To the University of Wyoming:

The members of the Committee approve the thesis of Bevin McCormick presented on November 14, 2023.

Brant Schumaker, Chairperson

Jennifer Malmberg, Co-advisor

Kevin Monteith, Outside Member

Paul Cross, External Member

APPROVED:

Jonathan Fox, Department, Division, or Program Chair, Department of Veterinary Sciences.

Barbara Rasco, College Dean/Provost

McCormick, Bevin, A, Master of Science, Animal and Veterinary Sciences, December, 2023.

Chronic wasting disease (CWD), a fatal disease in Cervidae species (cervids), has spread throughout North America and Scandinavia. Identifying hot spots for CWD transmission is important to cervid conservation. Livestock mineral licks attract wildlife and likely serve as environmental reservoirs for CWD. Additionally, environmental factors, such as high clay soil content, may increase prion transmissibility. The objectives of my research were to assess impact of livestock salt and mineral licks on CWD transmission and test devices that may limit cervid access and visitation to mineral, while allowing cattle free access. We tested cattle mineral feeders as excluding devices in three Wyoming regions, predicting that cervid access and visitation time would decrease after deployment. We reviewed game camera images to assess site use by mule deer, elk, and moose before and after deployment of mineral feeders and in concurrently running control sites. Results showed that mineral feeders were 98.6% effective in excluding mule deer (Odocoileus hemionus) 74.0% for elk (Cervus elaphus nelsoni), 100% for moose (Alces alces), and allowed livestock access. Given presence, visitation time at mineral sites was reduced by 65.8 - 73.7% in mule deer, but was unable to be assessed in other species due to small sample size. Furthermore, mineral feeders were found to have no significant affect on cattle presence or visitation time to livestock mineral licks. Our findings indicate that mineral feeders use at livestock mineral licks may reduce cervid use of livestock salt and mineral resources, providing a potential management technique that could reduce CWD transmission that should be further investigated.

Transmission of Chronic Wasting Disease Associated with Livestock Mineral Licks

By

Bevin A. McCormick

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DEDICATION PAGE

I would like to dedicate this thesis to my family for all their unwavering support throughout my education.

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CHAPTER 1: REVIEW OF LITERATURE

Introduction

Disease plays an important role in all ecosystems and among all taxa. With increased disturbance from anthropogenic effects like cross-continental movement of organisms and organic substances, urban development resulting in habitat fragmentation, global climate shifts, and increased agricultural operations, new and emerging diseases are an increasingly prominent threat to humans and wildlife (Daszak et al., 2001). There is evidence that these human-caused effects may also increase zoonotic potential of diseases and the probability of disease causing extirpation or extinction in wildlife (Daszak et al., 2001), which has significant implications for public health and conservation. This paper will explore a particular class of diseases called transmissible spongiform encephalopathies and a disease of strong concern in wildlife management, chronic wasting disease. I will discuss the history of discoveries of such diseases, developing knowledge of pathways of transmission, management implications, and the potential of increased transmission from agricultural operations.

Prion Diseases

Transmissible spongiform encephalopathies (TSEs) are a class of neurodegenerative diseases caused by prions. Prion, an abbreviated form of the phrase "proteinaceous infectious particle," was first coined by Stanley Prusiner, who won the Noble prize in Physiology or Medicine in 1997 for his work in prions (Zabel & Reid, 2015; "Stanley B. Prusiner…", 2023). Prior to the identification of prions as an infectious agent, a disease described as scrapie in 1732 was an ongoing problem in domestic sheep but had no identified etiological agent. Scrapie caused neurological symptoms, including changes in posture and movement, wasting, and, classically, compulsive scratching and rubbing on fence posts or similar objects (Zabel & Reid, 2015). The first disease later identified as a TSE infecting humans was described in 1920 by Hans Creutzfeldt and Alfons Jakob and called Creutzfeldt-Jakob Disease (CDJ). Like scrapie in sheep, CDJ caused neurological symptoms and always ended in fatality. While scientists were able to identify these diseases as slow-acting and infectious, the then contemporary knowledge of infectious agents could not explain their pathology. In the 1960s, continuing through the 1980s, researchers experimented with the stability of scrapie and found that it was resistant to radiation, extreme heat and pressure, and chemicals that generally serve as disinfectants (Zabel & Reid, 2015). This and similar research led to the postulation by John Stanley Griffith in 1967 that scrapie is an infectious protein (Griffith, 1967). Researchers following Griffith's work added support to this idea. Through their experimentation showing that scrapie could be neutralized using agents that denature proteins, Stanley Prusiner and his colleagues described and lent strong support to the prion hypothesis—that scrapie, and diseases in its class, were caused by a proteinaceous infectious particle, coined "prions" (Zabel & Reid, 2015).

Since the identification of prions as transmissible agents, scientists continue to explore prion properties and pathogenesis of TSEs. We now know that prions naturally occur as non-infectious glycoform proteins in animal cell membranes. This non-infectious form is designated as PrP^{C} (Lee et al., 2013). Transmissible scrapie prions (and often other disease-causing prions) are designated as PrP^{Sc}. While the amino acid makeup is identical in both prion forms, PrP^{Sc} is a misfolded isoform of PrP^{C} and has beta-pleated sheets in contrast to the alpha-helix structure of PrP^{C} (Goldfarb & Brown, 1995). When an infectious prion comes into contact with PrP^{C} in the body of a susceptible host, it causes the PrP^{C} to misfold into PrP^{Sc}. In this way, PrP^{Sc} replicates in the host, generally forming amyloid fibrils that aggregate as amyloid plaques which eventually cause spongiform changes to central nervous system tissue, inducing disease (Goldfarb & Brown, 1995; Lee et al., 2013). Researchers are still investigating other molecules that assist in transformation of prions and prion-induced infection.

Chronic Wasting Disease and its Effects

Chronic wasting disease (CWD) is a TSE that affects Cervidae family species (cervids), including mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), elk (*Cervus elaphus*), and moose (*Alces alces*) in North America (Rivera et al., 2019; Spraker et al., 1997). The first documented cases of CWD were seen in mule deer and black-tailed deer in a captive breeding facility in Colorado in 1967 (William & Young, 1980). By the 1980s, CWD had spread to wild cervids. While CWD had a perceived slow geographic spread prior to 2000 possibly due to low surveillance efforts, it is now found in free-ranging populations in 31 US states, three Canadian providences, Sweden, Norway, and Finland. Additionally, CWD has been found only in captive populations in Quebec, Canada, and South Korea (Cordova, 2019; Miller & Fischer, 2016; "Expanding distribution…", 2023). Specifically, in the state of Wyoming, CWD had been observed in 84% of all mule deer herds, 25% of elk herds, and in white-tailed deer throughout the state as of 2020 ("Wyoming CWD Management Plan", 2020). Additionally, as of April 2022, Colorado Parks and Wildlife had detected CWD in 74% of mule deer herds, 40% of elk herds, and 22% of moose herds ("About Chronic Wasting..", 2022).

CWD infection is always fatal and there is no known cure. Late stage clinical signs include excessive thirst, drooling, difficulty swallowing, loss of body condition, changes in gate, loss of balance, and drooping of the head (Rivera et al., 2019; Williams, 2005). Earlier signs of the disease may include behavioral changes, but no definitive diagnostic clinical sign has been identified. Aspiration pneumonia may appear at any time in CWD progression (Williams, 2005). Like all TSEs, CWD has a long slow clinical onset. Incubation periods occurring in naturally infected free-ranging cervids have been estimated to be a minimum of 16-17 months and likely average between two to four years (Williams, 2005; Williams & Miller, 2002). Some evidence suggests that incubation may be influenced by concentration and route of inoculation. (Williams, 2005). Strong evidence now links incubation period to host genetics in a species-specific manner (DeVivo et al., 2017).

At the population level, there is strong evidence that CWD has can cause notable declines. Several studies have shown evidence of CWD-induced population decline in endemic areas. David Edmunds and colleagues found reduced survival in CWD-positive white-tailed deer in South Converse County, WY. This, combined with high population prevalence (23.8% average annual prevalence), led them to conclude that CWD was likely the cause of the local white-tailed deer population declines between 2003-2010 (Edmunds et al., 2016). Similarly, Devivo et al. found in their study population (24% average annual CWD prevalence) that CWD-positive mule deer were more susceptible to hunter harvest and lion predation (Devivo et al., 2017). Additional studies have concluded that CWD has the potential to cause declines in elk populations as well (Monello et al., 2014). These correlated declines in mule deer populations are particularly concerning with the notably high percentages of mule deer herds where CWD has been detected in both Wyoming and Colorado discussed earlier in the section ("About Chronic Wasting..", 2022; "Wyoming CWD Management Plan", 2020). There are also notable difference in prevalence by sex. While early research involving captive populations saw little difference in infection rates, studies of free-ranging cervids have found significantly higher prevalence among males (Miller & Connor, 2005; Miller & Fischer, 2016).

Chronic Wasting Disease Transmission

Routes of Transmission

While routes of transmission are still being researched, studies have shown that susceptible species are likely to contract CWD naturally through ingestion or inhalation (Saunders et al., 2012; Nichols et al., 2013). To better understand the circumstances under which this might occur, researchers have examined how cervids may come into contact with CWD prions (PrP^{Sc}). Individuals infected with CWD shed PrP^{Sc} through bodily fluids such as urine, feces, saliva, and blood (Henderson et al., 2015; Haley et al., 2015; Mathiason et al., 2006). PrP^{Sc} has also been detected in many tissues, including antler velvet (Angers et al., 2009; Saunders et al., 2012). Transmission can then occur through direct contact with infected individuals or, indirectly, through contact with surfaces in the environment contaminated by deposition of bodily fluids or tissues of CWD-infected individuals (Miller & Williams, 2003; Mathiason et al., 2009; Miller et al., 2004; Moore et al., 2016). There is also evidence that vertical transmission of CWD occurs in Reeve's muntjac (*Muntiacus reevesi*), though, due to high neonatal mortality in many Cervidae species, in-utero transmission is not currently considered a major driver of transmission in most populations (Rivera et al., 2019; Nalls et al., 2013).

Because PrP^{Sc} is incredibly durable in the environment and an effective eradication method for natural environments has yet to be developed, indirect transmission of CWD is a major challenge to managers (Wiggins, 2009; Saunders et al., 2008). Numerous studies have shown that PrP^{Sc} on surfaces in the environment has high transmissibility, potentially, for years.

Mathiason et al. (2009) demonstrated that healthy deer exposed to feeding buckets and bedding utilized by CWD positive deer became infected. Miller et al. (2004) showed that mule deer can become infected with CWD when kept in pens that had held CWD positive deer 2.2 years prior. Similarly, carcasses of CWD positive cervids can create a significant point source of PrP^{Sc} that may persist in the landscape for months and be consistently visited by other cervids (Jennelle et al., 2009). Modeling suggests that environmental transmission may play a more important role, as compared to direct transmission, in the spread of CWD (Almberg et al., 2011).

Abiotic and Biotic Landscape and Ecosystem Factors

Another area of developing research focuses on abiotic and biotic environmental factors that may influence the CWD prevalence in populations. Soil is one abiotic factor that has strong evidence of influencing CWD transmission and local prevalence. Studies have shown that mule deer ingest soil at varying rates but continually throughout the year (Arthur & Alldredge, 1979). Infectious prions adsorb to soil particles, remain bioavailable, and, in some case, have higher transmissibility when bound to soil (Johnson et al., 2007; Johnson et al., 2006). Soil characteristics that influence this adsorption include mineral makeup, humic content, pH, and texture (Giachin et al., 2016; Ma et al., 2007; Dorak et al., 2017). Specifically, higher clay content and elevated pH have been shown to be associated with higher transmissibility and population prevalence, both in laboratory experiments and in models looking at the spatial relationships between soil (Dorak et al., 2017; Johnson et al., 2007; Johnson et al., 2006). Furthermore, there is evidence that some minerals increase prion transmissibility. In an in vitro study, higher manganese content in soil has been shown to prolong scrapie prion persistence by ten times and increase transmissibility to culture cells (Davies & Brown 2009). One study also showed a positive association of manganese liver concentrations and CWD infection in mule deer (Wolfe et al., 2010). Nichols et al. (2016) demonstrated that environmental magnesium and copper may influence CWD infection, but, in their study, transgenic mice with higher magnesium/copper ratio diets were significantly more likely to be negative to CWD.

Limited research reflects that plants may play a role in CWD circulation through the landscape. A study working with wheat grass showed that prions can bind to leaves and roots and remain infectious, that leaves sprayed with scrapie-positive obex homogenate continued to test positive for PrP^{Sc} for several weeks, and that plants uptake prions from infected soil and transport them to stems and leaves (Pritzkow et al., 2015). Conflicting research has shown no evidence of prion uptake by wheat (Rasmussen et al., 2014).

Predator and scavenger populations may significantly influence CWD transmission in ecosystems, but their influence is not yet well understood. Since carcasses of CWD positive cervids serve as potential source of highly transmissible PrP^{Sc} (Jennelle et al., 2009) and PrP^{Sc} is found in tissues frequently consumed by predators and scavengers such as cervid muscular skeletal tissues and fat (Angers et al., 2006, Race et al., 2009), it's important to understand how scavenging and predation affect prion movement within a landscape. Studies show evidence that CWD positive deer are more susceptible to predation by mountain lions (Devivo et al., 2017; Krumm et al., 2010; Miller et al., 2008). Given a higher probability of mortality due to predation, researchers strive to understand the extent to which prions endure digestive processes. A study in which coyotes were inoculated orally with CWD positive brain homogenate showed that PrP^{Sc} continues to be present and transmissible in coyote feces (Nichols et al., 2015). Additionally, the feces of mountain lions that have consumed CWD positive meat have been shown to contain infectious prions, but at much lower quantities than the meat that was consumed (Baune et al., 2021). Similar research has been completed involving avian scavengers. Feces from American crows that consumed scrapie positive meat were found to transmit scrapie to scrapie susceptible mice (VerCauteren et al., 2012). This research supports that predators and scavengers are capable of moving infectious prions within the environment, but that some predators may reduce the amount of infectious prions that remain on the landscape. Beyond prions ability to withstand digestive processes, predators influence how long infected individuals shed prions in the landscape. As prions continue to replicate within a host, host prion loading and shedding in the environment potentially increase with since time of infection (Davenport et al., 2018). Under some conditions predators may shorten both the amount of time infected individuals shed prions in the environment and the amount of prions that replicate within an infected individual and eventually end up in the environment, thus reducing overall transmission (Brandell et al., 2021).

Developing research is investigating the relationship between anthropogenic land use and CWD prevalence. For example, Farnsworth and colleagues identified that models using GPS data of mule deer with known CWD status in northern Colorado fit best when they incorporated whether a deer was found within 1 km of urban areas. Furthermore, their results showed that CWD prevalence increased two-fold in developed areas as compared to undeveloped areas (Farnsworth et al., 2009). In an another study overlaying land use spatial layers with geographical CWD prevalence across North America, it was found that agricultural land use was associated with higher CWD prevalence in both white-tailed and mule deer as compared to deer found in within areas with natural land cover (Miller, 2022). General changes in cervid movement associated with agricultural land may have larger implications for CWD spread. For example, greater dispersal distances have been observed in male juvenile white-tailed deer in areas with a high proportion of agricultural land (Gilbertson et al., 2022). Additional studies are needed to distinguish significant factors among the complex variables involved in land use, such as changes in hunting occurrence, possible changes in deer density, connectivity, etc. (Nobert et al., 2016).

Genetics and Species Barriers

Similar to other TSEs in other species, variation in the PRNP gene in cervids appears to influence incubation period and correlate with time since initial CWD detection in a given region (LaCava et al., 2021; Lloyd et al., 2011; Monello et al., 2017; Robinson et al., 2012). Many studies show evidence that cervid populations with a known history of CWD exposure see disease-driven selection of specific PRNP genes (LaCava et al., 2021; Monello et al., 2017; O'Rourke et al., 2007). Polymorphisms in the PRNP gene change how non-infectious prions (PrP^C) are encoded within each individual (Robinson et al., 2012). The differences in PrP^C structure are associated with differences in incubation times and time to eventual fatality (Robinson et al., 2012; O'Rourke et al., 2007). Multiple studies of mule deer in Wyoming and Colorado have shown evidence of slower clinical onset of CWD in mule deer with 225F PRNP genotype (Jewell et al., 2005; DeVivo et al., 2017). Similarly, PRNP polymorphisms have been shown to affect CWD progression in elk, white-tailed deer, caribou, and other cervid species not native to North America (Green et al., 2008; Johnson et al., 2011, Velásquez et al., 2015; Monello et al., 2017; Moore et al., 2018; O'Rourke et al., 2007; O'Rourke et al., 2004; Cheng et al., 2017; Robinson et al., 2012). CWD pathogenesis becomes a more complex consideration as researchers study the interactions between the varying PrP^C structures resulting from PRNP gene polymorphisms and various CWD strains. Research studies on transgenic mice and hamsters have shown evidence for multiple CWD strains (Raymond et al., 2007). Additionally, how varying strains interact with different PrP^{C} structures influence which strains are successful in infecting hosts (Bian et al., 2019). This was further corroborated by inoculating mice with brain homogenates from deer with different PRNP genotypes (Velásquez et al., 2015). These findings have greater implications related to the possibility of CWD crossing to non-cervid species.

Whether by direct ingestion of infected tissue or contact with infected surfaces in the environment, there is strong potential that other species frequently come into contact with PrP^{Sc}. As such, some research targets understanding under what circumstances CWD may cross species barriers and infect species outside of the Cervidae family. Zoonotic potential has been of concern due to human susceptibility to bovine spongiform encephalopathy and human consumption of cervid meat. Studies attempting to determine human susceptibility to CWD have shown mixed results, complicating our understanding of transmission potential. For example, studies in which transgenic mice and macaques were experimentally inoculated with CWD have shown no evidence of transmission potential (Kurt et al., 2015; Race et al., 2009; Tamguney et al., 2006; Waddell et al., 2018; Wilson et al., 2012). Yet, intracranial inoculation of squirrel monkeys suggest that zoonosis may be possible (Race et al., 2009; Waddell et al., 2018). Additionally, in vitro studies of have shown evidence that PrP^{Sc} may convert human PrP^{C}. (Davenport et al., 2015; Kurt et al., 2015; Waddell et al., 2018). Limited observational studies have shown that we have not yet detected any increased prion-disease among humans that have consumed CWD positive meat (Waddell et al., 2018). Similarly, CWD in cattle (*Bos taurus*) would pose strong implications for public health. A ten-year study of orally inoculated cattle and cattle housed in CWD positive enclosures showed strong evidence that CWD cannot infect cattle through natural exposure at this time (Williams et al., 2018). The susceptibility of predators, scavengers, and non-target species remains an important area of study (Jennelle et al., 2009). Canine species have shown strong resistance to CWD infection, while feline species have demonstrated resistance to natural exposure but susceptibility to experimental inoculation (Mathiason et al., 2013; Vidal et al., 2020; Wolfe et al., 2022). Other studies show evidence that mink (*Mustela vision*), voles (*Microtus pennsylvanicus and Myodes gapperi*) and mice (*Peromyscus spp.)* are susceptible to contracting CWD via intracerebral inoculation, though mink showed resistant through oral innocculation *(*Harrington et al., 2008; Heisey et al., 2010). There's also evidence of susceptibility in beaver (*Castor canadensis*), raccoon (*Procyon lotor*), and swine (*Sus scrofa*) (Herbst et al., 2022; Moore et al., 2017; Moore et al., 2022). Sequencing of PRNP of common mammal species in Canada identified pronghorn antelope (*Antilocarpa americana*) as likely one of the most susceptible species genetically, closely followed by bighorn sheep (*Ovis canadensis*), and humans and other primates as least susceptible of the tested species (Cullingham et al., 2020).

Cervid Movement and Ecology

The relationships among host movement, interactions and behavior and disease dynamics are important in assessing transmission rates and probabilities. However, in wild, free-ranging cervids, these relationships are difficult to establish. Researchers have created models to help inform us if CWD transmission increases with host density—density-dependent (DD)—or if density has little effect on transmission—frequency-dependent (FD). While establishing this relationship could allow for implementation of informed management strategies, it has been noted that DD and FD could be considered two extremes of a scale, with most host-disease relationships falling in between (Storm et al., 2013). Models have shown evidence of both DD and FD transmission effects in CWD, dependent on the scale and parameters incorporated in each model (Conner et al., 2007; Habib et al., 2011; Mysterud & Edmunds, 2019; Storm et al., 2013; Wasserberg et al., 2009). A study examining contact rates among female elk in Wyoming showed that contact rates among pairs of individuals declined as group size increased but per capita contact rates were positively correlated with group size (Cross et al., 2013). This suggests that scale and pairwise versus per capita contact rates must be carefully considered in CWD models. Similarly, in their review of CWD transmission models, Storm et al. (2013) posited that in a Cervidae species within-group interactions constitute the majority of transmission, but between-group interactions were likely dependent on density, further bolstering the likely significance of scale in models aimed at understanding CWD transmission (Habib et al., 2011; Storm et al., 2013). This complex relationship between disease and social structure, along with the non-linear relationship with host-density, creates formidable challenges to wildlife disease management, in which assumptions of density-dependence have historically pushed culling—controlled removal of individuals of the affected species—to be a predominant disease management technique (Miguel et al., 2020).

Though many North American cervid populations migrate seasonally, there have been relatively few studies geared toward understanding the effects of migration and season on CWD transmission. Likely, this is due to both to the complexity of behaviors and landscapes associated with migration and gaps in knowledge about transmission. A few studies have explored season-specific effects on CWD transmission (Mysterud & Edmunds 2019). Oraby et al. (2014) used mathematical models to see if transmission could be more accurately predicted using seasonality, first suggesting that whether CWD transmission is DD or FD may change between summer and winter ranges. The model showed that transmission was more likely in summer than winter, but the authors noted this has been argued against in other studies (Oraby et al., 2014). Seasonal-dependent effects would likely have broad implications for in management for CWD if they were understood.

Conversely, many patterns of natural aggregation and spatial overlap in cervids are somewhat well-known and, at least geographically, often influenced by season. For example, family groups of mule deer, white-tailed deer, and elk join to form larger groups on winter ranges (D'Eon & Serrouya, 2005; Janousek et al., 2021; Sauvé & CǑTÉ, 2007; Youmans, 1979). Additionally, spatial overlap is likely to occur among individuals with similar ranges near shared resources. A study specifically looking at overlap between elk and mule deer at natural salt licks in their summer range showed both that there is spatial overlap between species at these locations and that behaviors that would transmit CWD were common in these areas (Lavelle et al., 2014).

Chronic Wasting Disease Diagnostic Techniques

While there are only two CWD diagnostic tests currently used for surveillance, great strides in laboratory tests for detecting CWD have created additional, timely techniques that are currently used in research. Prior to the identification of CWD's infectious agent, CWD could be diagnostically identified postmortem by characteristic lesions in central nervous system tissue (Haley & Richt 2017). Among these lesions, amyloid plaques were characteristic, though not well understood, and could be identified through histochemical staining with Bodian silver and Congo red (Haley & Richt 2017). The identification of prions role in CWD allowed for the development of other testing techniques. Two techniques are currently used for diagnostic testing for surveillance: immunohistochemistry (IHC) and enzyme-link immunosorbent assay (ELISA).

IHC testing of obex and retropharyngeal lymph node tissue remains the “gold standard” test for CWD diagnostics, though ELISA is also a well-used diagnostic test and often considered comparable (Haley & Richt 2017; Kramm et al., 2020). IHC, ELISA, and Western blot (WB) are all an antibody-antigen dependent assay, or immunoassays, that allow for differentiation between PrP^{C} and PrP^{Sc} with high specificity (Haley & Richt, 2017). IHC uses acid treatment combined with enzyme digestion and staining and microscopy for prion detection (Haley & Richt, 2017; Magaki et al., 2019). ELISA uses enzyme digestion or other binding components and observed color for prion detection (Haley & Richt, 2017).

The first test employing seeded amplification used in CWD diagnostics was the protein misfolding cyclic amplification assay (PMCA) (Haley & Richt, 2017). In PMCA, the seed CWD sample and additives are introduced to a substrate of PrP^{C}, usually made of brain homogenate of a susceptible species, and allowed to run through cycles of *in vitro* PrP^{Sc} replication (Barria et al., 2012; Haley & Richt, 2017). After sufficient conversion of PrP^{C} to PrP^{Sc}, tests such as Western blot or ELISA are used to detect PrP^{Sc} (Barria et al., 2012). Advances in PMCA, such as series PCMA, have led to the ability to detect very low levels of prions (Haley & Richt, 2017). As with all amplification assays, careful avoidance of cross-contamination is essential for accurate results (Barria et al., 2012). Additionally, protocols must be carefully selected to gain the necessary sensitivity but avoid over amplification, which can lead to spontaneous conversion and loss of specificity (Haley & Richt 2017). Real time quaking induced conversion (RT-QuIC) is a more recently developed amplification assay for CWD detection. The amplification process in RT-QuIC differs from PMCA in that recombinant substrate can be rapidly derived, often from *E coli*, and easily adapted to the specific materials in which the seed sample is found (Haley & Richt 2017). RT-QuIC does not require Western blot or ELISA for detection in PrP^{Sc}, but instead incorporates fluorescent dye and provides an assay read-out (Haley & Richt 2017). Additionally, a major advantage to researchers of RT-QuIC over PMCA is that amplified PrP^{Sc} is not infectious. Researchers are continuing to develop CWD detection techniques using RT-QuIC that allows for the quantification of prions (Orrú et al., 2015; Minikel 2015), antemortem testing (Tewari et al., 2022; Ferreira et al., 2021), and detection in abiotic substrates, such as soil (Walsh et al., personal communications. January 2021).

Management of Chronic Wasting Disease

With the potential of CWD greatly impacting charismatic cervid species that have ecosystem and economic significance, managers are continuously developing best practice standards for CWD management. To date, there is no effective vaccine or cure for CWD, and very few options for preventing its introduction to new areas (Mysterud & Edmunds 2019). As a first tool in control, many state fish and game agencies have made surveillance a priority. Opportunistic sampling—collecting samples from roadkill or other mortalities—provides inconsistent data, often at small sample sizes. In many areas in the United States, surveillance primarily occurs through sampling of human harvested deer ("Surveillance strategies…" 2003). In many states, state fish and game agencies have implemented mandatory testing of specified species in designated hunting units. Additionally, voluntary testing in hunting units without testing mandates is encouraged. These data frequently are used to produce regional CWD prevalence estimates ("Surveillance strategies…" 2003; "Wyoming CWD Management Plan" 2020).

With regular surveillance as an important tool, most CWD management plans have been primarily focused on controlling the spread of CWD. Culling of wildlife is one of the most frequently and historically used management strategies for managing wildlife disease throughout the world (Miguel et al., 2020) and has sometimes been considered an effective control measure in diseases with positive density-dependent effects (Mysterud et al., 2020). Theoretical models and models based on observational field data have been created to study management practices and have largely targeted the effectiveness of culling (Uehlinger et al., 2016). Models indicated that selective culling based on CWD status of free-ranging deer, male-targeted harvest, and selective predation may be effective in reducing CWD prevalence but results were very dependent on parameters (Uehlinger et al., 2016). Additionally, models evaluating non-selective and seasonal culling showed effective results given specific scenarios, but, with many factors—like the DD and FD effects—unknown, it's difficult to judge their accuracy (Wasserberg et al., 2009; Uehlinger et al., 2016). Some models show that determining a level of culling that effectively reduces prevalence, but allows for robust host populations could be a substantial challenge (Rogers et al., 2022). Some longer term field observational studies have shown effective mitigation of increased CWD prevalence in the presence of consistent non-selective culling (Manjerovic et al., 2014; Uehlinger et al., 2016) or increased male mule deer CWD prevalence in areas with decreased annual hunting licenses (Miller et al., 2020). There have also been occasional attempts for regional eradication, but eradication of CWD in free-ranging cervids is generally considered infeasible (Holsman et al., 2006; Williams, 2005).

In the state of Wyoming, CWD management is centered on intense surveillance, changes in hunter-harvest and occasional culling events responsive to prevalence estimates, and minimizing artificial congregation. In the 2020 Wyoming CWD Management Plan, the Wyoming Game and Fish Department (WGFD) outlined a plan to collect 200 samples from each herd unit in the state over three years, facilitate removal of likely positive cervids through increase hunter-harvest of male cervids and implementing necessary culling, and reduce artificial congregation points. ("Wyoming CWD Management Plan" 2020). Additionally, WGFD have implemented hunting regulations for the transport and disposal of deer, elk, and moose carcasses: carcasses maybe transported to designated businesses, hunting camps, or private residences for meat processing but "the head and all portions of the spinal column" must remain at the location of the kill unless otherwise disposed of at an WGFD approved site or incinerator. Additionally, "whole deer, elk and moose carcasses cannot be transported out of Wyoming" ("Proper Carcass Disposal and Transport").

Comparatively, CWD management in Colorado by Colorado Parks and Wildlife (CPW) focuses on surveillance, culling, and hunter harvest. In 2002, CPW conducted extensive state-wide surveillance using samples from hunter harvest (over 10,000 mule deer and over 14,000 elk) ("Colorado Chronic…", 2018). Having then established CWD's geographic range was much more extensive than previously thought, they temporarily scaled back surveillance. Early on, to reduce prevalence and spread, culling was a predominant strategy ("Colorado Chronic…", 2018). Culling efforts were later discontinued in many areas due to cost, difficulty in measuring the strategy's effectiveness, and very limited stakeholder support ("Colorado Chronic…", 2018). Early aggressive culling in Colorado has been used for insights into the effectiveness of culling. Some studies show a possible inverse relationship between hunting pressure and CWD prevalence using Colorado data (Conner et al., 2021; Miller et al., 2020). In 2018, CPW created an adaptive management plan that is still being used today. In the plan CPW established a 15-year surveillance plan of mandatory testing of hunter harvested mule deer rotating yearly among designated herds and repeating testing of herds every approximately 5 years ("Colorado Chronic…", 2018). In areas of low or unknown prevalence, CPW plans to prioritize opportunistic sampling of symptomatic individuals and mortalities. Additionally, voluntary testing of all hunter harvested Cervid species is heavily encouraged (“Colorado Chronic…”, 2018). The adaptive management plan also designated a prevalence threshold for some herds that when exceed necessitates management action which will determined by CPW staff managing said herd (“Colorado Chronic…”, 2018).

One of the largest issues in CWD management is stakeholder support. In the case of CWD, stakeholders often include landowners, hunters, wildlife enthusiasts, cattle producers, and others. Attitudes around chronic wasting disease in Wyoming can span from in disbelief of its existence to highly-concerned about its impact, but no found studies have quantified or published this information. One study that surveyed deer or elk hunters in eight states in the US found that very few hunters would consider ceasing hunting as a result of current prevalences, but that 42-54% would stop hunting in their state if there was a prevalence of 50% (Needham et al., 2004). With so many vested interests and the need for so many groups to be involved in effective management plans, communication and public support is vital. Both Wyoming and Colorado clearly outline plans for communicating with the public in their CWD response plans (“Colorado Chronic…”, 2018; “Wyoming CWD Management Plan”, 2020).

CWD and artificially-elevated points of cervid congregation

At the intersection of cervid ecology and behavior, anthropogenic land use, and management for CWD lies interesting questions about how human activity influences both local CWD transmission and CWD spread across landscapes and what management strategies may offset human exacerbation of this daunting conservation challenge. With the limited effectiveness of current CWD management strategies, the recent rapid geographic spread of

CWD, and quantifiable regional declines in some deer populations (as discussed in the "Chronic Wasting Disease and its Effects" section) , the urgency for these factors to be incorporated into management strategies is apparent. Research that aims to investigate the role of artificial cervid congregation in CWD transmission and discourage or mitigate congregation is therefore important to management.

Artificial aggregation resulting from anthropogenic activity results in higher potential transmission due to both higher rates of direct contact between individuals and higher deposition of prions in areas that serve as a significant attractant to cervid species. A classic and contentious example is areas of baiting and supplemental feeding of wildlife (Sorensen et al., 2014). Supplemental feeding is generally used to provide nutrition to wildlife in addition to their natural forage to either artificially increase populations or make up for deficiency in available nutrition resulting from extreme weather, anthropogenic impacts, or similar such circumstances. Baiting sites are primarily used to draw in wildlife for hunting or other recreational purposes. The resulting areas have artificially elevated use and often higher densities of individuals, which has significant implications for disease transmission (Sorensen et al., 2014). Many studies have discussed the direct implication in disease management to such sites. For example, Sorensen and colleagues identified risks associated with bovine tuberculosis transmission to both livestock and wildlife at such sites (Sorensen et al., 2014). For these and similar reasons, feeding and baiting of cervids has been banned by state fish and game agencies in Wyoming, Colorado, and some Midwestern US ("Baiting and Feeding", "Wyoming CWD Management Plan" 2020; "Colorado Chronic…", 2018).

Another more debated example in Wyoming is supplemental feeding by the WGFD and US Fish and Wildlife Service at elk winter sites—commonly referred to "elk feedgrounds."

Supplemental feeding of elk on feedgrounds began in Wyoming in the early twentieth century ("Elk Feedgrounds…" 2021). With over a century of elk feedground use by some herds in Wyoming, wintering at these locations is well-established. Elk wintering at these feedgrounds has become a part of the culture, with many stakeholders very invested in feedground existence ("Elk Feedgrounds…" 2021). While supplemental feeding began as a way to counteract loss of elk in extreme winter conditions, elk feedgrounds are estimated to have 2.2 times the density of elk than that of winter areas with no supplemental feeding (Cook et al., 2023, "Elk Feedgrounds…" 2021). This has significant implications when it comes to brucellosis, CWD, and other diseases ("Elk Feedgrounds…" 2021). Cook and colleagues created models using WGFD elk feedground data that show evidence that continued feeding will lead to a reduction in elk populations in 20 years but will allow higher hunter harvest and lower brucellosis cost to nearby cattle producers (Cook et al., 2023). These results, and the results of similar studies, highlight the complex factors involved in the elk feedground debate (Maloney et al., 2020).

Beyond congregation points created to purposely attract cervids or sustain or elevate populations, other human activity may incidentally incentivize their spatial overlap or congregation. Agricultural land often serves as an attractant to cervids, leading humans to implement measures to attempt deter or exclude cervids, both as means of reducing damage and to mitigate disease (Agetsuma et al., 2016; Lavelle et al., 2015; Mejía-Salazar et al., 2018). Recent concerns have been raised in Wyoming about how supplementation of livestock diets creates artificial, unintentional cervid congregation points that may pose additional CWD transmission risk (Schumaker. personal communications. 2020).

It's been long acknowledged that sharing of resources between cattle and wildlife can lead to disease transmission. Wildlife and cattle use the similar food and water resources (Mejía-

Salazar et al., 2018; Stock et al., 1990). Zu Dohna et al. (2014) found in their study of mule deer, elk, and cattle that resource sharing led to significant increase in potential interspecies contacts. In Wyoming's Greater Yellowstone Ecosystem, brucellosis (*Brucella abortus*) has been consistently passed between wildlife and cattle. *B. abortus* causes symptoms that include arthritis, lethargy, and abortions in cattle, which can lead to economic loss for livestock producers (Rhyan et al., 2013). In the United States, there is a strict policy of eradication of *B. abortus* positive cattle herds (Olson, 2010). However, in the GYE, spillback to cattle from the wildlife reservoir, namely Rocky Mountain elk (*Cervus elaphus nelsoni*) and American bison (*Bison bison*), creates a serious management challenge (Kamath et al., 2016; Rhyan et al., 2013). Evidence suggests that elk are primarily responsible for transmission back to cattle (Kamath et al., 2016). Significant research effort has been invested to understand the relationship between cattle and wildlife reservoirs.

Cattle production is a very important and culturally significant part of the economy and land use in Wyoming. The Wyoming Farm Bureau Federation estimated that, in 2019, agriculture contributed $1.83 billion to Wyoming's economy, with 62.7% generated by the production of animals ("Cattle Industry of Wyoming"). The US Department of Agriculture estimates agriculture in Wyoming equates to $2 billion annually ("Wyoming Agriculture" 2022). In 2019, 46.5% of Wyoming's land was used for agricultural production ("Wyoming Agricultural Statistics" 2020). Having begun in the mid-1800s, cattle operations are so deep-rooted in Wyoming's identity that the state's emblem is a cowboy riding a bucking bronco, and it's known as the "Cowboy State" ("Wyoming Agriculture" 2022).

Feeding beef cattle supplemental salt and trace minerals has been shown to effectively enhance livestock production. Feeding gestating cows trace minerals to assist in fetal development has been shown to increase beef productivity (Harvey et al., 2021). Proper trace mineral nutrition also has implications in immune function of beef cows (Corah, 1996). Additionally, some studies have shown that supplemental mineral supplied to bulls in young age-ranges may have influence on reproductive (Pitts et al., 1966). Supplemental feeding is also used with grazing cattle to deter pests. Some feeds, which also include trace minerals, are designed to supply doses of pesticides that assist in the control of nuisance fly species ("Fly Control").

Cervids are also attracted to areas with high concentrations of salt and other important trace minerals (Lavelle et al., 2015). Salt and trace minerals are important to cervid health. Mineral consumption may influence immunity in cervids (Wolfe et al., 2010). Furthermore, evidence of sex-specific differences in type of mineral consumed and timing of consumption suggests that salt and minerals may assist in cervid reproduction (Atwood & Weeks 2003). However, mineral resources deployed for cattle are artificial mineral sources that may concentrate cervids in areas where they might otherwise not occur. Furthermore, Lavelle et al (2015) observed cervids licking and sniffing common surfaces and consuming soil at salt licks. These behaviors put cervids at risk for indirect CWD transmission in CWD positive areas (Lavelle et al., 2015). Plummer et al (2018) showed that water and soil at cattle salt and mineral lick areas serve as sources of PrP^{Sc}.

Better understanding dynamics of disease associated with anthropogenic environmental changes and finding ways to reduce any additional resulting disease transmission may assist in conservation related to diseases beyond CWD. Several other diseases exist in North America that affect Cervidae family species. Epizootic Hemorrhagic Disease virus (EHD) and bluetongue virus (BT) both cause hemorrhagic disease in white-tailed deer and mule deer ("Hemorrhagic Disease of…" , 2018). Both viruses are transmitted by midges (*Culicoides sp.*) and can result in rapid fatality ("Hemorrhagic Disease of…" , 2018). Similarly, Adenovirus Hemorrhagic Disease (AHD) results from *Odocoileus adenovirus* infection and most strongly affects mule deer. White-tailed deer, elk, and moose can also be infected ("Adenovirus Hemorrhagic Disease", 2018). AHD is transmitted by direct contact between infected and susceptible individuals and through contaminated environmental surfaces ("Adenovirus Hemorrhagic Disease", 2018). Bovine tuberculosis (bTB) is caused by infection by *Mycobacterium bovis* and primarily affects white-tailed deer and elk in the United States but has also been found in mule deer (Conner et al., 2008; Rhyan et al., 1995). This disease is primarily transmitted through aerosol inhalation during direct contact with an infected individual. *M. bovis* can persist in moist environments for long periods of time (Conner et al., 2008). bTB is a chronic respiratory disease. Individual hosts can show no clinical signs for months or years and later have disease reoccurrence (Conner et al., 2008). Anthrax, caused by the bacteria *Bacillus anthracis*, occurs in domestic cattle and grazing wildlife (Hugh-Jones & de Vos, 2002). In North America, cases have been seen in white-tailed deer, elk, and moose (Blackburn et al., 2010; Kellogg & Prestwood, 1970; Morris et al., 2016). Anthrax generally has rapid onset and ends in fatality (Hugh-Jones & de Vos, 2002). Like CWD, environmental transmission can play a significant role in anthrax. *Bacillus anthracis* can persist for decades as infectious spores in soil with potential to be unearthed by grazing wildlife or livestock (Carlson et al., 2018). A disease called hair loss syndrome (HLS) primarily affects black-tailed deer in Washington and Oregon, but has also been seen in mule deer populations in California in the United States (Bildfell et al., 2004; Bender & Hall, 2004; Marquez, 2020). HLS is caused by chewing lice but often co-occurs with other parasites and can result in poor body condition, co-infection, and death (Bildfell et al., 2004; Foreyt et al., 2004). Similar hair loss caused by winter tick infestation occurs in moose in North American (Samuel, 2007).

Elaeophora schneideri is an arterial worm that is transmitted by horseflies (Hibler and Metzger, 1974). Mule deer and black-tailed deer are considered definitive hosts, but *Elaeophora schneideri* has also been found in white-tailed deer populations in the Southeastern US (Couvillion et al., 1985; Gerhold, 2022; Hibler and Metzger, 1974). Additionally, elk and moose can act as incidental hosts and have shown lower resistance to infection (Gerhold, 2022). Meningeal worm, *Parelaphostrongylus tenuis,* is a nematode that starts its lifecycle in gastropods and matures with the its definitive host, white-tailed deer, after the host inadvertently consumes infected gastropods ("P. tenuis (Brainworm)", 2018). Many other Cervidae species, including mule deer, elk, and moose can be parasitized by *Parelaphostrongylus tenuis.* White-tailed deer do not often exhibit clinical signs, but higher infection rates in other species, such as moose, have been associated with slow population decline (Lankester, 20120). Similarly, Haemonchus contortus is a significant parasite of domestic livestock that also infects white-tailed deer (Davidson et al., 1980). It is transmitted through feces of infected hosts, such as livestock and white-tailed deer (Adduci et al., 2022). It is associated with malnutrition and reduced survival in white-tailed deer fawns, but clinical sign are rare in adults (Davidson et al., 1980).

Future Research Direction

The relationship between cervids and livestock salt and mineral licks has not been well studied. There is no research that shows whether these sites serve as a greater attractant to wildlife as compared to natural sites with high concentrations of salt and mineral. With limitations in PrP^{Sc} detection techniques, we are currently unable to quantify the prions in soil, making it difficult to distinguish if salt and mineral licks have higher PrP^{Sc} than surrounding areas. Additionally, we are still developing knowledge of how some minerals, like manganese, may potentially affect prion transmissibility or survival in the environment (Davies & Brown, 2009). There has also been very little research that documents cervid use or changes in behavior and movement related to livestock salt and mineral licks in general. Additional research is needed to understand livestock salt and mineral licks affect CWD transmission.

Even though great strides have been made in many areas of CWD in the last decade, there are still many questions about how best to manage the disease. Researchers and managers continue to collaborate to find innovative and effective management solutions to reduce CWD prevalence that are accepted by stakeholders.

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CHAPTER 2: EXCLUDING CERVIDAE SPECIES FROM LIVESTOCK MINERAL RESOURCES TO REDUCE CHORNIC WASTING DISEASE TRANSMISSION

Introduction

Agricultural land overlapping free-ranging wildlife habitat is a conservation issue that exists globally (Izumiyama et al., 2003; Odadi et al., 2011; Peralvo et al., 2005; Sukumar, 1990). Sometimes, these anthropogenic activities can have unintentional influence on the behavior and spatial selection of wildlife. In North America, we see such effects with Cervidae family species (cervids). Studies of white-tailed deer in the Midwestern US have shown evidence that areas with high proportions of agricultural land are associated with increases in juvenile male dispersal distances (Gilbertson et al., 2022) and an increased rate of dispersion among females (Nixon et al., 2007). Furthermore, resources on agricultural land can serve as an attractant to cervids species, causing humans to take measures to exclude them (Lavelle et al., 2015; Mejía-Salazar et al., 2018; Pruvot et al., 2014).

Production of livestock can result in additional complex dynamics due to interspecies interactions between livestock and cervids. For example, zu Dohna et al. (2014) observed resource partitioning among elk, mule deer, and cattle, competitive displacement of cervids, and significant potential interspecies contacts when these species co-occurred on rangeland. Additionally, alterations to the landscape and resource availability resulting from livestock operations, such as the addition of fencing or food and water resources, may alter how cervids move and behave on the landscape (Lavelle et al., 2015; Rich et al., 2019; Xu et al., 2021).

Proximity of cervids to livestock becomes especially significant for management when an incidental consequence is increased disease transmission. Sharing of resources between cattle and wildlife can lead to disease transmission (Miller et al., 2013). A classic example of the effects of cervid proximity to livestock on disease dynamics exists in Wyoming's Greater Yellowstone Ecosystem (GYE), where brucellosis (*Brucella abortus*), a zoonotic disease that primarily affects cattle, has been consistently passed between wildlife and cattle (Rhyan et al., 2013). In cattle, *B. abortus* causes arthritis, abortions, and other symptoms leading to reproductive loss that results in economic loss to cattle producers (Rhyan et al., 2013). Because of its significant impacts, federal and state agencies have a strict program to eradicate the disease in cattle (Olson, 2010). In the GYE, at least five spillovers from domestic cattle to wildlife have occurred (Kamath et al., 2016). Since the initial spillovers, Rocky Mountain elk (*Cervus elaphus nelsoni*) and American bison (*Bison bison*) serve as reservoirs, with elk acting as the primary source of new infections in cattle in the GYE area (Kamath et al., 2016; Rhyan et al., 2013). While *B. abortus* is an example of transmission between domesticated species and wildlife, some research investigates the affect agricultural operations may have on transmission of diseases only affecting wildlife species.

Chronic wasting disease is an invariably fatal disease of Cervidae family species that has no known treatment or cure (Rivera et al., 2019; Williams, 2005). It is not known to naturally infect any other species (Rivera et al., 2019). It was described in 1967 in mule deer and black-tailed deer in captive facilities in Colorado, and began sparking global concern in the last two decades (Benastad et al., 2016; Riviera et al., 2019; William & Young, 1980). CWD falls into a class of diseases called transmissible spongiform encephalopathies (TSE), which are neurodegenerative diseases caused by prions (Williams, 2005). Infectious prions (Prp^{Sc}) are misfolded isoforms of noninfectious cellular proteins (Prp^{C}) that exist on cell surfaces in all mammals and in many other species (Lee et al., 2013). When Prp^{Sc} comes into contact with Prp^{C}, it causes the Prp^C to misfold, thereby resulting in aggregates of misfolded proteins that form amyloid plaques in the central nervous system (CNS) (Lee et al., 2013). Clinical signs of CWD include ataxia, polydipsia, polyuria, hyper-salivation, and drooping head and ears (Williams, 2005). While routes of transmission are still being researched, studies have shown that susceptible species are likely to become infected with CWD naturally through oral and nasal contact with infected individuals and with infected surfaces in the environment (Miller & Williams, 2003; Miller et al., 2004; Saunders et al., 2008). Because prions are extremely difficult to eradicate from surfaces in the environment, environmental transmission is of major concern and creates substantial management challenges (Wiggins, 2009, Saunders et al., 2008).

The effects of agricultural land use on CWD have been broadly considered in studies that look at factors like changes in dispersion (Gilbertson et al., 2022), but how human land use affects CWD transmission is continuing to be researched. While there is strong evidence that CWD cannot currently infect cattle (Williams et al., 2018), cattle production may have effects on intra- and inter-species transmission among cervids due to current livestock feeding practices.

Cattle production is a very important and culturally significant part of the economy and land use in Wyoming. The Wyoming Farm Bureau Federation estimated that, in 2019, agriculture contributed $1.83 billion to Wyoming's economy, with 62.7% generated by the production of animals ("Cattle Industry of Wyoming"). The US Department of Agriculture estimates agriculture in Wyoming equates to $2 billion annually ("Wyoming Agriculture" 2022). In 2019, 46.5% of Wyoming's land was used for agricultural production ("Wyoming Agricultural Statistics" 2020). Having begun in the mid-1800s, cattle operations are so deep-rooted in Wyoming's identity that the state's emblem is a cowboy riding a bucking bronco, and it's known as the "Cowboy State" ("Wyoming Agriculture" 2022).

Feeding beef cattle supplemental salt and trace minerals has been shown to effectively enhance livestock production. Feeding gestating cows trace minerals to assist in fetal development has been shown to increase beef productivity (Harvey et al., 2021). Proper trace mineral nutrition also has implications in immune function of beef cows (Corah, 1996). Additionally, some studies have shown that supplemental mineral supplied to bulls in young age-ranges may have influence on reproductive (Pitts et al., 1966). Supplemental feeding is also used with grazing cattle to deter pests. Some feeds, which also include trace minerals, are designed to supply doses of pesticides that assist in the control of nuisance fly species ("Fly Control").

Cervids are also attracted to areas with high concentrations of salt and other important trace minerals (Lavelle et al., 2015). Salt and trace minerals are important to cervid health. Mineral consumption may influence immunity in cervids (Wolfe et al., 2010). Furthermore, evidence of sex-specific differences in type of mineral consumed and timing of consumption suggests that salt and minerals may assist in cervid reproduction (Atwood & Weeks 2003). However, mineral resources deployed for cattle are artificial mineral sources that may concentrate cervids in areas where they might otherwise not occur. Furthermore, Lavelle et al (2015) observed cervids licking and sniffing common surfaces and consuming soil at salt licks. These behaviors put cervids at risk for indirect CWD transmission in CWD positive areas (Lavelle et al., 2015). Plummer et al (2018) showed that water and soil at cattle salt and mineral lick areas serve as sources of PrP^{Sc}.

CWD has impacted Cervidae species in Wyoming. CWD has been detected in 84% of all Wyoming mule deer herds, 25% of Wyoming elk herds, and in white-tailed deer throughout the state as of 2020 (WGFD 2020). Some herds of mule deer (DeVivo et al. 2017) and white-tailed deer (Edmunds et al. 2016) in Wyoming have had observed populations declines that were correlated with CWD prevalences of 23-25%. Wildlife managers are trying to find cost effective management tools with broad stakeholder support (Landon et al., 2023; WGFD, 2020). Excluding cervids from livestock salt and mineral licks (hereon, referred to as "livestock mineral licks") while allowing cattle free-access to these resources could reduce CWD transmission by discouraging cervid congregation and spatial overlap. This would support efforts of wildlife managers, hunters, wildlife enthusiasts, and other such CWD interest groups using a method potentially well-accepted by cattle producers.

Cattle mineral feeders are produced in many designs and sizes, but are generally containers designed to dispense salt and mineral for cattle (Barn World, 2023; Soiux Steel Company, 2023). Some models are cylindrical polyethylene containers (approximately 97 X 36 cm) with heavy rubber lids that prevent wind and rain from depleting the salt and mineral inside (Barn World, 2023; Soiux Steel Company, 2023). While cattle freely access the contents of these mineral feeders, some observations indicated that cervids do not. The objective of this study was to better understand free-ranging cervid use of livestock mineral licks and test an on-the-market cattle mineral feeder as a potential strategy for excluding cervids from these resources. Based on limited anecdotal observations of these species interacting with mineral feeders prior to this study, we hypothesized that mule deer (*Odocoileus hemionus*) and moose (*Alces alces*) would not access mineral feeder contents but that Rocky Mountain elk (*Cervus elaphus nelsoni*) would learn to use the mineral feeders. Furthermore, we hypothesized that there would be a decrease in visitation time among all cervid species to livestock mineral licks where all salt and mineral were dispensed inside these feeders, thereby reducing opportunity for CWD transmission.

Study Area

Fieldwork was conducted within three regions of Wyoming, referred to here as the Northern Bighorns, Southern Bighorns, and South Casper regions (Figure 1). The Northern Bighorn sites were on US Forest Service land near US-14 between the towns of Dayton and Lovell in the Bighorn Mountain Range. Site elevations ranged from 2289 m to 2549 m in this region. Inter-Mountain Basins Montane Sagebrush Steppe, Rocky Mountain Aspen Forest and Woodland, and Southern Rocky Mountain Dry-Mesic Montane Mixed Conifer Forest are the primary habitat types at these sites (USGS, 2011). The Southern Bighorn sites were located on private land west of I-25 and southwest of Kaycee, WY. Sites ranged in elevation from 1749 m to 2128 m. Habitat at these sites primarily consisted of Northwestern Great Plains Mixed-grass Prairie and Inter-Mountain Basins Montane Sagebrush Steppe (USGS, 2011). The South Casper sites were located on private ranches and BLM land near WY-48, approximately 60 miles south of Casper. Habitat at these sites was primarily Inter-Mountain Basins Big Sagebrush Steppe, Northwestern Great Plains Mixed-grass Prairie with interspersed Western Great Plains Riparian Woodland and Rocky Mountain Foothill Limber Pine-Juniper Woodland (USGS, 2011). Elevations ranged from 1997 m to 2311 m. The majority of field sites were located in the South Casper region due the larger cattle production operations at these ranches and an additional willing landowner. A detailed breakdown of sites can be found in Figure 1. Individual livestock mineral sites within each region were pre-selected by each rancher and identified during our first visit to each ranch or grazing allotment.

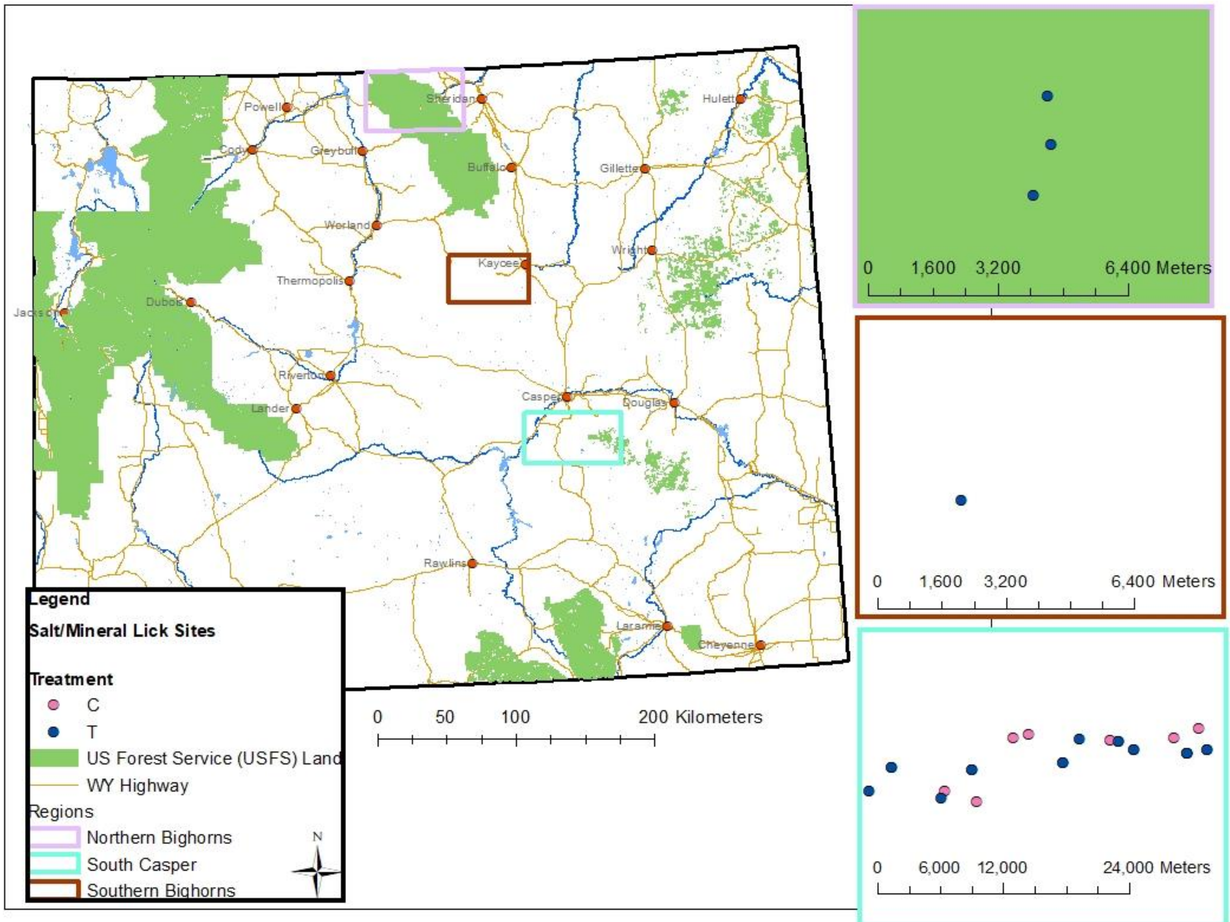

Figure 1. Map of Wyoming, USA, with all mineral sites used in analysis by region. Pink points represent control sites where mineral was placed on the ground, and navy points represent treatment sites where all mineral was placed inside of mineral feeders.

Methods

Control and treatment implementation

We dispensed salt and mineral at sites where livestock producers had regularly dispensed salt, mineral, or both to supplement the diets of their livestock. For all sites at each livestock operation, we dispensed the same salt, mineral, or salt-mineral combination that the livestock producer usually provided to their livestock.[1] We divided sites into control and treatment sites. Control sites consisted of the usual salt and mineral combination provided by the specific livestock producer, dispensed in the method the livestock producer usually dispensed it: placed on the ground or in an open container and openly accessible to all livestock and wildlife. We monitored all salt and mineral present at each of these sites during the monitoring period using the game camera set-up described in "Site monitoring." Treatment sites were similar to control sites, except that we placed all salt and mineral inside a device that might exclude cervids (hereafter called "mineral feeders"). After brief, pre-study testing of several on-the-market cattle mineral feeders, we selected Sioux Steel Ground Mineral Feeders for our test device (Figure 2).

Figure 2. Photos of Sioux Steel Ground Mineral Feeders that were tested on this project. Photos showed evidence that livestock including cattle (cows, bulls, and calves) and horses were able to access mineral feeder contents with no training.

Throughout the study, we monitored a total of fifty sites: thirty-nine in the South Casper region, six in the Southern Bighorns region, and five in the Northern Bighorns region. Though

[1] In this study, livestock producers' supplemental feeding practices included the use of NaCl blocks, blocks containing both NaCl and other minerals, mineral-molasses-flax seed mixtures pre-poured into steel drums, or some combination thereof.

the designated monitoring period was May through September in 2021 and 2022, we experienced intermittent periods of data loss due to factors including camera malfunction, weather, and interference with the cameras by cattle or wildlife. Sites with low quality data arising from logistical issues were excluded from the analysis. We randomly assigned sites as control or treatment via computer algorithm, with approximately fifty percent of the sites allocated to each group. During the study, livestock producers requested that a total of four specified sites remain control; therefore, these sites were not assigned randomly. Our research questions were: 1) Do cervids gain access to mineral feeder contents?; and 2) Does the deployment of mineral feeders result in changes in cervid visitation time? For each research question, we used different, but overlapping, data sets which are described below.

Access

To assess access, we used data exclusively from treatment sites in all three regions. These are represented as the navy-colored points in Figure 1. In the Northern Bighorns and Southern Bighorns regions, we monitored four and two treatment sites, respectively. We deployed cameras in each region July 7 and Jun 16, 2021, respectively, and monitored them through September 2021. In 2022, we monitored Northern Bighorn region sites from June 29 to September 7 and the Southern Bighorn region sites from Jun 14 to September 7. The monitoring schedule for treatment sites in the South Casper region, which were also used to assess access, is described in the section below.

Visitation

To assess changes in visitation, we used data from control and treatment sites only in the South Casper region. In 2021, all active sites were control sites and the earliest camera was deployed on July 15 and last camera was deployed on August 1. We ended all monitoring in 2021 by September 26th. In 2022, we deployed cameras at sites starting the second week of May at which time they were all controls. After three weeks, we implemented the treatment by deploying the previously described mineral feeders at half of all sites, which were randomly designated via computer algorithm as treatment. We then continued camera monitoring of all sites through the first two weeks of September.

Site monitoring

Generally, each site consisted of a single game camera (Browning), mounted three to fifteen meters away from the salt, mineral, or mineral feeder. We mounted game cameras on sturdy trees, fence posts, or other artificial structures where available. Since many sites were in open sagebrush steppe and far from standing structures, we mounted most cameras in the study on temporary posts approximately 1.5 meters above the ground. Single posts in open landscape attracted cattle as something to rub against; therefore, we erected exclosures with barbed wire to protect the cameras. The supplemental feeding practices of some cattle producers in the study involved multiple blocks of salts or mineral placed in areas up to 40 meters across. At these sites, we mounted two to three game cameras in a line along the site with all cameras facing the same compass direction to monitor the entire site. All cameras were motion activated and set to take bursts of five rapid photos, with a five second delay between bursts if constant motion was detected.

Photo processing

We used Timelapse 2.0 software (Greenberg et al., 2019) to record information in camera trap photos. We batched photos in the Timelapse software by episodes, with an episode defined as a group of photos in which there was less than a two-minute gap between any two sequential photos. For each photo, we recorded site ID, species present, and count of elk, moose, mule deer, white-tailed deer, and unidentified *Odocoileus* species. We also documented the following: cervid naso-oral contact with salt, mineral, or mineral feeders; cervid soil consumption; and presence of a cervid head inside any mineral feeder.

We maintained quality assurance and control through a two-tiered system, where each set of data (associated with a photo set) was created by one technician and then checked by a second technician (Figure 3). Additionally, we collected any variables showing issues with data quality in that monitoring period. If a camera was knocked (generally by cattle or wildlife) or if cattle pushed any salt or mineral out of the camera's view—either of which prevented full monitoring of all salt or mineral in that site—we marked photos accordingly and did not use them in analysis. Lastly, we also collected data that provided information about challenges in implementing treatment. Mineral feeders sometimes sustained damage from cattle. For example, sometimes cattle feeding caused the mineral feeder lids to slowly unscrew from the top of the mineral feeder, eventually allowing the lid to be pushed off. Also, cattle producers occasionally placed mineral or salt outside the mineral feeders. We recorded these variables.

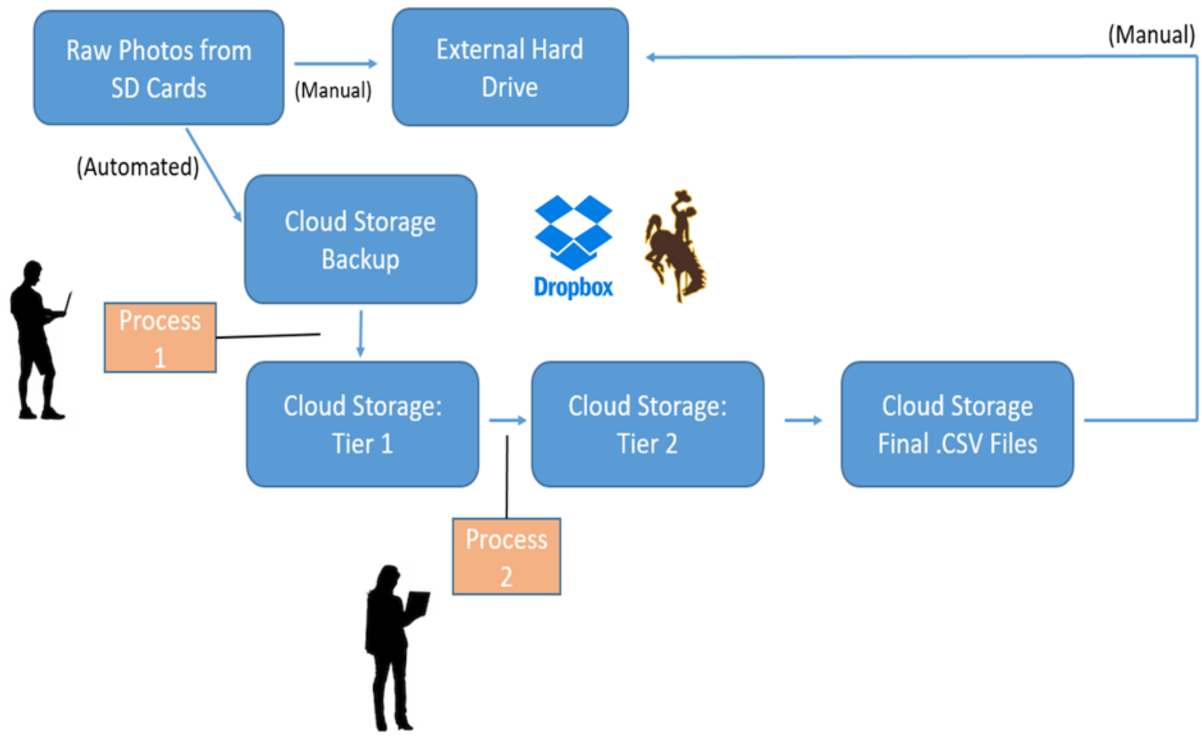

Figure 3. We maintained quality control of photo processing data through the workflow depicted here. Through the use of cloud-based storage (Dropbox and the University of Wyoming) and careful documentation, remote technicians were able to assign themselves photo sets to process through the Timelapse photo processing software. Process 1 denotes the initial recording of all variables in all photos in a single photo set, while Process 2 denotes the stage where a second technician checked the data that was recorded in Process 1.

Analysis

Access

To determine the effectiveness of the mineral feeder's exclusion of cervids, we used all available data from treatment sites in all three regions after implementation of treatment. We removed data from analyses when any of the following were true: 1) Salt or mineral was placed outside of the mineral feeder by a cattle producer, because, with easily accessible salt or mineral, cervids were likely unmotivated to get into the feeder. 2) The lid of the mineral feeder was damaged or missing, because this could allow cervids to gain unencumbered access to mineral feeder contents. 3) The mineral feeder was no longer in view of the camera. For sites with multiple cameras, all visits among all cameras were summed.

We recorded the total number of visits per species to each site and the number of visits in which an individual was successful in accessing the salt or mineral within the mineral feeder. In order to reduce possible bias due to cervids passing through some sites located along frequently used corridors, we defined a visit as an episode in which mineral-seeking behavior was observed in any photo of that episode. A photo was considered to include mineral-seeking behavior if it included cervid naso-oral contact with salt, mineral, mineral feeder surfaces, or soil. We defined a visit with access to salt or mineral as a visit in which a cervid was able to successfully lift the flap of the mineral feeder and place its mouth and nose inside. We calculated the effectiveness of treatment by calculating the percentage of unsuccessful visits (i.e. (total visits – visits with access) / total visits x 100).

Visitation

To determine if mineral feeders reduce cervid presence and visitation to salt and mineral licks intended for livestock, we used a Before-After Control-Impact (BACI) study design and compared species-specific time spent at sites before and after the implementation of treatment (i.e. the impact) in site designated as control and treatment in the South Casper region in 2022. We defined species-specific visitation time as the number of minutes per day that individuals of a species spent at a livestock mineral lick during which we observed any mineral-seeking behavior by individuals of the species. We calculated visitation time by subtracting the timestamp of the first photo of an episode from the timestamp on the last photo of an episode and summing visitation time among all episodes per day at each site. We eliminated partial days from the data to avoid bias due to possible diurnal patterns in cervid visitation to the sites. Days were calculated as 24 hour periods starting at 6:00:00 PM. If an episode was transected by the start of a new day, we divided the episode among the two days at the 6:00:00 PM timestamp. To better understand if visitation time post implementation of treatment was determined by a few persistent individuals, we created a similar model evaluating covariate effects on species-minutes. We calculated species-minutes by multiplying visitation time in each episode by the average number of individuals of that species among all photos in the episode and summing species-minutes by day.

Due to the supplemental feeding practices of some of the cattle producers in the study, we needed multiple cameras to monitor some very large livestock mineral sites. To avoid counting individuals more than once among the cameras, we averaged visitation time and species-minutes during the same day for all cameras across multi-camera sites.

We created generalized linear mixed models (or GLMMs) with site as a random effect for all models (Figure 4) using the R package glmmTMB (Brooks et al., 2017). In our models, we included covariates for control and treatment, before or after impact, time-step, and daily visitation time by cattle. To account for differences over the course of treatment due to seasonal effects on cervid movement and nutritional needs, we broke data into monthly time-steps (May, June, and August). The August time-step included some data from days in early September due to the maintenance schedule of some field sites. Due to limited photo processing resources, the May and June time-steps only include three control and three treatment sites and there was no July time-step. Daily cattle visitation time was calculated using the same method as visitation time for cervids but did not restrict data to periods where mineral-seeking behavior by cattle was observed. We incorporated this into our models after anecdotal observations showed that cattle presence may dissuade cervids from accessing these sites. We also considered including the effect of days since the implementation of treatment, but high correlation with the time-step variables caused us to eliminate this variable from analysis.

Due to the very large number of zeros in the visitation time and species-minutes variables in our final data sets, finding well-fitted models was challenging. We first used a zero-inflated negative binomial model, which required that we round all data for dependent variables (species-specific visitation time and species-minutes) to the nearest integer (Figure 4). We also evaluated the effects of the implementation of treatment on species-specific visitation using a hurdle model that answered two questions: 1) does implementation of treatment effect species presence? 2) Given a species is present, is there a change in visitation time? Hurdle models consisted of two smaller models each answering one of the questions above. We used similarly structured GLMMs with daily species-specific presence-absence as the dependent variable in one and one was a conditional model evaluating effects on species-specific visitation time given species presence (Figure 4). We used two hurdle models that varied only in the second part of the model. In Hurdle 1, we evaluated covariate effects on visitation time given presence using GLMM a negative binomial distribution and log link. In Hurdle 2, we evaluated covariate effects on visitation time given presence using GLMM with a Gaussian distribution and with the dependent variable log transformed (Figure 4).

With the BACI study design, we evaluated the statistical relationship of differences in dependent variables for control and treatment sites pre-implementation of treatment, control and treatment sites post-implementation of treatment, control sites pre- and post- implementation of treatment, and treatment sites pre- and post-implementation of treatment. We used the interaction term in the GLMMs referenced above and algebra to determine coefficient estimates of the

BACI comparisons. We used contrast and variance-covariance matrices to determine p-values for BACI comparisons.

Lastly, we assessed if the use of mineral feeders affected the amount of time cattle spent at livestock mineral licks. For these models, we used the zero-inflated negative binomial model for in Figure 4, but we dropped cattle visitation as a covariate and instead used it as the dependent variable.

Zero-inflated negative binomial (ZINB 1 and ZINB2):

$$logit(P_{ij}) = \beta_0 + \beta_1 T_{ij} + \beta_2 I_{ij} + \beta_3 S_{Jij} + \beta_4 S_{Mij} + \beta_5 T_{ij} * I_{ij} + \beta_6 C_{ij} + site\ random\ effect$$

$$log(\mu_{ij}) = \beta_0 + \beta_1 T_{ij} + \beta_2 I_{ij} + \beta_3 S_{Jij} + \beta_4 S_{Mij} + \beta_5 T_{ij} * I_{ij} + \beta_6 C_{ij} + site\ random\ effect$$

Hurdle (Hurdle 1 and Hurdle 2) Presence - Absence:

$$Y_{ij} \sim B\left(n_{ij},\ p_{ij}\right)$$

$$n_{ij} = 1$$

$$logit(p_{ij}) = \beta_0 + \beta_1 T_{ij} + \beta_2 I_{ij} + \beta_3 S_{Jij} + \beta_4 S_{Mij} + \beta_5 T_{ij} * I_{ij} + \beta_6 C_{ij} + site\ random\ effect$$

Hurdle 1 Visitation Time, Given Presence:

$$V_{ij} \sim NB(\mu_{ij}, \varphi)$$

$$\log(\mu_{ij}) = \beta_0 + \beta_1 T_{ij} + \beta_2 I_{ij} + \beta_3 S_{Jij} + \beta_4 S_{Mij} + \beta_5 T_{ij} * I_{ij} + \beta_6 C_{ij} + site\ random\ effect$$

Hurdle 2 Visitation Time, Given Presence:

$$V_{ij} \sim N\left(\mu_{ij}, \sigma^2\right)$$

$$\mathrm{E}[\log(\mu_{ij})] = \beta_0 + \beta_1 T_{ij} + \beta_2 I_{ij} + \beta_3 S_{Jij} + \beta_4 S_{Mij} + \beta_5 T_{ij} * I_{ij} + \beta_6 C_{ij} + site\ random\ effect$$

Figure 4. Generalized linear mixed models used to compare before-after control-impact (BACI) effects, with i = site, j = day, V = species-specific visitation time, Y = presence-absence, T= control-treatment indicator variable, I = before-after impact indicator variable, S = monthly time-step indicator variable (M = May, J = June), and C = cattle visitation time. In ZINB2, V was substituted

for species-minutes. Note, in visitation time given presence for Hurdle 1 we used a negative binomial distribution with a log link function and, in visitation time given presence for Hurdle 2, we used a Gaussian distribution and only the dependent variable was log transformed. In final models, C was dropped (see results). We used $e^{\beta} - 1*100$ *to back transform coefficients of categorical covariates to gain the average percent change in odds of the dependent variable or average percent change in the dependent variable, associated with a logit link or log link and log(Y), respectively.*

Results

We collected over six million photos among the 50 monitored livestock mineral sites. The large number of collected photos necessitated that we prioritize the processing of photos from some sites for analysis. We eliminated photos from some time periods due to earlier described issues with site access and monitoring. We then prioritized sites with the least amount of data loss due to these issues in 2022 and further prioritized processing photos from sites with available 2021 data. We processed a total of 1,054,800 photo during this study and 137,956 of the photos contained observations of cervids (13.1%).

Access

We used data from 14 treatment sites within the three regions in 2021 and 2022 for a total of 267.88 days of monitoring (21.9 days and three sites in the Northern Bighorns, 38.2 days and one site in the Southern Bighorns, and 208.0 days and ten sites in South Casper). We found the treatment to be 98.6% (n = 360 total visits) effective in excluding mule deer, 74.0% (n = 50 total visits) effective in excluding elk, and 100% (n = 15 total visits) effective in excluding moose (Figure 5). We observed at least one individual mule deer accessing mineral feeder contents in each of the three regions. Similarly, elk accessed mineral feeder contents in all regions where elk were documented in the photos (Northern Bighorns and South Casper). Moose presence was documented only in the Northern Bighorns. We did not have a sufficient sample size of days with observations of elk at any site to assess changes in percentage of successful visits over time.

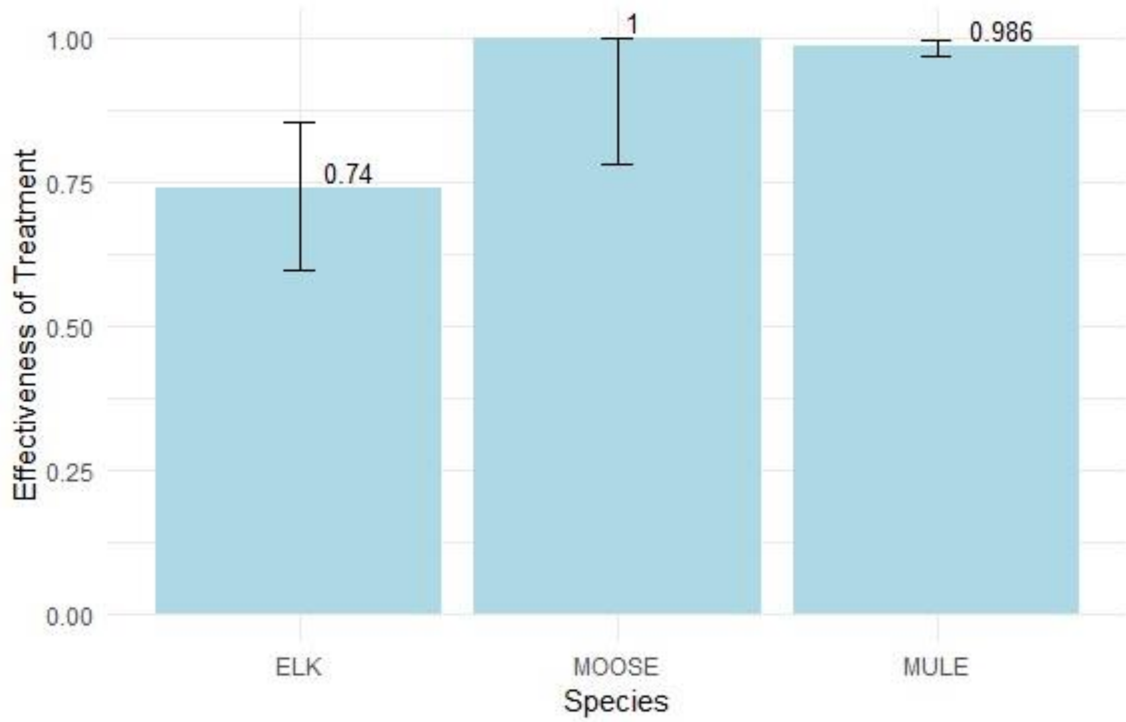

Figure 5. Plot displaying the effectiveness of treatment by species summed among all days at all sites. This data comprises a total of 267.88 days of monitoring among 14 sites within three regions of Wyoming in two years (2021 and 2022). Elk accessed mineral feeder contents in 13 of 50 observed visits, moose in 0 of 15, and mule deer in 5 of 360.

Visitation

We used 14 sites from the South Casper region in 2022 to assess visitation. We monitored the seven control sites for a total of 118 days of monitoring and seven treatment sites for a total of 183 days of monitoring. For the May and June time-steps, we only had data from three control and three treatment sites. We collected sufficient data to gain estimated effects of implementation of treatment on mule deer presence and visitation time (Tables 1 and 2);

however, there were too few observations of all other species among sites in the South Casper region in 2022 to evaluate changes in visitation due to implementation of treatment. We dropped cattle visitation time as a covariate from all models, because in all models it was not significant, did not affect the significance of other variables, and further increased Akaike information criterion (AIC) values.

In the zero-inflated negative binomial model with mule deer visitation time as the dependent variable (ZINB 1), we found a 94.2% (p = 0.00367) decrease in realtive odds of mule deer absence in control sites post-impact compared to control sites pre-impact. Conditional on mule deer visiting a site, the time they spent at post-impact treatment sites as compared to post-impact control sites decreased by an 65.8% (p = 0.017) on average. All other BACI comparisons for ZINB1 had p-values greater than 0.05 (Table 2). Comparisons from ZINB and hurdle models with mule deer-minutes as the dependent variable showed similar results (Table 2).

The hurdle model approach yielded similar results (Tables 1 and 2). In Hurdle 1 and 2, we found 15.7 (p = 0.00336) times greater relative odds of mule deer presence in control sites post-impact compared to pre-impact. In Hurdle 1, we found that, given mule deer presence at sites, there was a 64.7% (p = 0.0300) mean decrease in mule deer visitation in post-impact treatment sites compared to post-impact controls. In Hurdle 2, we found a 73.7% (p = 0.0205) decrease in mule deer visitation in post-impact treatment sites compared to post-impact controls assuming mule deer presence. All other BACI relationships in both hurdle models had p-values greater than 0.05 (Table 2). All GLMMs were driven by some sites that experienced much more frequent mule deer visitation than other sites (Figure 6).

We found the implementation of treatment to have no significant effect on presence of cattle (p = 0.423) or the amount of time cattle spent at mineral licks (p = 0.839) at post-impact treatment sites compared to post-impact control. We also found no effect on cattle presence (*p* = 0.565) or cattle visitation time (*p* = 0.623) in treatment sites post-impact compared to pre-impact. In photos, we observed instances of horses and cattle including cows, calves, and bulls accessing mineral feeder contents. We observed cattle breeds included Texas longhorn at mineral feeders. While we did not observe any livestock having difficulty accessing mineral feeder contents, we recorded only limited observations of very horses and very young calves at mineral feeders.

Table 1: Table of GLMMs with mule deer visitation data used to make comparisons to establish the effect of treatment and impact on dependent variables. T= control-treatment indicator variable, I = before-after impact (i.e. implementation of treatment) indicator variable, S = monthly time-step indicator variable (M corresponding to May and J to June), and TxI = interaction between control-treatment and before-after impact. ZI signifies the zero-inflated portion and Cond the conditional portion of ZINB models. Effects with a p-value ≤ 0.05 were considered significant. Significant p-values are in bold and marked with *.

Model	Method	Estimates (β)	Standard Error (SE)	Significance (p)	AIC value
ZINB 1--Visitation time as dependent variable	Zero-inflated negative binomial, logistic regression, logit link (ZI), log link (Cond), *(ZI models absence as success)*	ZI: T: -1.83 I: -2.86 S_J: -0.0971 S_M: -1.36 TxI: 2.73 Cond: T: 0.306 I: 0.940 S_J: 0.229 S_M: -0.100 TxI: -1.38	ZI: T: 1.20 I: 0.985 S_J: 0.536 S_M: 0.957 TxI: 0.947 Cond: T: 0.769 I: 0.668 S_J: 0.357 S_M: 0.548 TxI: 0.665	ZI: T: 0.127 I: **0.00367*** S_J: 0.856 S_M: 0.156 TxI: **0.00399*** Cond: T: 0.690 I: 0.159 S_J: 0.522 S_M: 0.856 **TxI: 0.0382***	1068.2
Hurdle 1 and 2--Presence-absence as dependent variable	Binomial, logistic regression, logit link, *(Models presence as success)*	T: 1.67 I: 2.76 S_J: 0.00628 S_M: 1.074 TxI: -2.63	T: 1.070 I: 0.940 S_J: 0.493 S_M: 0.904 TxI: 0.882	T: 0.118 **I: 0.00336*** S_J: 0.990 S_M: 0.234 **TxI: 0.00286***	337.9

Hurdle 1--Conditional model assuming presence, with visitation time as dependent variable	Negative binomial, logistic regression, log link	T: 0.245 I: 0.870 S_J: 0.307 S_M: 0.00949 TxI: -1.29	T: 0.755 I: 0.641 S_J: 0.350 S_M: 0.536 TxI: 0.633	T: 0.745 I: 0.174 S_J: 0.380 S_M: 0.986 **TxI: 0.0422***	773.7
Hurdle 2--Conditional model assuming presence, with visitation time as dependent variable	Gaussian, logistic regression, log transformed dependent	T: 0.433 I: 0.743 S_J: 0.485 S_M: 0.00444 TxI: -1.77	T: 0.920 I: 0.809 S_J: 0.418 S_M: 0.695 TxI: 0.801	T: 0.638 I: 0.359 S_J: 0.246 S_M: 0.995 **TxI: 0.0274***	345.8

Table 2: Table of all GLMMs assessing mule deer visitation and their estimated coefficients for all BACI comparisons. Each cell below model names contains: 1) raw estimated coefficient, 2) the transformed coefficient, and 3) the p-value. (+) signifies an increase and (-) signifies a decrease. Significant p-values are in bold and marked with *.

BACI Comparison	Model Component	ZINB 1– Visitation Time	Hurdle 1— Visitation Time	Hurdle 2 – Visitation Time	ZINB 2– Species-Minutes
Treatment compared to Control, Pre-Impact (β_1)	Presence-Absence *(-ZINB models show estimated changes in log(odds) and relative changes odds of absence. -Hurdle models estimated changes in log(odds) and relative changes odds of presence.)*	-1.83259 84.0% (-) p = 0.127	1.673027 433% (+) p = 0.118	Same as Hurdle 1	-1.6236 80.3% (-) p = 0.522
Treatment compared to Control, Post-Impact ($\beta_1 + \beta_5$)		0.89269 144% (+) p =0.312	-0.957779 61.6% (-) 0.203	Same as Hurdle 1	1.0831 195% (+) p = 0.188
Post-Impact compared to Pre-Impact, Control Sites (β_2)		-2.86098 94.2% (-) **p = 0.00367***	2.755740 1470% (+) **p = 0.00336***	Same as Hurdle 1	-2.8620 94.3% (-) **p =0.00398***
Post-Impact compared to Pre-Impact, Treatment Sites ($\beta_2 + \beta_5$)		-0.1357 12.7% (-) p = 0.863	0.124934 13.3% (+) p = 0.870	Same as Hurdle 1	-0.1553 14.4% (-) p = 0.846
Treatment compared to Control, Pre-Impact (β_1)	Visitation time, given some probability of presence	0.3060381 35.8% (+) p = 0.691	0.245004 27.8% (+) p = 0.746	0.43284 54.2% (+) p = 0.638	0.560896 75.2% (+) p = 0.521

Treatment compared to Control, Post-Impact (β1 + β5)	*(-Probability of presence is 1 for all Hurdle models. -All transformed coefficients show estimated changes in mean visitation time.)*	-1.073268 65.8% (-) **p = 0.0166***	-1.04153 64.7% (-) **p = 0.0300***	-1.33438 73.7% (-) **p = 0.0205***	-1.131087 67.7% (-) **p = 0.0149***
Post-Impact compared to Pre-Impact, Control Sites (β2)		0.9400634 156% (+) p = 0.159	0.8703741 139% (+) p =0.175	0.74271 110% (+) p = 0.359	1.086126 196.0% (+) p = 0.155
Post-Impact compared to Pre-Impact, Treatment Sites (β2 + β5)		-0.4392423 35.5% (-) p = 0.311	-0.41616 34.0% (-) p = 0.325	-1.02451 64.1% (-) p = 0.0840	-0.605857 45.4% (-) p = 0.268

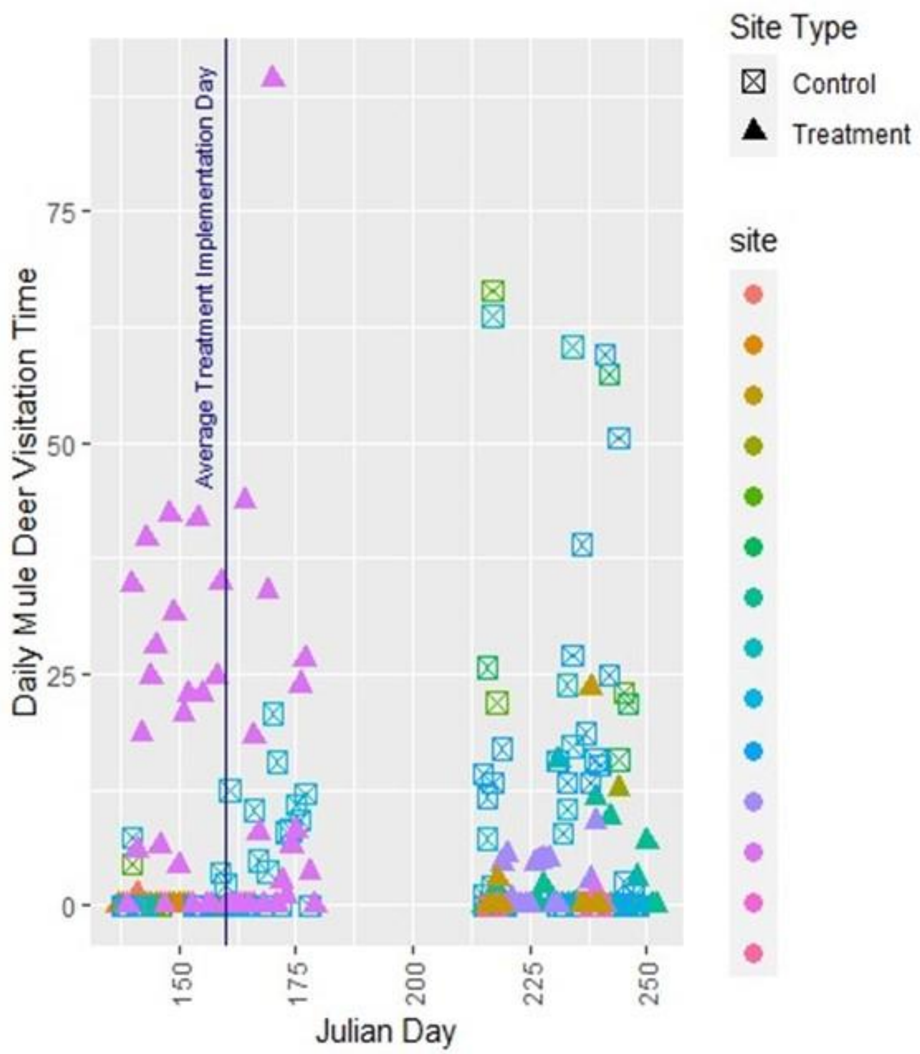

Figure 6. Plot of the dependent variable (mule deer visitation time) in the South Casper region in the 2022 season. This plot shows that the models were disproportionately affected by some sites that mule deer visited more frequently. There was no processed photo data for July (Julian days 182 – 212).

Site monitoring

During visits to sites by members of the research team, we recorded game cameras knocked down by livestock or wildlife or pushed to an angle that would likely prevent proper data collection in 93 of 458 site visits (20.3%) among both 2021 and 2022. During the 2021 season, we made 107 site visits and recorded cameras as knocked 35 times (32.7%). In July through early August of 2021, we erected barbed-wire exclosures to prevent data loss due to knocked cameras. We carefully maintained all exclosures during sites visits in the 2022 season. In 2022, we recorded knocked cameras in 58 of 351 of site visits (16.5%). Additionally, we recorded mineral feeder lids as missing or partially pushed into the mineral feeder in 19 of 145 (13.1%) of site visits to treatment sites. We recorded mineral supplements placed outside of mineral feeders by livestock producers in 9 of 145 (6.2%) of site visits to treatment sites.

Discussion

In this study, we found mineral feeders to be very effective (98.6%) at excluding mule deer from accessing mineral feeder contents, thus reducing the frequency with which mule deer have contact with artificial salt and mineral resources. We also found that, given mule deer presence, the use of mineral feeders significantly reduced mule deer visitation time—by an estimated 64.7-73.7% dependent on the model—greatly reducing the time mule deer spend at these sites when they may potentially contaminate surfaces with CWD, contract CWD from contaminated surfaces, or have direct contact with infected individuals. Additionally, this study allowed us to test mule deer among multiple regions in Wyoming, providing evidence that mineral feeder use could be an effective solution among diverse habitats and on land with varying livestock production practices. Since the mineral feeders are designed to prolong the life of livestock mineral resources (Barn World, 2023), and our findings indicated that cattle visitation to these sites remained unchanged with the use of mineral feeders, livestock producers have incentive to use mineral feeders in their cattle operations without expected detrimental effects to livestock production. Through these results, we found that mineral feeder use at livestock mineral licks may be an effective method to reduce potential CWD transmission in mule deer in some circumstances and is in an option that merits further exploration.

It is important to note that all mule deer models were site driven. As seen in Figure 6, mule deer visitation time in treatment sites in the May (Julian days 121-151) and June (Julian days 152-181) was almost completely restricted to visitation time at a single site, while mule deer visitation time among treatment sites during August (Julian days 213 – 243) was divided among a greater number of sites. Similarly, control site data was predominantly from a few sites. This effect maybe be corrected in future studies by including a greater number of sites.

Additionally, all GLMMs showed that there was significantly higher presence of mule deer at control sites, post-implementation of treatment as compared to control sites pre-implementation of treatment (Table 2 and Figure 6). There are several possible explanations of this effect. First, it is possible that this effect is a result of the implementation of treatment, in that, mule deer may have been displaced from sites with mineral feeders to control sites where mineral was more freely accessible. If true, this could have strong disease transmission implications. Specifically, if implementation of treatment causes mule deer to congregate at or more frequently use sites without mineral feeders, we could see increased disease transmission at livestock mineral licks without mineral feeders. However, it's also possible that the higher presence of mule deer at post-impact control sites results from a covariate that we did not record and incorporate in this study. For example, seasonal differences in water availability, proximity to higher elevation areas, or change in livestock production due to cattle rotation could influence where mule deer spend time. Lastly, this effect could be a result of the smaller sample size (three control and three treatment sites for the May and June) that our analysis incorporated in the early time steps.

In order to evaluate the overall effectiveness of mineral feeder use in reduction of potential CWD transmission among elk and moose, further research would need to be conducted. We found mineral feeders to be somewhat effective in excluding elk (74.0%) and, within a small sample size, completely effective in excluding moose (100%), but this study recorded limited data of elk and moose activity over a short timeline, so we were unable to assess changes in visitation time after the deployment of mineral feeders. An additional study evaluating changes in elk and moose visitation time would provide evidence of whether there is reduced opportunity for CWD transmission. The results that access to mineral feeder contents was dependent on species also suggests that differences in species behavior and learning should be considered when assessing management plans to reduce CWD transmission across a landscape.

This study provided insight into possible challenges that should be considered when implementing use of mineral feeders as a CWD transmission management strategy or conducting similar studies. First, livestock producers must have compatible supplemental feeding practices. One livestock producer in our study used a poured molasses-mineral mixture that we had to special order to fit within the mineral feeders. Second, livestock producers need to have sufficient number of mineral feeders. At some sites, livestock producers needed to deploy more mineral than they had anticipated which led to the placement of supplements outside mineral feeders and eliminated the use of these sites during these time periods from our analysis. We recorded this occurring in 6.2% of visits that research team members made to sites. Third, livestock producers must occasionally visit and maintain feeders. During at least two site visits, we observed mineral feeder lids had been slowly unscrewed from the top of the mineral feeder, likely, during cattle use. Mineral feeder lids were pushed into the feeder by cattle or lids were missing in 13.1% of site visits. Fourth, livestock mineral licks would likely need to be accessible by vehicle for mineral feeder use to be feasible. Mineral feeders are large and heavy and may not be feasible to deploy at remote livestock mineral licks where salt and mineral must be packed in. Furthermore, in this study, recording cervid behaviors at sites very frequently visited by cattle resulted in the collection of millions of photos, and only approxiately13.1% of photos contained cervids. This caused photo processing time to be one of the limiting resources within the study and should be considered in future studies of this type.

It remains unclear how much CWD transmission occurs at livestock mineral sites. There is limited data about how often transmission may occur at these sites as a result of direct contact between cervids as compared to contact with contaminated surfaces, such as soil. Furthermore, we do not yet know how interspecies interactions may influence cervid use of these sites and result in changes in disease transmission dynamics. Several avenues of research would provide further insights into the relationships evaluated in this study. As previously mentioned, future studies could evaluate if the use of mineral feeders at some sites increases cervid use of nearby mineral sites without mineral feeders. Other research might explore differences in CWD transmission risk based on size of livestock operations and timing of mineral deployment. We anecdotally observed that, in some cases, cattle would arrive at mineral immediately after it was deployed and remain at sites until it was completely devoured, potentially providing less opportunity for cervids to use these resources and spread CWD. Future research might investigate the longevity of minerals in soil after mineral blocks have been removed, which would provide us with better understanding of the long-term impacts of these sites, including how long cervids may continue to use them. Lastly, some research has been conducted showing that some minerals within soil may cause prions to become more infectious or last longer in soil (Davies & Brown, 2009), but there is little knowledge about how artificial surfaces may change prion infectivity or persistence. Future studies could assess how artificial surfaces, like surfaces of mineral feeders, may influence CWD transmission.

Management Implications

As livestock and wildlife continue to share the landscape, it's important to conservation that we strive to understand how agriculture-driven changes affect wildlife survival and what management techniques might mitigate any adverse effects. CWD is a fatal, highly transmissible disease that can persist in the environment for years (Rivera et al., 2019), and few management strategies exist to mitigate transmission. Strategies that discourage congregation and spatiotemporal overlap of cervids resulting from anthropogenic activities, such as livestock production, have potential to limit CWD transmission by reducing direct and indirect contact among individuals of these species. While livestock mineral licks are used to supplement the diets of livestock, they also attract wildlife, including cervids, and may result in artificially increased use of these sites. We found that the use of mineral feeders during livestock supplemental feeding both reduces mule deer contact with mineral and reduces the amount of time mule deer spend at these sites when present. Our findings indicate that, in some circumstances, mineral feeder use may be a potential strategy for reducing mule deer use and mitigating contact, thereby reducing the risk of CWD transmission among mule deer, and this tool also has the potential to be well-accepted by livestock producers.

Techniques that reduce the influence of livestock production on wildlife behavior could have broader management implications beyond CWD. For example, if mineral feeders prove effective at reducing elk use of and visitation to livestock mineral licks, they could potentially reduce potential transmission of diseases passed between cattle and elk, such as brucellosis.

Photo data collected through this study showed evidence that livestock mineral licks are frequented by other wildlife species, such as pronghorn (*Antilocarpa amaericana*), which are susceptible to fatal pneumonia resulting from *Mycoplasma bovis*, a bacterial pathogen with a livestock reservoir (Malmberg et al., 2020). Investing in the development of tools that decrease artificial congregation and discourage contact between wildlife and cattle could provide strategies for mitigating disease transmission among wildlife and between wildlife and livestock.

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