De Leeuw I, Van Campe W, De Clercq K, Thiry E, Saegerman C. Assessment of Cross-Protection Induced by a Bluetongue Virus (BTV) Serotype 8 Vaccine Towards Other BTV Serotypes in Experimental Conditions. *Vet Res* (2018) 49: doi: 10.1186/s13567-018-0556-4

44. Fay PC, Jaafar FM, Batten C, Attoui H, Saunders K, Lomonossoff GP, Reid E, Horton D, Maan S, Haig D, et al. Serological Cross-Reactions Between Expressed vp2 Proteins from Different Bluetongue Virus Serotypes. *Viruses* (2021) 13: doi: 10.3390/v13081455

45. Bissett SL, Roy P. Impact of VP2 Structure on Antigenicity: Comparison of BTV1 and the Highly Virulent BTV8 Serotype. *J Virol* (2024) 98: doi: 10.1128/jvi.00953-24

46. Jiménez-Cabello L, Utrilla-Trigo S, Lorenzo G, Ortego J, Calvo-Pinilla E. Epizootic Hemorrhagic Disease Virus: Current Knowledge and Emerging Perspectives. *Microorganisms* (2023) 11: doi: 10.3390/microorganisms11051339

47. Janowicz A, Caporale M, Shaw A, Gulletta S, Di Gialleonardo L, Ratinier M, Palmarini M. Multiple Genome Segments Determine Virulence of Bluetongue Virus Serotype 8. *J Virol* (2015) 89:5238–5249. doi: 10.1128/jvi.00395-15

48. van den Brink KMJA, Brouwer-Middelesch H, van Schaik G, Lam TJGM, Stegeman JA, van den Brom R, Spierenburg MAH, Santman-Berends IMGA. The Impact of Bluetongue Serotype 3 on Cattle Mortality, Abortions and Premature Births in the Netherlands in the First Year of the Epidemic. *Prev Vet Med* (2025) 239: doi: 10.1016/j.prevetmed.2025.106493

49. Barua S, Rana EA, Prodhan MA, Akter SH, Gogoi-Tiwari J, Sarker S, Annandale H, Eagles D, Abraham S, Uddin JM. The Global Burden of Emerging and Re-Emerging Orbiviruses in Livestock: An Emphasis on Bluetongue Virus and Epizootic Hemorrhagic Disease Virus. *Viruses* (2025) 17: doi: 10.3390/v17010020

50. Ferrara G, Improda E, Piscopo F, Esposito R, Iovane G, Pagnini U, Montagnaro S. Bluetongue Virus Seroprevalence and Risk Factor Analysis in Cattle and Water Buffalo in Southern Italy (Campania region). *Vet Res Commun* (2024) 48:579–584. doi: 10.1007/s11259-023-10215-w

51. Eschbaumer M, Eschweiler J, Hoffmann B. Long-Term Persistence of Neutralising Antibodies against Bluetongue Virus Serotype 8 in Naturally Infected Cattle. *Vaccine* (2012) 30:7142–7143. doi: 10.1016/j.vaccine.2012.08.030

52. WOAH. “Epizootic Haemorrhagic Disease (Infection with Epizootic Hemorrhagic Disease Virus).,” *WOAH Terrestrial Manual 2021*. (2021)

CHAPTER 4 – EVALUATION OF A P48 ELISA FOR *MYCOPLASMA BOVIS* IN NORTH AMERICAN BISON (*BISON BISON*): INFERIOR PERFORMACE COMPARED TO A COMMERCIALLY AVAILABLE ELISA

Introduction

North American bison (*Bison bison*) are a species of ecological, cultural, and economic significance. As a keystone species, they maintain the balance of grassland ecosystems and play a vital role in supporting Indigenous food sovereignty (1–3). However, bison populations continue to face challenges from *Mycoplasma bovis*, a bacterial pathogen first detected in the United States in the late 2000s (4,5). By 2013, *M. bovis* had emerged as a significant threat to both conservation and production herds, with a rapid increase in the number of affected herds and geographic spread noted in 2021 (4–7). Unlike in cattle, where *M. bovis* is associated with the polymicrobial bovine respiratory disease complex, bison experience severe primary infections characterized by caseonecrotic pneumonia, septic arthritis, and high mortality rates, with outbreaks leading to losses of 10–45% of adults (6,8,9).

Due to the challenges associated with the safe capture and handling of bison, effective diagnostic tools are essential for disease management, particularly for identifying asymptomatic and chronic carriers. In cattle, chronic carriers can intermittently shed *M. bovis*, complicating detection, and similar subclinical or persistent infections are suspected in bison (10–12). These undetected infections likely contribute to disease persistence within herds (8,13).

The lack of species-specific diagnostic tools for wildlife uncovers the need for more targeted and effective diagnostic methods specifically designed for bison. Current antemortem diagnostic methods include PCR for detecting active *M. bovis* infections and commercially developed ELISAs for serological surveillance, yet both approaches have limitations (12,14,15). Widely used commercial ELISA kits were originally developed for cattle and may not accurately reflect *M. bovis* exposure status in bison (8,14). Previous studies evaluated the suitability of these kits for bison sera and demonstrated that their performance was influenced by the antigen source and the conjugate used for IgG detection (14). Additionally, these commercial kits may fail to detect low antibody titers in infected but asymptomatic animals, leading to underestimation of antibody prevalence (10). This challenge is compounded by uncertainty surrounding the interpretation of existing serologic data, which has led to conflicting conclusions about the true extent of *M. bovis* exposure in bison populations.

A previous report initially suggested widespread exposure of bison to *M. bovis* based on seropositive results (16). However, later research revealed significant cross-reactivity between *M. bovis* and other *Mycoplasma* species in serologic assays (8). This misinterpretation emphasized the urgent need for more accurate diagnostic tools to distinguish between true *M. bovis* infections and exposure to other *Mycoplasma* species. The implications of resolving this ambiguity are profound: either *M. bovis* exposure is far more geographically and numerically extensive than previously thought, or it remains a primary pathogen limited to herds with documented mortality events (12). Clarifying this distinction is critical for shaping future bison

management strategies, whether focused on broad surveillance or targeted interventions in high-risk herds.

To address these limitations, alternative antigen targets have been explored to improve ELISA specificity and sensitivity in bison. The P48 protein, a conserved surface antigen of *M. bovis*, is homologous to the P48 protein in *Mycoplasma agalactiae*, which has proven to be a highly sensitive and specific antigen in *M. agalactiae* assays (17). Given the high number of other *Mycoplasma* species known to occur in cattle and bison, assays for both *M. bovis* and *M. agalactiae* require a high degree of specificity to minimize cross-reactivity (17,18). Therefore, this study aimed to develop an indirect ELISA test for the detection of antibodies against the *M. bovis* P48 protein and compare the performance of this assay to a commercially available test kit.

Materials and Methods

Purification of Recombinant M. bovis P48 Protein

The sequence of the P48 gene from *M. bovis* PG45 was codon-optimized for expression in *E. coli*, synthesized, then cloned into the pET-28a(+) plasmid (GenScript, Piscataway, NJ, USA). Recombinant plasmids were used to transform BL21 *E. coli* (ThermoFisher, Waltham, MA, USA) following manufacturer's instructions and positive clones were selected by plating on LB agar plates with kanamycin (100µg/ml) at 37 °C for 18 hrs. Colonies were used to inoculate 100ml cultures of LB broth with kanamycin incubated with shaking at 37 °C until the OD600 reached 0.5. Protein production was induced by the addition of isopropylthiogalactoside to the culture medium to a final concentration of 0.3mM. Following a 2 hr incubation, bacterial cells were harvested by

centrifugation at 4000 x g, 10 min at 4 °C. The supernatant was discarded and the cell pellet resuspended in binding buffer. *E. coli* cells were disrupted via sonication, details, and the insoluble material was pelleted via centrifucation at 10,000 x g for 10 minutes at 4 °C. Histidine tagged P48 protein were purified from the supernatant by filtering through a nickel nitrilotriacetic acid resin (Millipore Sigma, Burlington, MA, USA) on a rocking platform for 1 hr at 4 °C. Bound protein was eluted by addition of 0.5 ml elution buffer on ice for 5 minutes. The resin was removed from the eluted protein by centrifugation at 13,000 rpm for 2 min at 4 °C. Supernatant from the elution step were further purified by dialysis using a dialysis cassette (ThermoFisher) in 50mM Tris-HCL pH 8.0 and stored at -80 °C.

P48 Indirect ELISA Development

An indirect ELISA targeting the *Mycoplasma bovis* P48 gene was developed following a standardized protocol. 96-well microtiter plates (Thermo cat #14-245-153) were coated with P48 antigen at a concentration of 4 µg/mL by diluting stock protein in 1X phosphate-buffered saline (PBS) and incubating overnight at 4°C. Plates were washed with PBS-T and then blocked with 3% milk in PBS-Tween 20 (PBS-T) for 1 hour at room temperature.

Samples were heat-inactivated for 1 hour at 56 °C. Serum samples were diluted 1:50 in PBS-T containing 1% milk and added in duplicate to wells, followed by a 2-hour incubation at room temperature. After washing, an HRP-conjugated secondary antibody (protein A/G HRP Thermo Scientific #32490) was diluted 1:10,000 in PBS-T containing 1% milk and incubated for 1 hour at room temperature. Plates were developed using SigmaFast OPD substrate, and the reaction was stopped with 3M HCl after 6 minutes.

Optical density (OD) was read at 490 nm using a microplate reader and the mean optical density of the sample triplicate or duplicate was used for classification. Positive control serum was obtained from a bison with confirmed *M. bovis* infection by PCR, culture, and commerical ELISA positivity. Negative control serum was sourced from long-term monitored herds with no history of *M. bovis* exposure, as determined by annual ELISA and PCR testing.

Cutoff Determination for P48 ELISA

Following optimization, the final cutoff values were applied as follows:

Negative	Indeterminant	Positive
< 0.30	0.30-0.43	>0.43

These values were calculated based on known positive and negative samples. When running optimization protocols, false negatives were found within the indeterminate range (0.30-0.43), along with many known negative samples. The lower limit of the indeterminate range is the standard deviation of the negative controls times three. The upper limit of the indeterminate range is the mean OD of negative control samples + (3 × standard deviation of negative controls).

Comparison of the P48 ELISA to the commercial ELISA

To assess the performance of P48 ELISA, results were compared to the commercially available BioX BioK260 *Mycoplasma bovis* ELISA (Rochefort, Belgium). The commercial ELISA was performed according to the manufacturer's instructions using the same serum sample set.

Three distinct sample groups were used to evaluate the assays:

1. Confirmed *M. bovis*-Positive Samples (n = 35): Defined as samples from bison that tested positive for *M. bovis* by PCR at least 3 months after the start of an outbreak.
2. Other *Mycoplasma* Samples (n = 30): Defined as samples that were culture positive for other *Mycoplasma* species (n=28 *M. bovirhinis*; one *M. dispar*; one *Enterobacter* spp.) but PCR and culture negative for *M. bovis*.
3. Negative Control Samples (n = 168): Obtained from bison herds with long-term annual testing and no historic positive results on commercial ELISA, culture and/or PCR for *M. bovis*.

All PCR samples in the present study were tested using the validated *uvrC* gene, which has demonstrated greater sensitivity than conventional PCR in cattle (12,19).

All serum samples were heat-inactivated at 56°C for 60 minutes as a biosecurity measure as noted in Schwartz et al. (1973) and did not impact results (20). Samples were then aliquoted into ~100 µL volumes to minimize freeze-thaw cycles and stored at -20°C until testing.

Samples classified as indeterminate were excluded from the final statistical analysis to ensure a clear distinction between positive and negative results. This approach was chosen to reduce potential misclassification bias and improve assay accuracy.

Sensitivity (Se) and specificity (Sp) were calculated along with their corresponding 95% confidence intervals (CIs) using the Wilson Score Interval method. These calculations were performed in R (v4.3.0) using the binom package (v1.1-1.1).

Additional diagnostic metrics calculated included positive predictive value (PPV), negative predictive value (NPV), false positive rate (FPR) and false negative rate (FNR), overall accuracy, receiver operating characteristic (ROC) curve and area under the curve (AUC), and Cohen's Kappa Statistic to assess agreement between ELISAs. These analyses were performed with the tidyverse (v2.0.0), binom (v1.1-1.1), pROC (v1.18.5), and irr packages (v0.84.1). DeLong's test was used to compare the statistical significance of the AUCs. A p-value < 0.05 was considered significant. Graphs were created using ggplot2 (v3.5.1).

Results

Sensitivity and Specificity

The P48 ELISA, commercial ELISA, and a combination of the two ELISAs (combined ELISA) were evaluated on their ability to detect *M. bovis* positive serum samples. The commercial ELISA demonstrated higher sensitivity (77.8%, CI: 61.9-88.3%) and specificity (97.5%, CI: 94.2-98.9) (Table 1) indicating a higher accuracy in identifying both positive and negative samples. In contrast, the P48 ELISA had a lower sensitivity (63.9%) but maintained a high specificity (94.9%). The combined ELISA, in which a sample was considered positive if either test was positive, yielded the highest sensitivity (83.3%, CI: 68.1-92.1%), at the cost of a slightly lower specificity (93.4%, 89.1-96.1%) (Figure 4.1, Table 4.1).

Predictive Values and Overall Accuracy

The Commercial ELISA had the highest PPV (84.8%, CI: 69.1-93.3%) compared to 69.7% (CI: 52.7-82.6%) for the P48 ELISA, indicating that the commercial ELISA had the highest probability that a positive result correctly identified an infected individual (Figure 4.1, Table 4.1). In contrast, the combined ELISA had a marginally higher NPV (combined 96.9%, CI: 93.3-98.6; Commercial 96.0% CI: 92.3-98.0%), minimizing false negatives and ensuring that negative results were more reliable. Additionally, the commercial ELISA had the highest overall accuracy at 94.4%, while the P48 and combined ELISA had an accuracy of 90.2% and 91.9% respectively (Figure 4.1, Table 4.1).

False Positive and False Negative Rates

False positive rate (FPR) and false negative rate (FNR) were evaluated to assess diagnostic reliability (Table 2). The commercial ELISA had the lowest FPR (2.53%), making it the most specific test with the combined ELISA having the lowest FNR (16.7%). The P48 ELISA had the highest FNR (36.1%). In the other *Mycoplasma* spp. group, the P48 ELISA detected 6/30 (20%) false positives while the commercial ELISA detected 5/30 (16.7%) false positives**.**

Comparison of AUC Values

The area under the curve (AUC) was computed for each test to measure overall diagnostic performance (Table 4.3, Figure 4.2). The commercial ELISA had the highest AUC (0.876), followed closely by the combined ELISA (0.884), while P48 ELISA had the lowest AUC (0.794), indicating reduced discriminatory power.

To determine whether differences in AUC were statistically significant, DeLong's test for two correlated ROC curves was applied. These findings confirm that commercial ELISA is statistically superior to the P48 ELISA (p-value: 0.048) but combining both ELISAs does not significantly outperform commercial alone (p-value: 0.713). These results confirm that the commercial ELISA alone is the most effective, and while the combined ELISA slightly improves AUC, the difference is not statistically significant.

Cohen's Kappa: Agreement Between Tests

The Cohen's kappa statistic was used to measure agreement between ELISA tests. The P48 compared to the commercial ELISA had a kappa value of 0.674 indicating moderate agreement. The combined ELISA verses the positive or negative sample categories had a stronger agreement of 0.711 indicating substantial agreement and improved diagnostic accuracy.

Discussion

Our findings indicate that the P48 ELISA did not surpass the commercial test kit in diagnostic performance. Additionally, the combination of the two ELISAs did not significantly improve serological detection of *M. bovis* antibodies in bison serum samples. The findings presented in this study also provide the first formal sensitivity and specificity estimates for the commercial ELISA in bison (Se: 77.8, Sp: 97.5, Figure 4.1, Table 4.1). While the commercial ELISA performed better than the P48 ELISA, both assays had limitations, showing the necessity for further refinement of *M. bovis* serological testing.

A key objective of this study was to evaluate the specificity of the commercial ELISA, given its suspected cross-reactivity with other *Mycoplasma* species and its previous use without validation leading to result misinterpretation (8,16). Cross-reactive antibodies produced in response to other ruminant *Mycoplasma* species introduce false positive serological diagnostic tests that can reduce diagnostic test accuracy and misinform disease management decisions. In this study, 30 serum samples from bison infected with other *Mycoplasma* species (e.g., *M. bovirhinis*) were tested to assess assay specificity. Ideally, a highly specific ELISA should classify these samples as negative though our data shows the P48 ELISA produced 6/30 (20%) false positives, while the commercial ELISA yielded 5/30 (16.7%), indicating that both assays may detect cross-reactive antibodies. This suggests that while P48 may be a strong antigen for detecting *M. bovis*-specific antibodies, it may share conserved epitopes with proteins of other *Mycoplasma* species resulting in false positives. Minimizing false positives is critical for effective diagnostic tests as incorrect identification of infected animals can lead to adverse events including unnecessary herd interventions, movement restrictions, or culling.

Although the specificity of both assays remained high (~95–97%), the high level of false positives in the other *Mycoplasma* spp. samples may have significant impacts to herd-level disease management in herds with high rates of these other *Mycoplasma* species. These findings are consistent with Register et al. (2021), which reported approximately 3% seropositivity among bison herds with no known history of *M. bovis*, matching the expected false-positive rate of the commercial ELISA in non-infected herds (16). This dramatically changes the current understanding of the ubiquity of *M.*

bovis and the importance of preventing *M. bovis* introduction into naïve herds and demonstrates that the current ELISAs available must be used alongside other confirmatory testing such as PCR, *Mycoplasma* culture, or repeated serological testing to ensure accuracy.

Unlike ELISA, which detects antibodies, PCR identifies the pathogen, making direct comparisons difficult. Due to the lag in time between initial infection and the development of detectable antibodies, it is plausible that PCR positive animals might test negative on ELISA simply due to the timing of sample collection. This, in turn, could lead to an underestimation of ELISA sensitivity, as truly infected animals may not yet have circulating antibodies at the time of testing. This challenge is compounded by the fact that timing and consistency of antibody production in bison infected with *Mycoplasma bovis* have not been well characterized. Bison appear to exhibit variability in humoral immune responses, with some animals failing to seroconvert despite confirmed infection through multiple positive nasal swab PCR tests (21). However, findings from the present study, in which samples were collected from PCR-positive animals in herds that experienced *M. bovis*-associated mortality at least three months prior to sampling, suggest that sufficient time had likely elapsed for the development of detectable antibody levels.

Establishing an appropriate P48 ELISA cutoff was critical for balancing sensitivity and specificity. Unlike some ELISA validation studies that use dilution series to determine antibody detection thresholds, this study optimized cutoff values using known PCR-confirmed positive and negative samples (22). Given the lack of a universal serological gold standard for *M. bovis*, an indeterminate range (0.30–0.43 OD) was

implemented to account for borderline cases, reducing false positives while flagging weakly reactive samples for retesting. The final positive cutoff was set at >0.43 OD, ensuring a conservative threshold for classifying positive sera. By using real sample distributions instead of serial dilutions, this method improves practical applicability for field diagnostics.

One important direction for ELISA development is the exploration of *M. bovis* antigenic diversity, particularly variable surface proteins (Vsps), which undergo phase variation to evade immunity (23,24). Such antigenic variation predicts that an ELISA based on a single protein could miss strains or phases of the pathogen that do not prominently express that target. Future proteomic and immunologic comparisons between *M. bovis* isolates from bison and cattle could identify additional immunodominant markers, improving diagnostic accuracy. Given that cross-reactivity with other *Mycoplasma* species remains a significant challenge, targeted antigen modifications and epitope mapping could enhance assay specificity. Similar screening efforts in bison, utilizing convalescent sera, could identify new antigenic targets that improve sensitivity while reducing non-specific reactivity. Wynn et al. identified a core genome shared across *M. bovis* strains from both cattle and bison, which includes 38 secreted or outer membrane proteins with antigenic potential (25). Rather than pointing to major genomic differences between strains, these findings suggest that multiple antigens are consistently present and potentially recognized by the host immune system, even within a single strain. Therefore, a multi-antigen approach combining P48 with other immunogenic proteins may improve detection by compensating for antigenic and host immune response variation (26–28).

Ultimately, these findings bring to light the challenges in *M. bovis* diagnostics, particularly in improving sensitivity without compromising specificity and expanding the diagnostic toolkit to keep pace with *M. bovis*'s antigenic variability across time and host species. By targeting stable immunogenic proteins and implementing multiplex antibody detection, more robust serological assays for bison herd surveillance and management tools may be developed in the future. This study provides foundational data on the performance of the commercial ELISA in bison and expresses demand for next-generation serological diagnostics tailored to bison-specific *M. bovis* epidemiology. It also reinforces the importance of re-evaluating past findings and interpretations derived from the commercial ELISA, given its limitations in this host species.

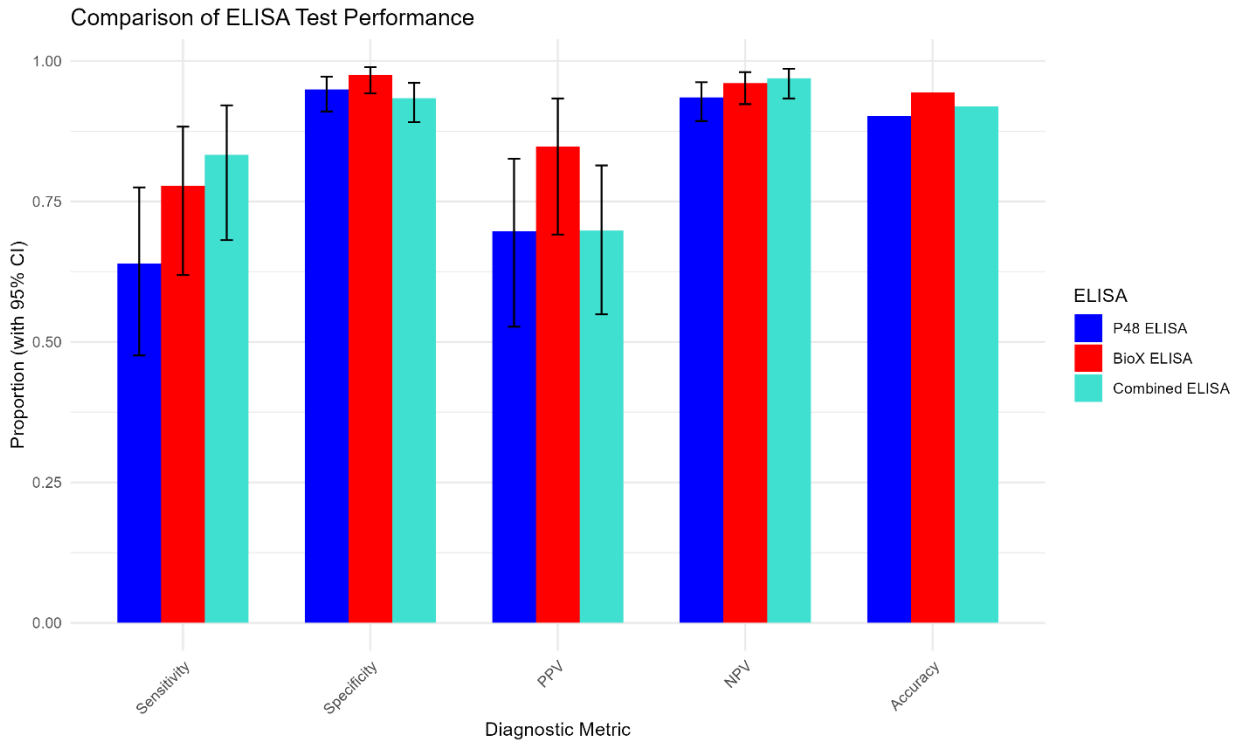

Figure 4.1. Comparison of sensitivity, specificity, negative predictive values (NPV), positive predictive values (PPV) with 95% confidence intervals (CI) and overall accuracy between two ELISAs and the combination of the two (combined ELISA) in detecting *Mycoplasma bovis* antibodies in bison serum.

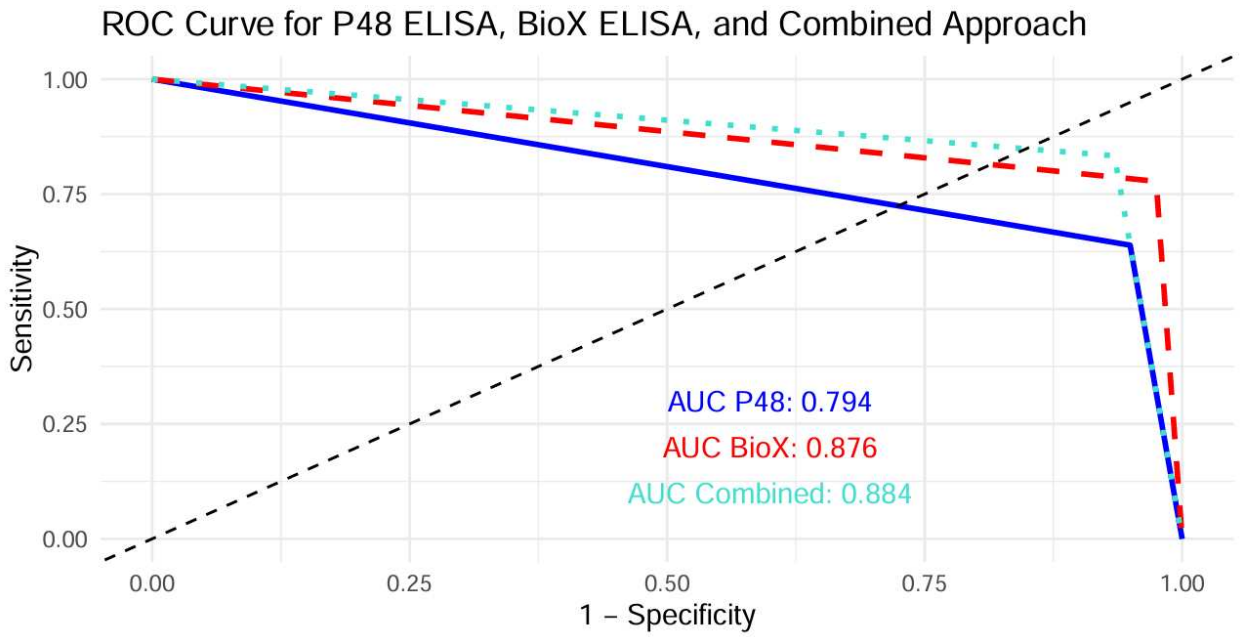

Figure 4.2. The area under the curve (AUC) computed for two *Mycoplasma bovis* ELISAs and the combination of the two to measure overall diagnostic performance in detecting *M. bovis* antibodies in bison serum.

Table 4.1. Comparison of the sensitivity, specificity, negative predictive values, positive predictive values with 95% confidence intervals) and overall accuracy of the P48, commercial, and a combination of the two (combined ELISA) in detecting *Mycoplasma bovis* specific antibodies in bison serum.

Test	ELISA	Estimate (%)	Lower CI (%)[c]	Upper CI (%)[c]
Sensitivity	P48	63.9	47.6	77.5
	Commercial	77.8	61.9	88.3
	Combined	83.3	68.1	92.1
Specificity	P48	94.9	90.9	97.2
	Commercial	97.5	94.2	98.9
	Combined	93.4	89.1	96.1
PPV[a]	P48	69.7	52.7	82.6
	Commercial	84.8	69.1	93.3
	Combined	69.8	54.9	81.4
NPV[b]	P48	93.5	89.3	96.2
	Commercial	96.0	92.3	98.0
	Combined	96.9	93.3	98.6
Accuracy	P48	90.2		
	Commercial	94.4		
	Combined	91.9		

[a], positive predictive values

[b], negative predictive value

[c], confidence interval

Table 4.2. False positive and false negative rates used to assess diagnostic reliability of two ELISAs and the combination of the two (Combined ELISA) for detecting *Mycoplasma bovis* antibodies in bison serum. The bold numbers indicated that the BioX ELISA was the most reliable in confirming true negative and the Combined ELISA reduced false negatives.

Metric	P48 ELISA	Commercial ELISA	Combined ELISA
False Positive Rate	5.05%	2.53%	6.57%
False Negative Rate	36.10%	22.20%	16.70%

Table 4.3. Area under the curve (AUC) and interpretation in assessing overall diagnostic performance two ELISAs and the combination of the two (Combined ELISA) for detecting *Mycoplasma bovis* antibodies in bison serum.

Test	AUC	Interpretation
P48 ELISA	0.794	Fair diagnostic ability
Commercial ELISA	0.876	Good diagnostic ability*
Combined ELISA	0.884	Slightly better than BioX**

*=Statistically significant improvement as compared to the P48 ELISA

**=Not statistically significant

REFERENCES

1. Ratajczak Z, Collins SL, Blair JM, Koerner SE, Louthan AM, Smith MD, et al. Reintroducing Bison Results in Long-Running and Resilient Increases in Grassland Diversity. Proceedings of the National Academy of Sciences. 2022 Sep 6;119(36).
2. Boyce AJ, Shamon H, McShea WJ. Bison Reintroduction to Mixed-Grass Prairie Is Associated With Increases in Bird Diversity and Cervid Occupancy in Riparian Areas. Front Ecol Evol. 2022 Mar 18;10.
3. Truett JC, Phillips M, Kunkel K, Miller R. Managing Bison to Restore Biodiversity [Internet]. Vol. 11, Plains Research. 2001. Available from: https://about.jstor.org/terms
4. Janardhan KS, Hays M, Dyer N, Oberst RD, DeBey BM. Mycoplasma Bovis Outbreak in a Herd of North American Bison (Bison Bison). Journal of Veterinary Diagnostic Investigation. 2010 Sep 1;22(5):797–801.
5. Sweeney S, Jones R, Patyk K, LoSapio C. Mycoplasma Bovis- An Emerging Pathogen in Ranched Bison. USDA APHIS Emerging Disease Notice. 2013;
6. Register KB, Olsen SC, Sacco RE, Ridpath J, Falkenberg S, Briggs R, et al. Relative Virulence in Bison and Cattle of Bison-Associated Genotypes of Mycoplasma bovis. Vet Microbiol. 2018 Aug 1;222:55–63.
7. NBA Mycoplasma bovis Fact Sheet- May 2022. 2022.
8. Register KB, Jones LC, Boatwright WD, Shury TK, Woodbury M, Hamilton RG, et al. Prevalence of Mycoplasma spp. In the Respiratory Tract of Healthy North American Bison (Bison bison) and Comparison with Serum Antibody Status. J Wildl Dis. 2021 Jul 1;57(3):683–8.
9. Bras AL, Barkema HW, Woodbury M, Ribble C, Perez-Casal J, Windeyer MC. Risk Factors for Mycoplasma bovis-Associated Disease in Farmed Bison (Bison bison) Herds in Western Canada: A Case-Control Study. Prev Vet Med. 2016 Jul 1;129:67–73.
10. Maunsell FP, Woolums AR, Francoz D, Rosenbusch RF, Step DL, Wilson DJ, et al. Mycoplasma bovis Infections in Cattle. J Vet Intern Med. 2011 Jul;25(4):772–83.
11. Biddle MK, Fox LK, Hancock DD. Patterns of Mycoplasma Shedding in the Milk of Dairy Cows with Intramammary Mycoplasma Infection. J Am Vet Med Assoc. 2003 Oct 15;223(8):1163–6.

12. Schwartz K, Schwalbe E, Buttke D, Bragg T, Killion H, Sondgeroth KS, et al. Evaluating Two Sampling Methods for Mycoplasma Bovis Diagnosis in American Bison (Bison bison). J Wildl Dis. 2024 Jul 1;60(3):584–93.

13. Bras AL, Barkema HW, Woodbury MR, Ribble CS, Perez-Casal J, Windeyer MC. Clinical Presentation, Prevalence, and Risk Factors Associated with Mycoplasma bovis–Associated Disease in Farmed Bison (Bison bison) Herds in Western Canada. J Am Vet Med Assoc. 2017 May 15;250(10):1167–75.

14. Register KB, Sacco RE, Olsen SC. Evaluation of Enzyme-Linked Immunosorbent Assays for Detection of Mycoplasma bovis-Specific Antibody in Bison Sera. Clinical and Vaccine Immunology. 2013 Sep;20(9):1405–9.

15. Bras AL, Suleman M, Woodbury M, Register K, Barkema HW, Perez-Casal J, et al. A Serologic Survey of Mycoplasma spp. in Farmed Bison (Bison bison) Herds in Western Canada. Journal of Veterinary Diagnostic Investigation. 2017 Jul 1;29(4):513–21.

16. Register KB, Parker M, Patyk KA, Sweeney SJ, Boatwright WD, Jones LC, et al. Serological Evidence for Historical and Present-Day Exposure of North American Bison to Mycoplasma bovis. BMC Vet Res. 2021 Dec 1;17(1).

17. Robino P, Alberti A, Pittau M, Chessa B, Miciletta M, Nebbia P, et al. Genetic and Antigenic Characterization of the Surface Lipoprotein P48 of Mycoplasma bovis. Vet Microbiol. 2005 Aug 30;109(3–4):201–9.

18. Fu P, Sun Z, Zhang Y, Yu Z, Zhang H, Su D, et al. Development of a Direct Competitive ELISA for the Detection of Mycoplasma bovis Infection Based on a Monoclonal Antibody of P48 Protein. BMC Vet Res. 2014 Feb 18;10.

19. Dudek K, Nicholas RAJ, Szacawa E, Bednarek D. Mycoplasma bovis infections—Occurrence, Diagnosis and Control. Vol. 9, Pathogens. MDPI AG; 2020. p. 1–21.

20. Schwartz PM, Shipman C, Carlson RH, Drach JC, Tritsch (L, Cheda GB, et al. Thermal Inactivation as a Means of Inhibiting the Serum-Associated Deamination of 9-f3-D-Arabinofuranosyladenine in Tissue Culture Media [Internet]. Vol. 5, ANTIMICROBIAL AGENTS AND CHEMOTHERAPY. 1974. Available from: https://journals.asm.org/journal/aac

21. Kaplan BS, Malmberg JL, Sondgeroth KS, Davila KS, Dassanayake RP, Sacco RE, et al. Serum IgG Immunoglobulin Levels are Associated with Reduced PCR Detection of Mycoplasma bovis in Naturally Infected American Bison (Bison bison). J Wildl Dis. 2024 Jul 1;60(3):594–604.

22. Moskaluk A, Nehring M, Vande Woude S. Serum Samples from Co-Infected and Domestic Cat Field Isolates Nonspecifically Bind FIV and Other Antigens in Enzyme-Linked Immunosorbent Assays. Pathogens. 2021 Jun 1;10(6).

23. Thomas A, Sachse K, Farnir F, Dizier I, Mainil J, Linden A. Adherence of Mycoplasma bovis to Bovine Bronchial Epithelial Cells. Microb Pathog. 2003 Mar 1;34(3):141–8.

24. Nussbaum S, Lysnyansky I, Sachse K, Levisohn S, Yogev D. Extended Repertoire of Genes Encoding Variable Surface Lipoproteins in Mycoplasma bovis Strains. Infect Immun. 2002;70(4):2220–5.

25. Wynn EL, Browne AS, Clawson ML. Diversity and Antigenic Potentials of Mycoplasmopsis bovis Secreted and Outer Membrane Proteins within a Core Genome of Strains Isolated from North American Bison and Cattle. Genome. 2024 Jun 1;67(6):204–9.

26. Wawegama NK, Browning GF, Kanci A, Marenda MS, Markham PF. Development of a Recombinant Protein-Based Enzyme-Linked Immunosorbent Assay for Diagnosis of Mycoplasma bovis Infection in Cattle. Clinical and Vaccine Immunology. 2014 Feb;21(2):196–202.

27. Perez-Casal J, Prysliak T. Detection of Antibodies Against the Mycoplasma bovis glyceraldehyde-3-phosphate dehydrogenase Protein in Beef Cattle. Microb Pathog. 2007 Nov;43(5–6):189–97.

28. Sun Z, Fu P, Wei K, Zhang H, Zhang Y, Xu J, et al. Identification of Novel Immunogenic Proteins from Mycoplasma bovis and Establishment of an Indirect ELISA based on Recombinant E1 Beta Subunit of the Pyruvate Dehydrogenase Complex. PLoS One. 2014 Feb 10;9(2).

CHAPTER 5- CONCLUSIONS AND FUTURE DIRECTIONS

Integration of Findings and Implications

The research presented in this dissertation advances our understanding of bison health through a multidisciplinary approach by integrating stakeholder perspectives, epidemiological studies, and diagnostic evaluations. By addressing gaps in disease surveillance, biosecurity, and diagnostics, these findings inform the development of more effective policies and management strategies for bison populations.

A key contribution of this work is the multisectoral definition of bison health developed through a Delphi survey. Bison health can be assessed by a population's ability to express natural behaviors, be resilient to external stressors that include disease, and display high levels of lifetime reproductive output within its environment with minimal intervention. This definition provides a foundation for health monitoring that extends beyond pathogen detection and integrates ecological and behavioral health indicators.

The high seroprevalence of BTV and EHDV in bison herds across multiple states highlights the importance of incorporating bison into existing vector-borne disease surveillance programs. The identification of multiple circulating serotypes suggests that bison could serve as incidental hosts for *Orbiviruses*, warranting further investigation into their role in disease transmission dynamics. Additionally, the study of age-related immunity patterns may have implications for herd-level disease monitoring.

The evaluation of *M. bovis* diagnostics in bison shows the persistent need for species-specific serological assays. The comparative analysis of a novel P48 ELISA

against a commercially available ELISA demonstrates suboptimal diagnostic performance, highlighting cross-reactivity issues and poor sensitivity in bison. This finding stresses the importance of tailoring diagnostic tools to bison-specific immune responses to enhance disease detection and management. The validation of bison-specific ELISAs should be prioritized to improve *M. bovis* detection. Further research on bison immunological responses is necessary to enhance diagnostic accuracy, and point-of-care diagnostic tools should be developed for field applications in conservation and production settings.

Cross-sectoral collaboration is essential for effective disease management. Communication between tribal, conservation, government, and private-sector stakeholders should be facilitated. The integration of Traditional Ecological Knowledge (TEK) into disease mitigation strategies can strengthen management practices. Additionally, interdisciplinary training programs should be developed for veterinarians, ecologists, and bison producers to enhance One Health capacity building.

Future Research Directions

Building on the findings of this dissertation, several key areas warrant further investigation. The Delphi survey uncovered the needs of the bison sector including the need for enhanced diagnostic research, biosecurity resources, and collaborative approaches to mitigate disease risks and support bison health.

Longitudinal studies on BTV and EHDV in bison are essential in order to better understand vector transmission dynamics. It remains unclear whether bison serve as incidental hosts or more, requiring a deeper evaluation of viremia over time. Further

research should examine the role of bison in BTV serotype reassortment and genetic exchange, as well as assess the risk of bison exposure to novel BTV/EHDV serotypes, particularly considering differences between young and old individuals. Understanding antibody cross-protection between different BTV serotypes in bison will provide insight into immune responses and disease resilience. Additionally, longitudinal vector studies, such as midge captures near bison wallows in conjunction with bison sampling, can help expand knowledge of *Orbivirus* prevalence in the midge and how that plays into the disease dynamics of bison.

M. bovis pathogenesis and host adaptation also require further study, particularly through genomic comparisons between bison and cattle *M. bovis* strains to identify host-specific adaptations. Research into the role of chronic carriers in disease persistence and transmission is necessary, as is the exploration of host immune response variations to guide the development of targeted vaccines and therapeutics. The role of IgG2 immune responses as a potential marker for chronic carriers also warrants further examination. Additionally, a multiplexed ELISA or multiple protein ELISAs incorporating new proteins need to be developed in order to improve diagnostic sensitivity and specificity. Proteomic analyses will be instrumental in identifying novel antigenic targets for diagnostic development. Alternative diagnostic methods such as PCR-based surveillance for early detection should be further assessed and used in conjunction with ELISA for antemortem testing.

Conclusion

This dissertation proves the importance of a multidisciplinary and One Health approach to bison disease surveillance and management in providing a comprehensive

framework for improving bison health policy. Moving forward, standardized surveillance, improved biosecurity, species-specific diagnostics, and cross-sectoral collaboration will be essential for mitigating disease risks and ensuring the sustainable management of bison populations. By addressing these priorities, this research contributes to the long-term conservation and health resilience of North American bison in the face of emerging disease challenges and environmental changes.