THESIS

EVALUATING THE ROLE OF NORTH AMERICAN SCAVENGERS IN THE TRANSMISSION OF CHRONIC WASTING DISEASE PRIONS TO CERVIDS

Submitted by

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ABSTRACT

EVALUATING THE ROLE OF NORTH AMERICAN SCAVENGERS IN THE TRANSMISSION OF CHRONIC WASTING DISEASE PRIONS TO CERVIDS

Many scavenging species have been categorized as pests or potential disease vectors, and as a result, management actions toward scavengers are frequently focused on removal. However, scavengers can play important ecological roles, and there is a growing need to define their impact on ecological systems and processes. In the context of disease ecology, their influence is inherently complex, as their interactions at and with carcasses can either mitigate or facilitate the pathogen transmission. Chronic wasting disease (CWD) is a transmissible prion disease afflicting cervids – a taxon that is important to multiple stakeholders. The disease is invariably fatal, and prions persist in the environment for years. Scavengers have been implicated in CWD transmission due to their interactions with infected carcasses, and recent studies have begun to quantify the extent of their involvement.

In Chapter 1, we explored scavenger activities at 20 cervid carcasses in northern Colorado, USA using data gathered from camera traps from January 2022 to May 2023. Specifically, we characterized the species community at cervid carcasses, described carcass utilization patterns, identified landscape factors associated with scavenger diversity at carcass sites, and determined correlates of scavenger foraging duration. Black-billed magpies (*Pica hudsonia*) and coyotes (*Canis latrans*) comprised 63.9% of species detections at carcasses. We found that site-level species diversity decreased as human modifications to the landscape increased and foraging bout duration decreased as carcasses aged and as temperatures increased.

Foraging time at carcasses varied by species, with common ravens (*Corvus corax*) and black bears (*Ursus americanus*) spending the greatest time foraging on carcasses. While observability of carcass tissues consumed was limited, we found that most scavengers consumed muscle (tissue with relatively low prion loads), except for turkey vultures (*Cathartes aura*) who preferred abdominopelvic cavity organs. This chapter underscores the complex interplay between temporal, environmental, and species-specific factors in determining scavengers' role in transmission dynamics, with broader implications for understanding the cascading effects of scavenger activity on ecosystem health.

In Chapter 2, we explored the contributions of coyotes to CWD epidemiology. This species is of particular interest in this system given their ubiquity across North America, proclivity for scavenging from cervid carcasses, and the retention of prion infectivity post-passage by coyotes. To further quantify coyotes' role in CWD ecology, we conducted controlled feeding experiments to examine the fate of three concentrations of CWD prions post-passage through the digestive tracts of eight adult coyotes. We utilized real-time quaking induced conversion (RT-QuIC) to serially determine the relative amount of prions in coyote feces after three levels of exposure: low (0.1% [4 $Log_{10}LD_{50}$/g] CWD positive brain homogenate [BH]), moderate (1% [6 $Log_{10}LD_{50}$/g] CWD positive BH), and high (10% [8 $Log_{10}LD_{50}$/g] CWD positive BH). We found that coyotes are remarkably efficient neutralizers of CWD; we recovered less than 1% of ingested prions from fecal samples across all tested exposure levels. Additionally, we found no evidence of prion seeding activity in the tissues of study animals after necropsy, indicating that coyotes neutralize, rather than sequester, ingested prions. Our findings demonstrate that coyotes provide an important ecosystem service by consuming CWD-positive

tissues, effectively mitigating the environmental accumulation of one of the most environmentally robust pathogens known at carcass sites.

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CHAPTER 1 – SCAVENGER DYNAMICS AND DISEASE TRANSMISSION IMPLICATIONS AT CERVID CARCASSES IN A CHRONIC WASTING DISEASE ENDEMIC ZONE[1]

INTRODUCTION

Carrion is a highly ephemeral, nutrient-dense resource that significantly influences ecosystem patterns and processes and the spatial distribution of species (Heinrich 1988, DeVault et al. 2003, Burkepile et al. 2006, Newsome et al. 2021, Barceló et al. 2022). Carrion is produced through various means, including hunting, predation, starvation, and vehicle strikes (Moleón et al. 2019). Infectious diseases are another primary source of mortality for wildlife and can cause carcasses to be transmission hotspots (Wilson and Wolkovich 2011, Le Sage et al. 2019, Vicente and VerCauteren 2019, Rietz et al. 2024). Indirect transmission potential is particularly relevant for long-lived pathogens, the nutrient influx from decomposing carcasses can stimulate vegetation growth, attracting herbivores long after decomposition, thus facilitating the spread of environmentally persistent diseases like anthrax and African Swine Fever (Bump et al. 2009, Ganz et al. 2014, Turner et al. 2014, Fischer et al. 2020, Newsome et al. 2021, Zheng et al. 2023).

The role of scavenging species in disease transmission is complex. Their interactions at carcasses can either mitigate or facilitate pathogen spread depending on the disease system, scavenger assemblage, and species' unique ecology (Ćirović et al. 2016, Le Sage et al. 2019, Szcodronski and Cross 2021, Kemenszky et al. 2022). Changes in scavenging communities can elevate the risk of pathogen transmission, particularly if dominant scavengers are absent (Ogada

1 Bye. K.C., K.C. VerCauteren, and G. Wittemyer. 2025. Scavenger Dynamics and Disease Transmission Implications at Cervid Carcasses in a Chronic Wasting Disease Endemic Zone. Journal of Wildlife Management. *In review*.

et al. 2012). Prolonged feeding on carcasses, particularly on tissues with high pathogen loads, can further increase exposure risks for scavengers (Williams and Miller 2002, Xavier et al. 2009, Poester et al. 2013, Szcodronski and Cross 2021). Moreover, many pathogens capable of being transmitted at carcasses are typically ingested by scavengers directly rather than needing to exit the host (Vicente and VerCauteren 2019, Newsome et al. 2021). Collating information on species assemblages using carcasses, their relative consumptive rates of carcasses, and the parts of carcasses ingested is crucial to diagnosing factors influencing pathogen transmission (Beasley et al. 2015, Vicente and VerCauteren 2019).

Cervids are abundant throughout much of North America and are an ecologically, culturally, and economically valuable taxon (Bishop 2004, Komppula and Gartner 2013, Cavaliere and Viscidy 2018). In this region, the family includes mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), elk (*Cervus canadensis*), moose (*Alces alces*), and caribou (*Rangifer tarandus*). Cervids provide significant recreational enjoyment through wildlife watching, hunting, and tourism and serve as a source of commercial and personal meat procurement (Hewitt 2011, Carrasco-Garcia et al. 2018). However, they also present sources of conflict due to human-wildlife interactions, including crop damage and vehicle collisions (Bissonette et al. 2008, Walter et al. 2010). Furthermore, cervids can carry several diseases capable of being transmitted through interactions with infected carcasses, including bovine tuberculosis, tularemia, anthrax, and chronic wasting disease (CWD) (Miller et al. 2004, Turner et al. 2012, Gürcan 2014, Devi et al. 2021).

Chronic wasting disease is a wildlife disease of particular concern for stakeholders. First identified in captive deer in Colorado, USA, in 1967, it was later detected in free-ranging deer and elk in Wyoming and Nebraska and is now widespread across North America (Williams and

Young 1980, Miller et al. 2000, Richards 2024). This disease is invariably fatal and has caused significant deer population declines in high-prevalence areas, impacting resilience and long-term demographics (Williams and Miller 2002, Miller et al. 2004, Almberg et al. 2011, Edmunds et al. 2016, DeVivo et al. 2018). Chronic wasting disease prions are highly resistant to degradation, with long-term persistence in the environment which poses risks to naïve hosts over time (Miller et al. 2004). While direct transmission mechanisms of CWD, such as deer-to-deer contact, are well understood, elements of indirect environmental transmission remain unclear (Miller and Williams 2003). Studies have increasingly focused on the role of scavenging species in CWD transmission but have not investigated the interplay between temporal, landscape, and species-specific factors at cervid carcasses that could influence transmission dynamics (Jennelle et al. 2009a, Jennelle et al. 2009b, VerCauteren et al. 2012, Fischer et al. 2013, Nichols et al. 2015, Baune et al. 2021).

Here, we explored scavenger activities at cervid carcasses in northern Colorado, USA, within the broader context of CWD and disease transmission. Our objectives were to: (1) characterize species assemblages utilizing cervid carcasses, (2) identify landscape factors associated with scavenger diversity at carcass sites, (3) describe species' tissue foraging preferences, and (4) assess the correlates of foraging bout duration. We discuss the implication of these results in relation to the possible disease transmission processes at carcasses.

METHODS

Study Area

The South Platte watershed defined our study area in northern Colorado, USA, specifically focusing on the Cache de la Poudre, Boulder Creek, Big Thompson, and Saint Vrain

tributaries of the South Platte River (Figure 1.1). National landcover database (NLCD) 2019 habitat types in our study area include woody wetlands, emergent herbaceous wetlands, cultivated crops, and grassland or herbaceous vegetation (Dewitz and US Geological Survey, 2024). Agricultural use and varied human disturbance and development intensities further characterize this region. Additionally, this region is the endemic epicenter for CWD, and prevalence varies from less than 5% to greater than 20% (in hunter harvested bucks from 2017-2022) across Colorado Parks and Wildlife's (CPW) management units (Williams and Young 1980, Prenzlow 2022).

Carcass Acquisition

From January 2022 to May 2023, twenty carcasses, within two days of death, were acquired opportunistically as road-killed or hunter-killed animals. We placed carcasses on properties managed by Larimer County, Boulder County, CPW, and Colorado Department of Transportation (CDOT) or on private land with landowner permission. Exact placement locations were determined through coordination with the respective management bodies.

Camera Trap Data Collection

Two remote camera traps (RECONYX PC800, RECONYX, Inc., Holmen, WI, USA) were deployed per carcass. Cameras were mounted at a height of 1-1.5 meters on a combination of t-posts and opportunistically located trees to maximize the observability of the focal carcass. The hind-view camera was placed 2-2.5 meters from the caudal end of the carcass and aimed inwards towards the abdominal cavity of the carcass to capture a more detailed view of what tissues species might be consuming. The front-view camera was placed 3-3.5 meters from the cranial end of the carcass, adjacent to the sternum, and aimed to capture the entirety of the

carcass and the surrounding site. Cameras were programmed to take a burst of 5 images at a shutter speed of 1/1000 (RapidFire™ mode) with no cool-down period. To minimize habituation of carcass visitors and maintain site independence, we ensured a minimum distance of one kilometer between active sites and implemented a six-week buffer between repeat use of the same location (Allen et al. 2014, Inagaki et al. 2020, Enari and Enari 2021). Carcasses were staked down with 10-inch-long tent stakes connected with a length of braided wire to reduce the likelihood of scavengers moving carcasses out of the field of view of cameras (Allen et al. 2014, Inagaki et al. 2020). Cameras were checked weekly to replace memory cards and batteries. Monitoring continued until the carcass was removed from the camera's field of view or reduced to bones and hide.

Image Processing

For each image captured by the front-view camera, we identified the species present, recorded the number of individuals per species, extracted the date and time associated with each image, and identified the stage of carcass decomposition (Figure S1.1). We also documented time to initial carcass discovery, time to first forage, and lag time between discovery and first forage. Tagged images from the front-view camera were also used to assess correlates of species diversity. For images captured by the hind-view camera, we recorded consumption events per species (Figure S1.2). Tagged images from the hind-view camera were then used to investigate species foraging preferences and examine factors associated with foraging duration. All image data recording was completed in Timelapse Image Analyzer (v2.2.6.0; Greenburg et al. 2019). Data cleaning and analysis were executed in R (v4.3.2; R Core Team, 2023).

Species Detections, Interactions, and Shannon's Diversity Index

We defined species detection as a camera trigger event containing evidence of wildlife presence at the carcass, with a 30-minute interval distinguishing separate detection events (O'Brien et al. 2003, Kelly and Holub 2008, Wang et al. 2015). Detections were recorded per species until the carcass was removed from the frame, only bones and hide were left, or for the first thirty days a carcass was active, whichever came first. Camera trap nights were calculated as the total number of days that all carcasses were actively monitored. We then used the number of detections per species to calculate site-level Shannon's diversity index with the "vegan" package (Oksanen et al. 2024).

We classified the carcasses into four states based on the degree of tissue accessibility to scavengers: intact, partially open, open, and removed or remnants (Figure S1.1), and recorded the species responsible for facilitating the transition from one carcass state to the next. For each carcass state, we calculated the percentage of species detections and detections across all states. Additionally, we documented interactions between domestic cattle (*Bos taurus*), mule deer, white-tailed deer, elk, domestic dogs (*Canis lupus familiaris*), and carcasses that could lead to disease transmission.

We then fit a series of Generalized Linear Models (GLM) (Gamma family; log-link function) with site-level Shannon's diversity index values as the response variable and site-level characteristics as covariates. Fixed effects include distance to water, distance to road, aspect, roughness, canopy cover, NLCD habitat classifications, global human modification index (GHM), average temperature, coefficient of variation of temperature, and season (Table S1.1). To gather these landscape covariates we used the "terra" (Hijmans 2024), "sf" (Pebesma 2018, Pebesma and Bivand 2023), and "sp" (Pebesma and Bivand 2005, Bivand et al. 2023) packages. We tested the correlations between our continuous covariates in a Spearman correlation matrix

and evaluated the log-likelihood values of single models for the correlated coefficients to decide which covariates to include. We then grouped those covariates into the following sub-models: anthropogenic (GHM and distance to road), abiotic (coefficient of variation for temperature and season), habitat (NLCD and canopy cover), and landscape (distance to water and aspect). We guarded against multicollinearity by ensuring that variance inflation factors were less than five for the sub-models that included categorical covariates (Dormann et al. 2013). We used Akaike Information Criteria for small sample sizes (AICc) for model selection within each sub-model, using an a priori cutoff of two for the delta AICc value (Burnham and Anderson 2004). If the null model was the top model, we concluded the covariates in the sub-models were not informative.

Foraging Behavior and Bout Duration at Carcasses

We defined foraging bouts as sequential images of a species where one or more individuals were observed foraging from the focal carcass. We compared the start and end times of each sequence to determine the length of that foraging bout and used a 10-second interval where all individuals of a species were observed not foraging to differentiate unique foraging bouts. We then calculated the average and standard deviation for foraging bout durations in seconds. We assessed species foraging bouts, rather than focusing on specific individuals of a species, because many of the species in our study area are not individually distinguishable. When the tissue type consumed was identifiable, we determined the frequency of foraging records for each tissue by each species and within each body cavity for species observed consuming organs (Figure S1.2).

Variations in the duration of foraging bouts relative to carcass age, species, and temperature were investigated for species with greater than ten observations. We fit a series of

additive Generalized Linear Mixed Models (GLMM; Gamma family; log-link function) with foraging bout duration in seconds as the response variable. Fixed effects included days since carcass placement, species, and temperature. As species was a categorical covariate, we used the "emmeans" package to compute estimated marginal means of foraging bout duration for each species and performed pairwise contrasts using the Tukey method for multiple comparisons (Tukey 1949, Lenth 2024). We included carcass site as a random effect to account for the repeated foraging bouts at each carcass. At the start of each foraging bout, we derived temperature from NLDAS-2 using the "terra" package (Hijmans 2024). We used AICc selection criteria ($\Delta AICc \leq 2$) to evaluate our candidate model set (Burnham and Anderson 2004).

Model Coding and Fit

The models for site-level species diversity and foraging bout duration were coded and fit in R (Version 4.1.2; R Core Team 2021) using the "AICcmodavg", "lme4", "lmerTest," and "emmeans" packages (Kuznetsova et al. 2017, Mazerolle 2023, Bates et al. 2024, Lenth 2024). Numerical covariates were centered and scaled (mean of 0, standard deviation of 1) before fitting the models.

RESULTS

Species Detections and Interactions

From the 20 carcasses monitored by cameras, we recorded 1,495 detections of 10 avian and 17 mammal species (Figure 1.2; Table S1.2). Black-billed magpies (*Pica hudsonia*; hereafter referred to as magpies) were the most frequently detected avian species, accounting for 45.7% of detections (n = 688). Magpies also discovered carcasses most frequently (n = 7), were the most

frequent species to first feed from carcasses (n = 9) and were the fastest species to discover carcasses (avg. of 0.39 days; Table S1.3). The next most detected avian species at carcasses were turkey vultures (*Cathartes aura)*, accounting for 9.8% of detections (n = 148). This species discovered fewer carcasses than magpies (n = 2) and it took them longer on average to do so (0.89 days). Coyotes (*Canis latrans)* were the most frequently detected mammal at carcasses, comprising 18.3% of detections (n = 275) and discovered four of our twenty carcasses, taking an average of 3.81 days to do so. Red foxes (*Vulpes vulpes*) were the second most detected mammalian species at carcasses (4.9%, n = 73). This species discovered fewer carcasses than coyotes (n=1) but were quick to do so (0.29 days).

We observed the following carcass state transitions facilitated by the species in Table 1.3: "intact to partially open," "partially open to open," and "intact to open." Open carcasses were the most frequently visited by species, accounting for 72.6% of detections (n = 1,085) and having the greatest variety of species detections (n = 14). The more intact a carcass was, the fewer the detections (partially open, 162 detections; intact, 130 detections), aside from when the carcass was removed prematurely or if only remnants remained (107 detections). Smaller species, such as magpies and striped skunks (*Mephitis mephitis*), most frequently engaged with open carcasses (Figure 1.3). Coyotes, domestic dogs, mule deer, and turkey vultures visited carcasses regardless of carcass condition (Figure 1.3).

We documented 560 interactions between elk, mule deer, white-tailed deer, domestic cows, domestic dogs, and carcasses with disease transmission potential (Figure 1.4). Domestic cows accounted for most of these observed interactions (n = 442) and this species nosed different parts of the carcasses. Domestic dogs (n = 100) interacted with the carcasses in the most varied ways, the most notable being rolling on the carcasses or in carcass remnants and chewing on

carcass remnants (Figure 1.4A). Even though elk, mule deer, and white-tail deer had the fewest interactions with carcasses (n = 18), they were the only taxa we observed foraging near carcasses and nosing remnants of conspecifics (Figure 1.4B). Detailed interactions for domestic cattle and domestic dogs can be found in the appendix (Figure S1.3).

Shannon's Species Diversity Index Model

We monitored carcasses for 293 trap nights to analyze landscape drivers of species diversity at carcasses. The null model was the top model for the abiotic, habitat, and landscape sub-models (Table S1.4). For the anthropogenic sub-model, the top model only included the GHM covariate (Table S1.4). GHM had a statistically significant effect on site-level species diversity (t-statistic = -2.88, p-value = 0.01), and with each unit increase in GHM, site-level species diversity declined by 21.3% (Figure 1.5).

Foraging Behavior at Carcasses

From the 19 carcasses monitored by cameras, we recorded a total of 5352 foraging events over a total of 170 trap nights (Table 1.1). We had an additional carcass where there were no observed foraging events within the first ten days after placement, although species did investigate the carcass. As a result, we excluded it from the foraging behavior analyses. The average foraging event duration was approximately 21 seconds (Table 1.1). Magpies accounted for the greatest number of foraging events (n = 3122), with an average duration of approximately 14 seconds. Common ravens (*Corvus corax*) had the avian scavengers' longest average foraging duration (≈43 seconds) and the longest foraging duration across functional groups. In contrast, the mammalian scavenger with the longest average foraging duration was black bears at ≈31 seconds.

In most observations, the type of material consumed was not identifiable (~92%). Of the confirmed types, muscle tissue was most frequently consumed (~4%), followed by bones and hide (~2%) and organs (~2%) (Table 1.2). Common ravens and black bears predominantly consumed muscle, while red foxes and coyotes favored bones or hide. Turkey vultures and magpies mainly ate organ tissues, with domestic dogs exclusively consuming organs when identified. Turkey vultures and magpies mainly targeted organs from the abdominopelvic cavity, black bears preferred thoracic cavity organs, and coyotes and red foxes showed balanced consumption across both cavities (Table S1.5). Bald eagles (*Haliaeetus leucocephalus*) and golden eagles (*Aquila chrysaetos*) exclusively focused on organs from the abdominopelvic cavity.

Foraging Bout Duration Model

The global model was the best model for foraging duration, which included days since placement, species, and temperature as predictors and carcass site as random intercept (Table S1.6). Parameter estimates suggest that with each additional day since carcass placement (p-value < 0.001), and as temperatures increase (p-value < 0.001), there is a multiplicative decrease in foraging duration of 10.13% and 25.09% respectively (Figure 1.6; Table S1.7). Several species demonstrated significant differences in their time foraging at carcasses when adjusting for days since carcass placement and temperature (Table S1.8). Magpies foraged for significantly less time than common ravens (ratio = 0.42, p-value < 0.001), turkey vultures (ratio = 0.38, p-value < 0.001), and golden eagles (ratio = 0.51, p-value < 0.001). Black bears foraged 2.83 times longer than red foxes (p-value < 0.001) but did not demonstrate a significantly different foraging bout duration from coyotes (p-value = 0.05). Coyotes foraged for 3.07 times longer than magpies (p-

value < 0.001), but did not show a significant difference in their mean foraging bout duration from any of the other species considered in this analysis (Table S1.8).

DISCUSSION

Our study elucidated the dynamics of scavenger assemblages and the utilization patterns of cervid carcasses in northern Colorado, USA. Birds, due to aerial locomotion, exhibit higher efficiency in locating carcasses than many terrestrial scavengers (DeVault et al. 2003, Ruxton and Houston 2004, Cortés-Avizanda et al. 2009). Our findings reflected this, as we documented significant avian presence at carcasses (63.9% of detections) compared to mammals (36.1% of detections). Within the avian group, magpies approached carcasses faster, were detected more frequently, and foraged more frequently from study carcasses despite spending less time foraging than other species. Magpies will observe conspecifics and other scavengers to learn about potential food resources and will often wait in the vicinity of a resource for an opportunity to feed when a dominant species is present (McKinstry and Knight 1993, Trost 2020). Their near-ubiquitous presence at our carcasses could be attributed to these behaviors. Turkey vultures in northern Colorado, USA, migrate seasonally, and literature on vulture declines could have implications for disease transmission in our system when turkey vultures are absent. Vulture declines led to changes in scavenger community dynamics and increased interactions between mammalian scavengers, raising concerns about heightened transmission of diseases such as rabies, distemper, and anthrax (Mudur 2001, Markandya et al. 2008, Ogada et al. 2012).

As facultative scavengers, black bears demonstrated the longest foraging bout durations among mammalian scavengers, thereby presumably limiting carrion availability to others when not in hibernation. Other studies have documented frequent kleptoparasitism of mountain lion

kills and ungulate carrion biomass loss to black bears (Murphy et al. 1998, Allen et al. 2015, Walker et al. 2021). However, coyotes and red foxes foraged more frequently and were present at more carcasses than black bears in our study area. Canids may emerge as the dominant mammalian scavengers of cervid carcasses in regions with few or no black bears, or when black bears are in hibernation, due to their generalist nature and efficiency at locating carcasses within different parts of their home ranges (Hein and Andelt 1996, Wilmers et al. 2003, Bassi et al. 2018). The role of different scavenger assemblages, behavioral hierarchies, and temporal dynamics in the transmission of CWD and other diseases is an emerging area that requires further exploration.

Scavenger foraging behaviors and preferences can also shape their influence on disease transmission. Canids often disarticulate and carry carcass pieces away from the carcass site (Haglund et al. 1989, Cáceres and Nadal 2009, Young et al. 2015, Rietz et al. 2024). Corvids will also remove material from carcass sites, caching these items and returning to them generally within 1-2 days for magpies and up to two weeks for common ravens (Birkhead 1991, Boarman and Heinrich 2020, Trost 2020). If tissues contain infectious agents, canids and corvids likely play a larger role in disseminating or diluting pathogens on the landscape. Additionally, magpies were documented consuming necrophagous fly maggots from carcasses, while coyotes were observed consuming adult flies from carcasses in a different study (Root 1988, Trost 2020, Mason 2023). While these behaviors were not directly observed during our study, some consumption of invertebrate scavengers likely occurred, especially during the summer months.

Of carcass tissues that could be identified in images, most scavengers were observed consuming muscle tissue. Turkey vultures consumed nutrient-dense organ tissue more often other tissue types, consistent with their morphology (e.g., weaker and thinner beaks compared to

other vulture species) (Houston 1975, Linde-Medina et al. 2021, Steinfield et al. 2024). However, they demonstrated the ability to open intact carcasses and facilitate carcass state transitions in our study. Other research demonstrated raptors and large- and medium-bodied mammals efficiently opening carcasses, subsequently allowing smaller scavengers access to resources (Selva et al. 2005, Selva and Fortuna 2007, Blázquez et al. 2009, Allen et al. 2014, Mateo-Tomás et al. 2015, Elbroch et al. 2017). This process likely accelerates pathogen dissemination or sequestration depending on the disease.

Species that visit cervid carcasses are not only limited to ones that forage at them (Stiegler et al. 2020, Szcodronski and Cross 2021, McTee and Stone 2022). We observed domestic cows, mule deer, elk, and white-tailed deer investigating and making direct contact with our carcasses throughout the decomposition stages. These interactions were neither consumptive nor driven by mineral acquisition, as most herbivore osteophagy occurs long after the decomposition of soft tissues (Figure 4B, Figure S1.3B; Denton et al. 1986, Cáceres et al. 2011). While uncommon in our study, these observations are particularly concerning for CWD transmission. Direct contact between live cervids and contaminated carcasses could allow the disease to spread within the cervid population (Miller et al. 2004). Additionally, these interactions contradict the "landscape of disgust" hypothesis, where species avoid contexts with high potential for parasite exposure (Weinstein et al. 2018, Moleón and Sánchez-Zapata 2021).

Our findings highlight the significant role of human-modified habitats on scavenger communities, consistent with studies documenting human impacts on wildlife community composition (Trombulak and Frissel 2000, Erb et al. 2012, Sebastián-González et al. 2019, Lewis et al. 2021). Landscape changes captured in the GHM Index negatively affected species diversity at carcasses in our study, potentially leading to prolonged carcass persistence (Kennedy

et al. 2018, Wenting et al. 2022). Longer carcass persistence times associated with increases in anthropogenic activities could alter disease transmission dynamics (Beasley et al. 2015, Vicente and VerCauteren 2019). Additionally, domestic dogs were present at almost half of the carcasses (n = 9), and their interactions with cervid carcasses highlight mechanisms for human exposure to pathogens in carcasses (Figure 1.4A).

We found that foraging bout duration was affected by the species foraging, the age of the carcass, and average ambient temperature. Consistent with the rest of our findings and their relative body sizes, magpies foraged for significantly less time than larger avian species (i.e. golden eagles and turkey vultures), and black bears foraged for substantially longer time than red foxes and domestic dogs (Hutchinson 1959, Hutchinson and MacArthur 1959, Ernest 2005). Foraging bout duration across species decreased the longer a carcass was available on the landscape. Older carcasses may present scavengers with higher risks than fresh carcasses as noxious chemicals accumulate from microbial activity (Burkepile et al. 2006, Beasley et al. 2015, Olson et al. 2022). Seasonality was not a competitive predictor, as opposed to findings in other studies, possibly due to the low number (n = 3) of carcasses monitored during colder months in our research (DeVault and Rhodes 2002, Beasley et al. 2015, Turner et al. 2017). However, the daily average ambient temperature at carcass sites was influential. Lower temperatures reduce invertebrate, bacterial, and fungal activity at carcasses, thereby reducing competition for vertebrate scavengers and potentially allowing them to forage for longer periods (DeVault et al. 2003, Wikenros et al. 2013, Gomo et al. 2017).

Examining vertebrate species assemblages on carcasses, their consumption rates, and the tissues they preferentially consume is a critical first step in elucidating factors that influence pathogen transmission and sequestration. The species that most frequently forage from carcasses

likely have a more prominent role in disease transmission and sequestration than those that rarely scavenge (Vicente and VerCauteren 2019). Our findings suggest that magpies and coyotes – together comprising 63.9% of detections – have outsized potential for such roles in northern Colorado, USA due to the frequency with which they interact with cervid carcasses. Notably, studies have demonstrated that these taxa could serve as vectors for CWD transmission, as prions were detected in their feces after ingestion (VerCauteren et al. 2012, Fischer et al. 2013, Nichols et al. 2015). Other scavengers, such as large mammals (e.g., bears), birds (e.g., raptors) and invertebrates (e.g. blow flies and beetles), may also play significant roles in disseminating or mitigating long-lived pathogens like CWD prions.

RESEARCH IMPLICATIONS

Our study underscores the complex interplay between temporal, environmental, and species-specific factors in determining scavengers' role in the transmission of CWD and other diseases, with broader implications for understanding the cascading effects of scavenger activities on ecosystem health. Prevalent, long-lived wildlife diseases would benefit from research into how species' consumptive processes contribute to both *in situ* and *in vivo* pathogen transmission. Video-capable trail cameras may be better suited for research examining the tissues scavengers consume from carcasses as more information can be gleaned from a continuous clip, rather than the single image or rapid-fire burst of images we used. Developing assays capable of determining relative pathogen loads in different carcass tissues, soil samples, and invertebrates is vital for elucidating the transmission risks present at carcasses. Furthermore, understanding the roles of different scavenger species, the impact of anthropogenic changes, and seasonal

variations on the epidemiology of different wildlife diseases are essential for developing novel and effective management strategies.

ETHICS STATEMENT

Collection of mule deer, elk, and white-tailed deer were permitted under CPW Scientific Collection Permits (License 2645331641 and 2323199732).

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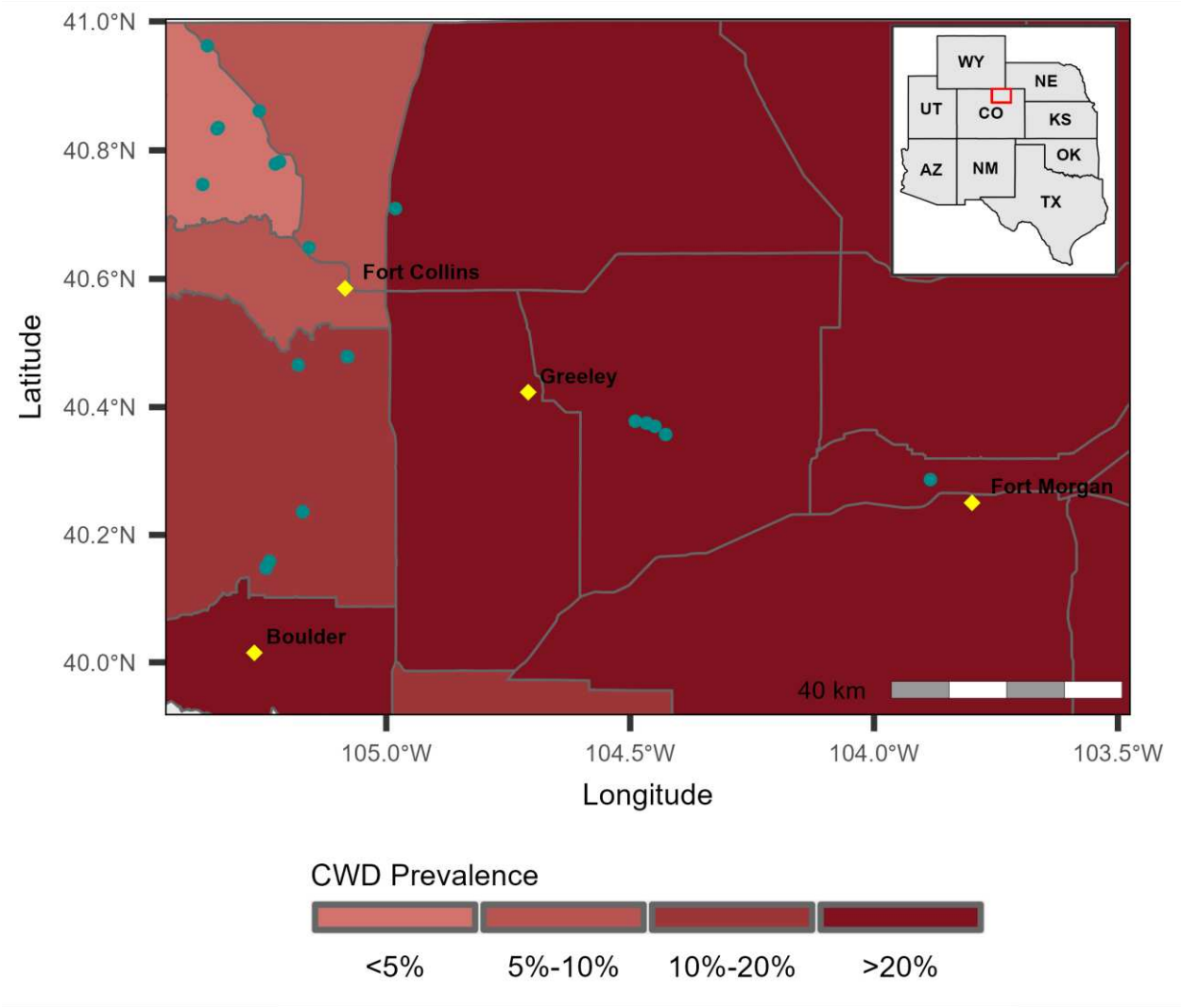

Figure 1.1 Map depicting the prevalence of chronic wasting disease (CWD) in hunter-harvested, adult male deer from 2017 to 2022 within the study area (Prenzlow, 2022). The prevalence data is shown across CPW's Game Management Units (GMUs), which are the boundaries dividing the sections of the map. Teal circles represent the locations of cervid carcasses (n = 20) monitored throughout the study period (January 2022 to May 2023*)*. Most monitored carcasses (n = 13) were located in GMUs with less than 10% CWD prevalence. The map was created using data from the "usmap" (Lorenzo, 2024a) and "usmapdata" (Lorenzo, 2024b) R packages.

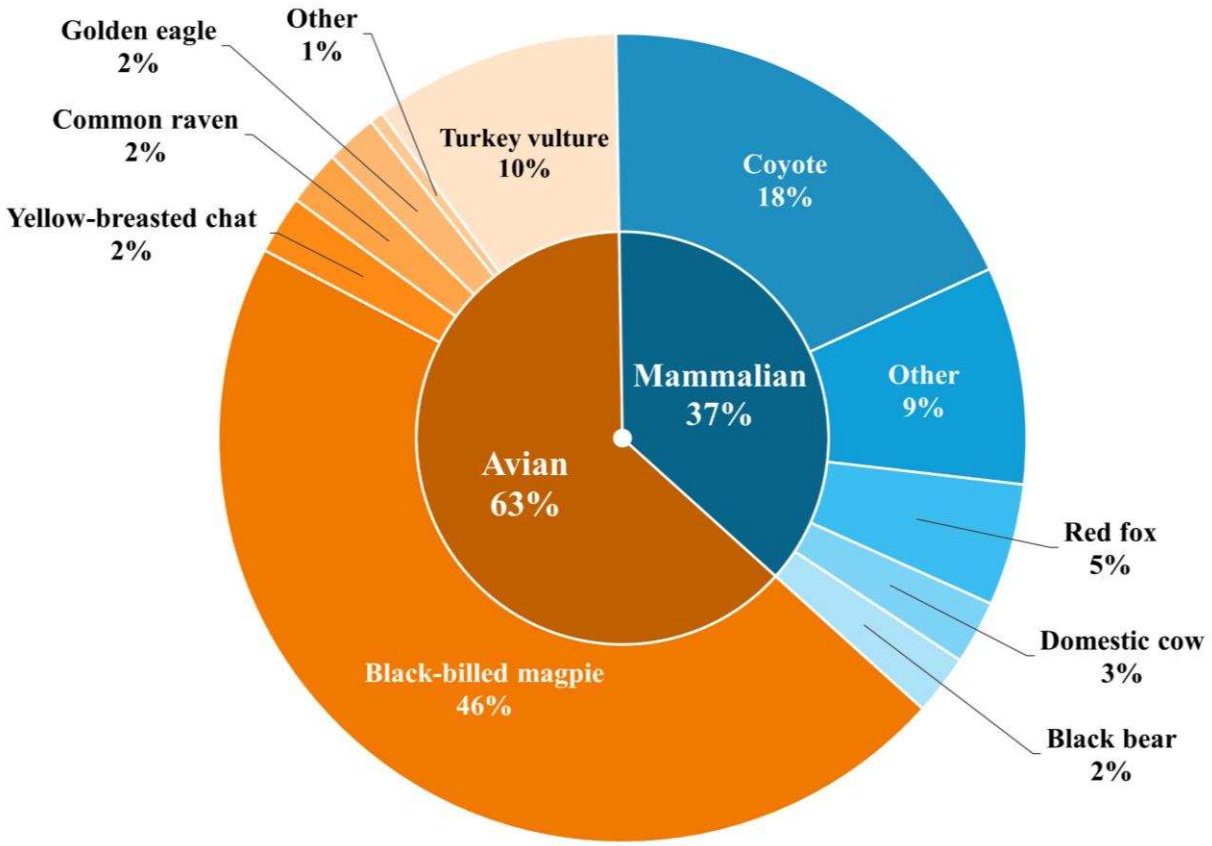

Figure 1.2. Identified species detections at cervid carcass sites (n = 20) in northern Colorado, USA, from January 2022 to May 2023. The "other" category for both functional groups is comprised of species representing less than 2% of detections. For a more detailed look at species detections, including the breakdown of "other" species detections, see Table S1.2.

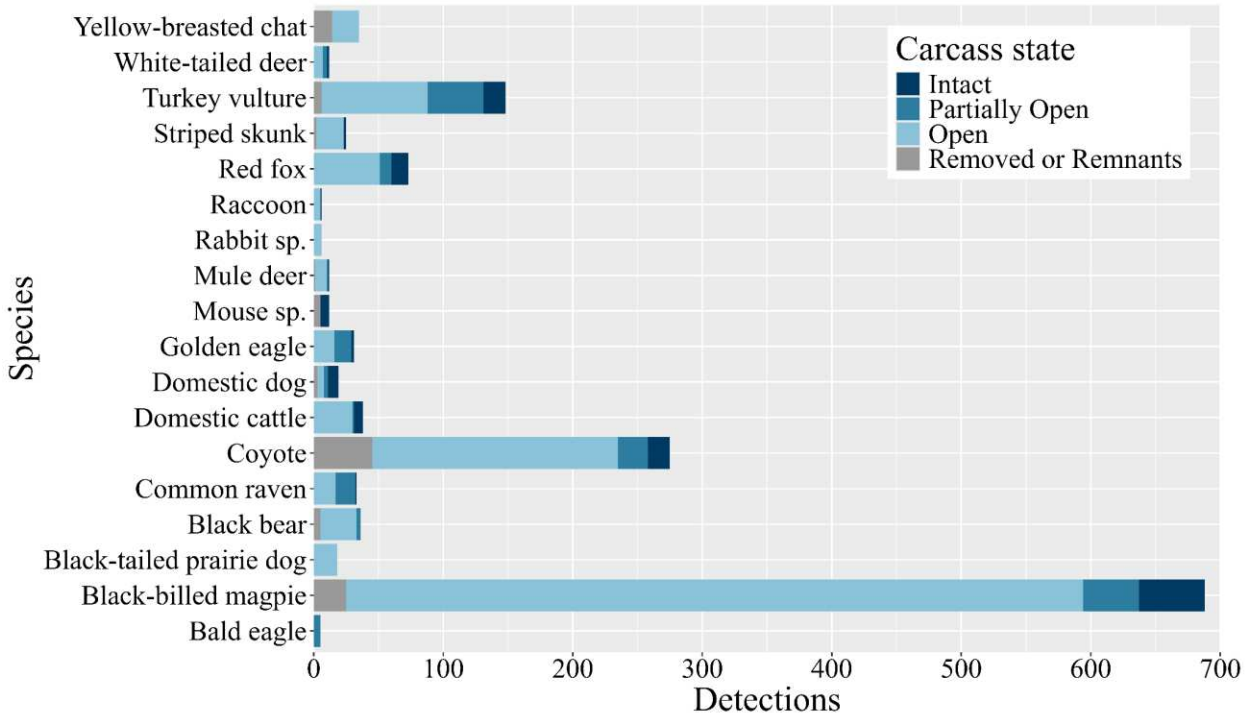

Figure 1.3. Carcass state detections for identified species with five or more detections across all monitored carcasses (n = 20) for identified species in northern Colorado, USA, from January 2022 to May 2023. Species with fewer than 5 detections include: mountain lions (3 detections), domestic horses (2 detections), elk (2 detections), domestic cats (1 detection), grackles (1 detection), red-tailed hawks (1 detection), spotted towhees (1 detection), and wild turkeys (1 detection). Intact carcasses had 130 detections, partially open carcasses had 162 detections, open carcasses had 1085 detections and removed carcasses or remnants of carcasses had 107 detections.

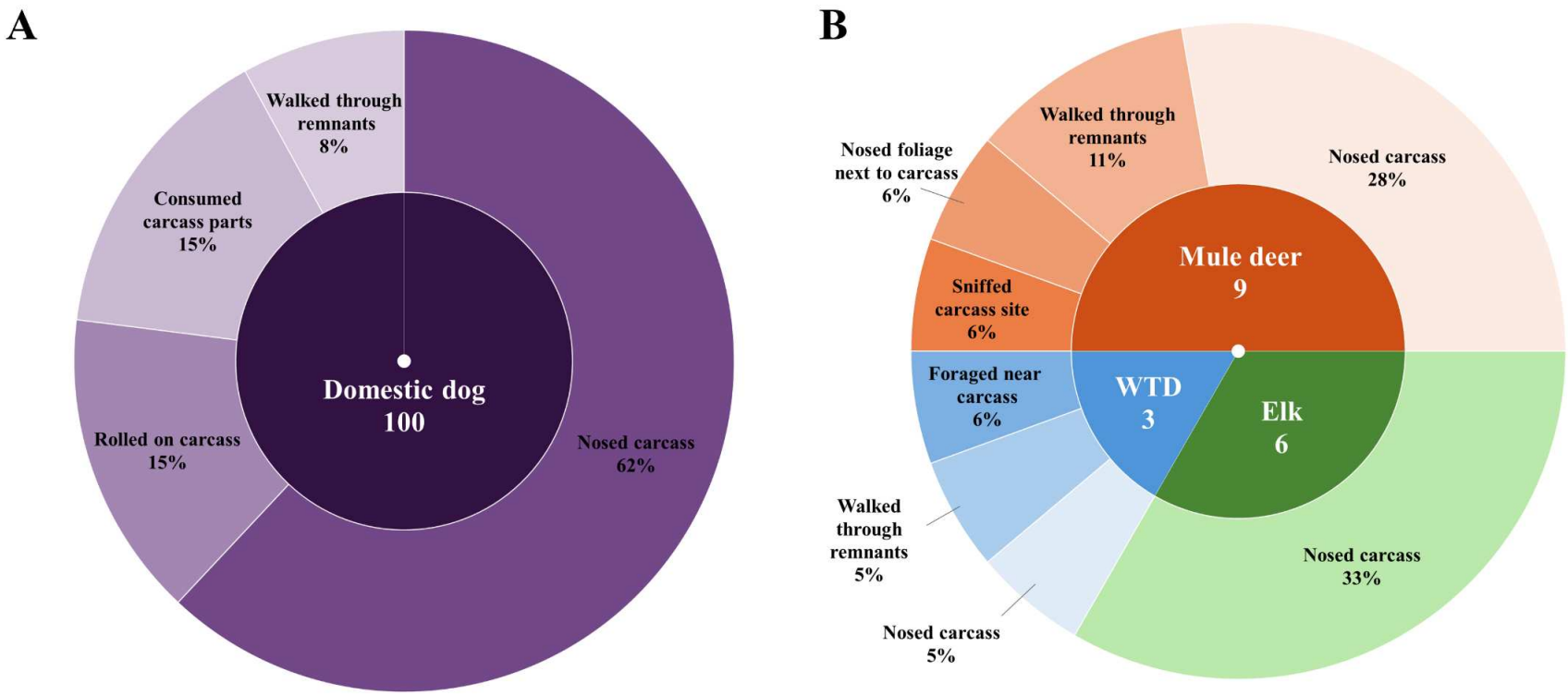

Figure 1.4. Observed interactions with disease transmission potential for species of interest (n = 541) in northern Colorado, USA, from January 2022 to May 2023. Domestic cattle interacted with cervid carcasses the most (n = 442) and only nosed different parts of the carcasses. **A)** We observed a total of 100 interactions with disease transmission potential for domestic dogs. **B)** We documented 18 interactions with disease transmission potential for wild cervids. WTD stands for white-tailed deer.

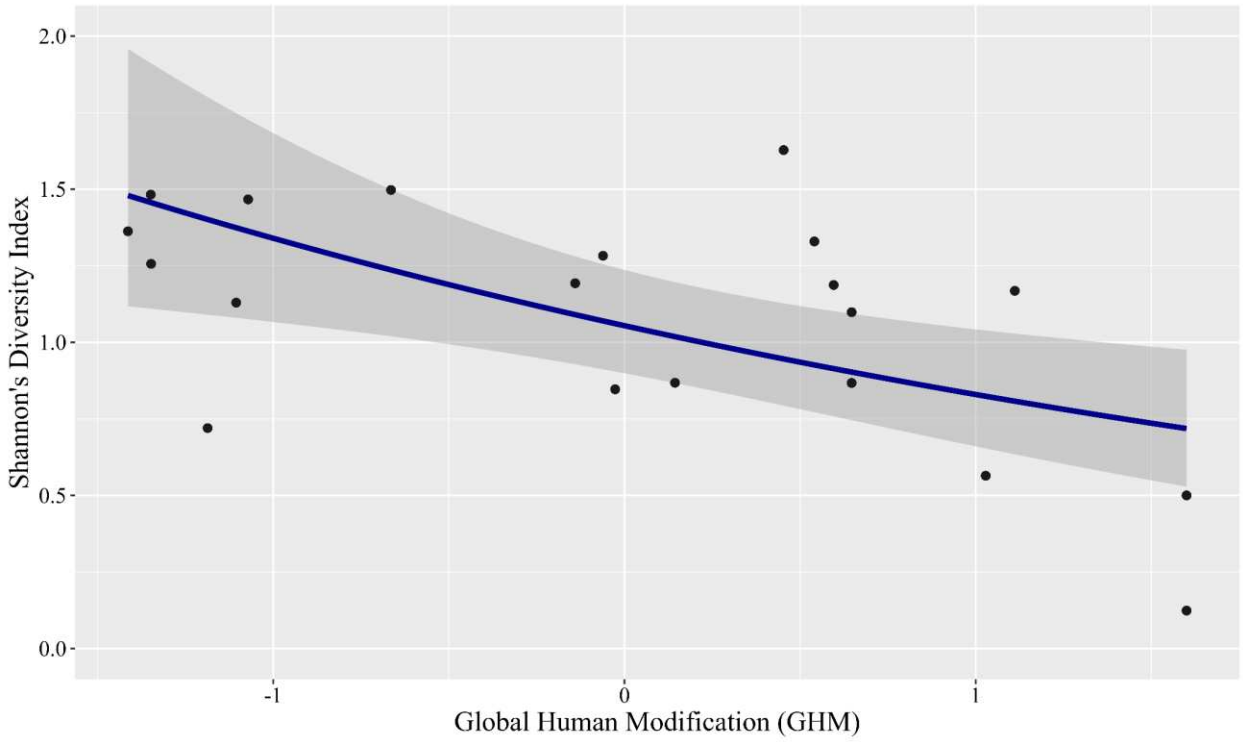

Figure 1.5. Exponentiated coefficient estimate and 95% confidence interval for the fixed predictor of the top site-level species diversity model (Gamma family; log-link function) (Akaike Information Criteria (AIC_c) = 28.36, weight = 0.13). Global human modification (GHM) index had a statistically significant effect on site-level species diversity (t-statistic = -2.88, p-value = 0.01). With each one-unit increase in GHM, there is a 21.3% decrease in site-level species diversity when adjusting for all other predictors in northern Colorado, USA from January 2022 to May 2023.

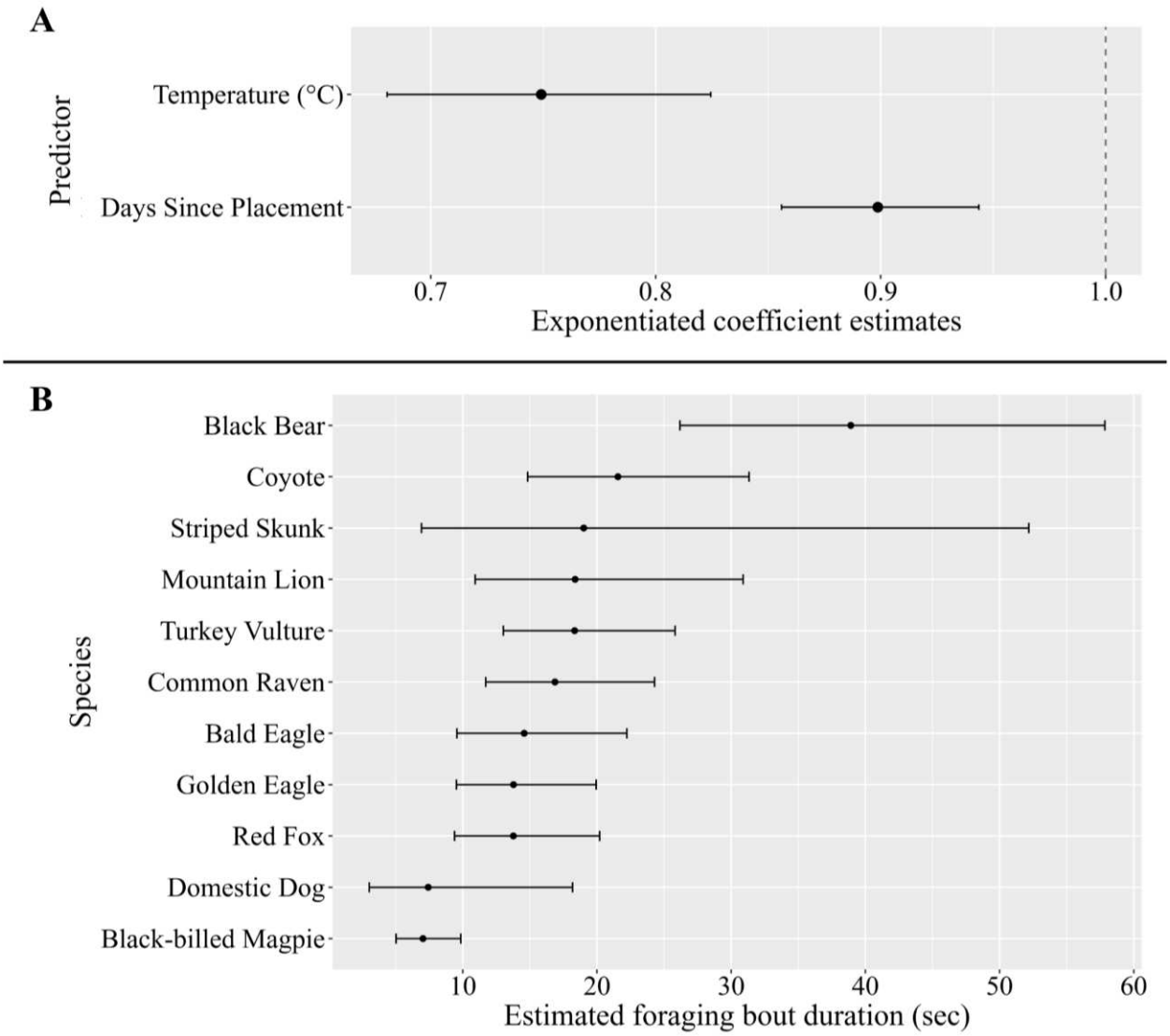

Figure 1.6. The top foraging bout duration model (Gamma family, log-link function) for studied carcasses in northern Colorado, USA, from January 2022 to May 2023 A) The continuous predictors days since placement (chi-square = 18.43, p-value < 0.001), and average temperature (chi-square = 34.87, p-value < 0.001) had significant effects on foraging bout duration. . B) The categorical predictor – *species* – also had significant effects on foraging bout duration (chi-square = 558.57, p-value < 0.001). We used the "emmeans" package to calculate the estimated marginal means for this predictor. Additional details of the fixed effect summary for this model and pairwise contrasts for Species can be found in the appendix (Table S1.7, Table S1.8).

Table 1.1. Observed species-level foraging bouts and associated durations at cervid carcasses (n = 19) in northern Colorado, USA over 170 trap nights from January 2022 to May 2023. We had an additional carcass where there were no observed foraging events within the first ten days after placement, although species did investigate the carcass. As a result, it was excluded from the foraging behavior analyses.

Species	Number	Average Duration (sec)	St. Dev. Duration (sec)
Black-billed magpie	2405	13.9	26.4
Turkey vulture	1485	32.6	80.3
Common raven	399	43.1	97.5
Golden eagle	324	31.6	54.0
Coyote	233	18.1	28.9
Red fox	173	19.2	25.5
Black bear	141	31.3	40.7
Bald eagle	111	33.1	44.4
Mountain lion	53	12.7	12.5
Domestic dog	10	9.1	9.6
Striped skunk	10	7.1	5.5
Mouse (*Peromyscus* sp.)	4	2.0	0.8
Red-tailed hawk	2	3.5	2.1
Domestic cattle	1	1.0	—
Rough-legged hawk	1	1.0	—

Table 1.2. Recorded carcass parts consumed by scavenging species over 170 trap nights at carcasses (n = 19) in North Colorado from January 2020 to May 2023. 92% of all recorded foraging was of an unknown type. Percentages were calculated from images where tissue type could be identified. The breakdown of which body cavity organs were being consumed can be found in the appendix (Table S1.5).

	Number of images					Percent		
Species	Total	Unknown	Muscle	Bones or hide	Organ	Muscle	Bones or hide	Organ
Turkey vulture	30045	27972	540	621	912	26.05	29.96	43.99
Black-billed magpie	19327	17953	657	322	395	47.82	23.44	28.75
Common raven	12864	12268	544	11	41	91.28	1.85	6.88
Golden eagle	6358	6226	86	43	3	65.15	32.58	2.27
Bald eagle	3079	3033	30	11	5	65.22	23.91	10.87
Coyote	2777	2064	426	253	34	59.75	35.48	4.77
Black bear	2336	1399	785	66	86	83.78	7.04	9.18
Red fox	2040	1912	6	109	13	4.69	85.16	10.16
Mountain lion	444	444	0	0	0	—	—	—
Domestic dog	71	62	0	0	9	—	—	100.00
Striped skunk	68	68	0	0	0	—	—	—

Table 1.3. The vertebrate scavenger species that facilitated observed carcass transitions in northern Colorado, USA from January 2022 to May 2023. For one carcass, we observed heavy invertebrate larvae activity that caused a carcass to transition from "partially open to open." While we did not directly observe the state transitions for two carcasses, evidence of heavy invertebrate larvae activity may have caused the carcasses to change states.

	Carcass state transitions		
Species	Intact to partially open	Partially open to open	Intact to open
Black bear	—	1	—
Common raven	1	—	—
Coyote	3	3	1
Golden eagle	1	1	—
Red fox	2	2	—
Turkey vulture	3	2	1

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CHAPTER 2 – COYOTES AS ECOLOGICAL ALLIES: REDUCING THE ENVIRONMENTAL BURDEN OF CHRONIC WASTING DISEASE

INTRODUCTION

The tissues of deceased animals, also known as carrion, are a highly ephemeral and nutrient-dense resource. Carrion foraging comes without the risks generally associated with predation events, and as such, many predators will take advantage of a carcass (DeVault et al. 2003). Many predators and scavengers are categorized as pests, threats to livestock or game animals, or potential disease vectors, and as a result, management generally focuses on their removal (Ogada et al. 2012, Suryawanshi et al. 2013, Morales-Reyes et al., 2017). However, recent insight into the ecological role of scavengers questions these assumptions and highlights the benefits provided by these species (Markandya et al. 2008, Maichak et al. 2009; Mateo-Tomás et al. 2017; O'Bryan et al. 2018). Thus, there is a growing need to identify and define their specific impacts on ecological systems and processes.

Chronic wasting disease (CWD) is a fatal neurodegenerative disease of cervids caused by an infectious misfolded protein, also known as a prion (Williams and Young 1980, Miller et al. 2004). Due to CWD's negative impacts on economically, culturally, and ecologically important cervid populations, there is strong interest in determining transmission pathways and the potential role scavengers may play as vectors for prions and other pathogens. The most efficient means of spread appears to be horizontal transmission routes (Miller and Williams 2003). Direct transmission (i.e., host-to-host) can occur when infected individuals contact susceptible individuals through grooming, rutting, and other social behaviors, while indirect transmission (i.e., environment-to-host) can occur in various ways. During both the prolonged asymptomatic and clinical stages of CWD, infected individuals shed prions in their fluids and excreta, including

saliva, blood, feces, and urine (Mathiason et al. 2009, Tamgüney et al. 2009, Haley et al. 2011, Haley and Hoover 2015, Plummer et al. 2017). Shed prions bind to clay-based soils, can be uptaken by plants, and have been detected in water sources, salt licks, and the tissues of infected cervids, and notably, can persist in their infectious states for years in the environment (Miller et al. 2004, Johnson et al. 2006, Nichols et al. 2009, Pritzkow et al. 2015, Plummer et al. 2018).

Chronic wasting disease eventually results in decreased attentiveness, ataxia, and a progressive decline in body condition. Mortality risks from hunters, vehicles, and predators increase, even during the asymptomatic period (Williams and Young 1980, Krumm et al. 2005). When a CWD-infected animal dies, their tissues become available to a suite of scavengers. These species are exposed to prions when they visit, consume, and otherwise interact with CWD-positive carcasses (Jennelle et al. 2009a, Vicente and VerCauteren 2019). The impact of these exposures on the ecology and epidemiology of CWD remains relatively unclear. Scavengers have been implicated in the transmission of CWD, and recent studies have begun to quantify the extent of their involvement (Jennelle et al. 2009a, Jennelle et al. 2009b, VerCauteren et al. 2012, Fischer et al. 2013, Nichols et al. 2015, Baune et al. 2021). Seminal work determined prions presence post-passage by American crows (*Corvus brachyrhynchos*) and coyotes (*Canis latrans)*, though relative prion amounts were not assessed (VerCauteren et al. 2012, Nichols et al. 2015). Studies within the last five years established fecal prion load reductions for mountain lions (~96%, *Puma concolor*) and bobcats (≤ 88%, *Lynx rufus*; Baune et al. 2021, Davis 2024). It is unknown whether scavenging activities spread or reduce environmental prion accumulation in comparison to letting intact carcasses decompose.

Given known prion survival post gut passage in coyotes, they are a scavenger of particular interest for CWD epidemiology. America: a widely distributed canid species in North

America; their range expanded by approximately 40% over the past 120 years and now substantially overlaps with the distribution of CWD across the continent (Hody and Kayes 2016, Jensen et al. 2022, Richards 2025). Coyotes opportunistically scavenge from cervid carcasses, primarily consuming muscle tissue, and can predate mule deer (*Odocoileus hemionus*) and white-tailed deer (*Odocoileus virginianus*) (Korschgen 1957, Pierce et al. 2000, Gese and Grothe 1995, Chitwood et al. 2014, Walker et al. 2021, Bye et al. in review).

To quantify coyotes' role in CWD ecology, we conducted controlled feeding experiments to examine the fate of three concentrations of CWD prions post-passage through the digestive tracts of eight adult coyotes. The three concentrations used in the feeding exposure approximate the prion loads found in different tissues of carcasses: (1) 0.1% (4 $\log_{10}LD_{50}$/g) CWD positive elk brain homogenate representing concentrations found in carcass muscle and liver tissues (Bosque et al. 2002, Angers et al. 2006) , (2) 1% (6 $\log_{10}LD_{50}$/g) CWD positive brain homogenate representing concentrations found in extraneural tissue such as lymph nodes (Zabel et al. 2007, Davenport et al. 2018), and (3) 10% (8 $\log_{10}LD_{50}$/g) CWD positive elk brain homogenate representing concentrations found in central nervous system (CNS) tissues (Wyckoff et al. 2016). We utilized real-time quaking induced conversion (RT-QuIC) to determine the relative amount of prions in coyote feces longitudinally over time for each exposure. Finally, we examined the tissues of the study coyotes postmortem by RT-QuIC to assess whether ingested prions were sequestered in their tissues, thereby potentially serving as another mechanism of indirect CWD transmission. We discuss the results of our work in relation to the ecological role played by coyotes in disease systems of conservation and management concern.

METHODS

Study Species

The eight coyotes obtained for our study were raised in captivity at the National Wildlife Research Center (NWRC) Millville Field Station in Logan, UT, USA. We received four female and four male coyotes of varying ages. They were transported via a climate-controlled vehicle to NWRC headquarters in Fort Collins, CO, USA. They were individually housed in elevated wire kennels with attached den boxes and grate-style flooring. Decking tiles were provided to encourage coyotes to move around their kennels upon arrival. Coyotes were fed a diet of Exotic Canine Diet (Mazuri®, Saint Louis, MI, USA) soaked in water and 78 grams of Friskies Paté Poultry Platter Wet Cat Food (Purina®, Saint Louis, MI, USA) once daily and given water *ad libitum*. Individuals were allowed to continue to acclimate to their surroundings. After their two-week quarantine period, we collected fecal samples from each individual to use as negative controls throughout the study. Once all individuals were eating and drinking regularly and had decking tiles removed from their kennels, study activities commenced.

Intestinal Transit Time

To estimate intestinal transit time, we offered each coyote 50mL of negative brain homogenate mixed with glitter (ArtSkills, Inc., Bethlehem, PA, USA) in their daily ration of food. We then collected feces from enclosures twice daily until the glitter was no longer detectable in the coyotes' feces. Nichols et al. (2015) demonstrated passage time to be 1 to 2 DPI (days post-ingestion) during their study when CWD-positive material was fed to coyotes, but passage time was not assessed outside of their experiment.

Feeding Trials

We previously determined the titer of our CWD-positive E2 elk brain isolate to be 10^8 Lethal $Dose_{50}$ (LD_{50}) units per gram of brain tissue by cervid PrP transgenic mouse bioassay (Wyckoff et al. 2016). 50 mL aliquots of E2 homogenates, hereafter known as positive brain homogenate (BH), were prepared before trials started and stored in a -80°C freezer (Table 2.1). Approximately 24 hours before the initiation of a trial, eight aliquots of positive BH were thawed in a 4° C refrigerator. Coyotes were also offered their daily ration of soaked kibble and cat food. The morning of each trial, one thawed aliquot was mixed with 155.9 grams of Friskies Paté Poultry Platter Wet Cat Food, placed in a clean bowl, and offered to each coyote (Nichols et al. 2015). Coyotes were monitored from behind two-way glass until they consumed their inoculum. The time of consumption was recorded, and their daily ration of soaked kibble was offered six hours later. Water was provided *ad libitum*.

Feeding trials began with a low exposure of 0.1% (4 $\log_{10}LD_{50}$/g) positive BH. We initiated the moderate exposure trial (1% [6 $\log_{10}LD_{50}$/g] positive BH) with the same eight coyotes after RT-QuIC revealed that all individuals produced multiple consecutive negative fecal samples from the low exposure trial. We proceeded with the high-exposure trial (10% positive BH [8 $\log_{10}LD_{50}$/g]) 43 days after the conclusion of the moderate-exposure trial. Due to logistical challenges, we initiated this trial before receiving confirmation via RT-QuIC that study individuals produced negative fecal samples.

Fecal Collections

Coyote feces were collected every 12 hours post-ingestion (HPI) for three days for the low-exposure trial and five days for the moderate and high-exposure trials. We collected coyote feces for all trials by turning new plastic bags inside out (Ziploc Quart Freezer Bags, SC Johnson, Huntingtown, MD, USA), and once we had the fecal samples in hand, turning the bag

right side out before sealing (Nichols et al. 2015). All feces deposited between collection rounds were placed in the same bag labeled with the protocol number, trial, coyote ID, time window, and date. We noted whether coyotes defecated between collection rounds, as not all coyotes produced a fecal sample every 12 hours. After collection, fecal samples were frozen at -80℃.

Contamination Precautions

Several precautions were taken to prevent potential cross-contamination of fecal samples between collection rounds and between trials. We used a 1:256 Environ LpH (eLpH) mixture for a wet contact time of 30 minutes to inactivate prions (Ernst and Race 1993). All waste generated during trials was placed into biohazard trash bags that were then sprayed with eLpH before incineration. Between each trial, coyote rooms, equipment, and caging were sprayed down with the eLpH mixture before being rinsed with water. Dirty food and water bowls were soaked daily in the eLpH mixture in the anteroom of each animal room before being thoroughly rinsed and reused.

Euthanasia and Necropsies of Study Animals

To investigate whether coyotes sequester prions in their tissues, euthanasia of study individuals was imperative. Coyotes were first sedated with isoflurane gas before being injected with phenobarbital directly into their heart 88 days after the initiation of the first feeding trials. Individuals were observed for signs of death, including the cessation of circulation and respiration with a stethoscope and a loss of intra-ocular tension. The specimens were then frozen at -20°C until necropsy. We collected the spleen, whole brain, obex, mesenteric lymph nodes, and retropharyngeal lymph nodes from study individuals 177 days after euthanasia. Necropsy tools were soaked in 40% bleach for five minutes, then thoroughly rinsed after we sampled each

tissue of interest. Tables and trays were also disinfected in the same manner between each animal.

Fecal and Tissue Homogenization

For fecal homogenization, we sampled three locations from each fecal sample: one 1 cm cross-section from the center and pieces shaved off from both ends of the sample. After weighing fecal samples, weights in milligrams were multiplied by 4 to calculate the volume in microliters of 1x phosphate-buffered saline (PBS) we added to the feces, then homogenized in a HOG-24 tissue homogenizer (MRC Laboratory Instruments, Harlow, UK) 3 times for 30 seconds at 6 m/sec with 2-minute incubation in ice between homogenizations. These 20% fecal homogenates were centrifuged at 200 x g for 8-10 mins in a microfuge (VWR). Supernatants were combined 1:1 with 1x PBS to produce the final 10% homogenate.

For necropsy tissue homogenization, we collected the spleen and lymph node (retropharyngeal and mesenteric) samples similarly to the fecal samples: one 1 cm cross-section from the center and sliced-off pieces from each end of the samples. We made brain cuts directly from the obex for five of eight coyotes, while we made cuts from the hindbrain region of the remaining three animals. Individual tissue samples were weighed, with their weights in milligrams multiplied by 4 to calculate the volume of microliters of 1x PBS that needed to be added. Before adding the 1x PBS 4-6 glass beads were placed into the tubes with the retropharyngeal and mesenteric lymph nodes to help better break apart the material in the homogenizer. The samples were then homogenized in a HOG-24 tissue homogenizer (MRC Laboratory Instruments, Harlow, UK) 3 times for 30 seconds at 6 m/sec with 2-minute incubation in ice between homogenizations. The 20% homogenates were centrifuged at 200 x g

for 5-8 mins in a microfuge (VWR). Supernatants were combined 1:1 with 1x PBS to produce the final 10% homogenate.

Real-Time Quaking Induced Conversion

We produced 10-fold serial dilutions of fecal homogenates in dilution buffer (VMRD, Pullman WA). We used 10e-3 dilutions for coyotes. Positive control, E2 known prion isolate, and negative control, normal, cervidized mouse brain homogenate, similarly were diluted either directly into RT-QuIC master mix (VMRD) or first spiked into control coyote feces then diluted into the master mix. RT-QuIC reagents were purchased and prepared according to manufacturer instructions (VMRD). 1X Master mix was then prepared and 98ul aliquoted into each well of a black optical 96-well plate. We added 2ul of diluted samples into each of the respective wells according to plate setup. We pooled samples for some plates to expedite preliminary results according to the method developed by Wagner et al. (2023). We sealed the plate, and loaded it into the shaking fluorometer (BMG Lab Tech, North Carolina). RT-QuIC reactions were run for 250 cycles (62 h) of 15-minute incubation at 37℃, and 30-second shaking at 700 RPM. Fluorescence was read for 60 seconds every 60 seconds, at excitation wavelength of 448-10, emission of 482-10. We report results as the reciprocal of the time to reach a fluorescence threshold at least 5 standard deviations above the mean fluorescence of our negative controls, expressed as arbitrary fluorescence units per hour (au/h).

For each sample dilution we ran at least six technical replicates and considered samples to be positive when its mean kinetic rate was statistically significantly different from our negative controls and seeding activity was identified in at least 35% of the replicates, which lies within the linear range of our detection assay. This threshold is more liberal compared to other CWD studies (50% threshold; Baune et al. 2021) but more conservative than studies on

Creutzfeldt-Jakob, a human prion disease (25% threshold; Groveman et al. 2019). We report all samples not meeting these two criteria likely containing seeding activity below an infectious dose of CWD.

Statistical Analysis

To determine statistical significance, we performed one-way ANOVA and multiple comparison student's t-tests (GraphPad Prism) comparing mean kinetic rates of test and positive control samples to negative controls (normal brain homogenate). Results are displayed as scatterplot graphs showing means with 95% confidence for each plate run.

Quantification of Passaged Prions

As we utilized RT-QuIC to process our fecal samples, we calculated the dilution of prions at which 50% of our technical replicates are positive [Seeding $Dose_{50}$ (SD_{50})], of our isolate using the Reed-Muench method (Figure 2.1A; Reed and Muench 1938). We then generated a SD_{50} titration curve to estimate SD_{50} titers based on the proportion of positive RT-QuIC reactions (Figure 2.1B). The proportion of RT- QuIC positive replicate wells to total replicate wells (x) was plotted on the SD_{50} curve to estimate the $\log_{10}$ SD_{50} titer for a fecal sample (y) using the equation y = 10.15x. We then calculated the $\log_{10}$ reduction and percent prion recovery (Equation 1) for each fecal sample.

$$\left(1 - \frac{10^{Inoculum\ titer} - 10^{Fecal\ titer}}{10^{Inoculum\ titer}}\right) * 100 = Percent\ prion\ recovery \quad (1)$$

Paired-Pooling

For sample sets where we expect few positives, we employed a paired-pooling approach based on a similar paired-pooling strategy used for high-throughput SARS-CoV2 testing during the COVID-19 pandemic (Wagner et al. 2023). Briefly, we constructed two sets of pools such that each sample tested was a member of two unique pools. The intersection of two positive pools indicates the individual positive sample present in both pools. We performed two paired-pooling experiments, one creating pools of fecal homogenates collected at 6 different time points for each of eight coyotes (8 total "coyote" pools, each pool containing 6 fecal samples) paired with a second set of 6 total "time" pools, each containing coyote 2-8 fecal samples collected at the same timepoint. In the second paired-pooled experiment, we created pools containing the same tissue type (of five tissues collected at necropsy) from each of 8 coyotes (8 coyote samples/pool, 5 total "tissue" pools) and pools containing all 5 tissue homogenates from each of 8 coyotes (5 tissue samples/pool, 8 total "coyote" pools). Both paired-pools from each experiment were run in triplicate, for a total of six replicates per sample.

RESULTS

Intestinal Transit Time

Glitter was observable in coyote feces on days one and two after ingestion. On the third day, no glitter was observed in seven of eight coyote feces. The eighth coyote did not produce a fecal sample on the third day, but we observed no glitter in their fecal sample on the fourth day after ingestion.

Titration Linear Range

The linear range for the titration of our positive inoculum was between 10^{-8} and 10^{-4} dilutions; 8 $\log_{10}SD_{50}$ equated to 78.6% positive replicate wells for a fecal sample, while 4

$log_{10}SD_{50}$ equates to 33.4% positive replicate wells. Samples with greater than 78.6% positive replicate wells fell outside our assay's linear range. Therefore, we only report the percent prion reduction for samples within the linear range, and the linear range further supports our 35% threshold for declaring a sample positive.

Low Exposure Trial

All coyotes consumed their inoculum. Based on preliminary RT-QuIC experiments showing no positive fecal samples from low dose prion exposure, we constructed paired-pooled samples by pooling samples from all collection times for each animal in one set of pools (6 "time" pools total), then pooling samples from all animals for a given timepoint in the second set (8 "animal" pools total, Figure 2.2). None of the pools contained 35% positive replicates (i.e., not discernable from negative controls) nor were their mean kinetic rates statistically different from the negative controls (Table 2.2). We proceeded with the moderate exposure trial as these fecal samples showed no evidence of prion seeding activity after 24-HPI (Figure 2.2).

Moderate Exposure Trial

We started this trial 29 days after the conclusion of the low-exposure trial. All coyotes consumed their inoculum. Three of eight coyotes produced fecal samples with positive replicate wells greater than the 35% threshold during different windows (50% positive wells at 0-12 HPI, 24-48 HPI, 48-60 HPI) with samples from the other coyotes showing no evidence of prion seeding activity (Figures 2.3 and 2.4; Table 2.3). The positive samples had mean fecal titers and prion reductions of 5.08 $log_{10}SD_{50}$ (95% CI: 3.81-6.34; Table 2.3). The percentage of consumed prions recovered from each of these fecal samples was 0.0008% (95% CI: < 0.001- 0.029%). The subsequent fecal collections for these coyotes were below the 35% threshold. All other fecal samples for the moderate dose trial were below the 35% threshold, or no evidence of prion

seeding activity was detected by RT-QuIC (Figures 2.3 and 2.4). The exception, however, was one of the five coyotes who had previously negative fecal samples until the last round of fecal collections (108-120 HPI). Their sample showed seeding activity in 60% of replicate wells, with a kinetic rate that was significantly different from the negative control. We calculated a mean fecal titer of 6.09 $\log_{10}SD_{50}$ (95% CI: 4.57-7.61; Table 2.3) and a prion recovery of 0.009% (95% CI: < 0.001- 0.288%) from this sample.

High Exposure Trial

All eight coyotes willingly consumed their inoculum. We did not detect evidence of prion seeding activity above the 35% threshold for samples from two of eight coyotes throughout the high-exposure trial. The remaining six coyotes produced fecal samples where prion seeding activity was detected via RT-QuIC. One coyote demonstrated prion seeding activity in 40% of replicate wells by 12 HPI. However, the mean kinetic rate was not statistically different from the negative controls (Figures 2.5 and 2.6), and we recovered minute amounts of prions from this sample (Table 2.4). By 24 HPI, five of six coyotes had samples exceeding the 35% prion seeding threshold. Three of these five had prion seeding activity detected in 56% of replicate wells with mean fecal titers of 5.71 $\log_{10}SD_{50}$ (95% CI: 4.12-7.30; Table 2.4). We recovered 0.003% (95% CI: < 0.001- 0.141) of ingested prions from these samples. Two of the five had prion seeding activity detected in 63% of replicate wells with mean fecal titers of 6.34 $\log_{10}SD_{50}$(95% CI: 4.67-8.01; Table 2.4). We recovered 0.016% (95% CI: < 0.001- 0.731) of ingested prions from these samples.

Of the six coyotes that produced a fecal sample by 36-HPI, none showed evidence of seeding activity (Figures 2.5 and 2.6). Four coyotes that produced a fecal sample by 48-HPI showed evidence of prion seeding activity (Table 2.4). Samples from two coyotes produced

between 36-48 HPI showed prion seeding activity in 50% and 56% of replicate wells with mean prion reduction of 5.08 $\log_{10}SD_{50}$ (95% CI: 3.54-6.62) and 4.44 $\log_{10}SD_{50}$ (95% CI: 2.85-6.03) respectively. These coyotes exhibited a slight increase in the number of positive replicate wells by 60-HPI, and by 72-HPI, the six coyotes that produced a fecal sample showed no seeding activity by RT-QuIC (Figures 2.5 and 2.6, Table 2.4). Samples from the two other coyotes produced by 48-HPI showed prion seeding activity in 75% of replicate wells with mean fecal titers of 7.61 $\log_{10}SD_{50}$ (95% CI: 1.90-5.71) and the highest prion recovery of the exposure trials (0.29%; 95% CI: 0.004 – 22.935; Table 2.4). The subsequent fecal samples from these coyotes showed no evidence of prion seeding activity.

We then ran the fecal samples from 72-96 HPI (one of eight coyotes) and 108-120 HPI (seven of eight coyotes). All tested fecal samples from these time windows were below the 35% threshold (Figures 2.5 and 2.6). The overall average fecal titer for samples with positive seeding activity above the 35% threshold was 6.17 $\log_{10}SD_{50}$ (SD: 0.99) reflecting a percent prion recovery of 0.05% (SD: 0.10).

Necropsy Tissues

Coyotes were euthanized two days after the conclusion of the high-exposure trial (88 days after the start of the first trial). When we tested their tissues – spleen, whole brain, obex, mesenteric lymph nodes, and retropharyngeal lymph nodes – with RT-QuIC using the pooling strategy described above, we did not observe any evidence of prion seeding activity originating from any necropsy tissues (Figure 2.7).

DISCUSSION

We found that coyotes are remarkably efficient at neutralizing ingested CWD prions. For much of the low exposure trial (4 $Log_{10}LD_{50}$/g; approximated carcass muscle and liver tissue prion loads) and the moderate exposure trial (6 $Log_{10}LD_{50}$/g; approximated extraneural prion loads), coyotes did not show evidence of prion passage in their feces, and when they did, prion recovery was much less than 1%. This holds significant ecological relevance. By consuming the tissues of deceased CWD-positive individuals, coyotes mitigate environmental CWD transmission risk associated with carcasses. Coyotes preferentially consumed muscle tissue and facilitated the decomposition of cervid carcasses in the CWD endemic zone in Northern Colorado (Bye et al. in review). When coyotes were observed consuming extraneural tissues, they primarily foraged on organs coming from the abdominal cavity (Bye et al. in review). Lymphatic tissues (e.g., Peyer's patches in the wall of the small intestine and B-cell follicles of the spleen) associated with abdominal organs are the first sites of prion deposition following oral exposure (Aguzzi and Heikenwalder 2006, Beekes and McBride 2007). Our results suggest that coyotes neutralize all prions from consumed muscle and liver tissues, and extraneural tissues.

The mean prion recovery from the high-exposure trial (8 $Log_{10}LD_{50}$/g; approximated CNS tissue prion loads) remained less than 1% despite an increase in prion excretion from more individuals than in the lower exposure trials. Our results suggest that the possible upper limit of fecal prion recovery (upper 95% confidence interval from samples with the highest prion recovery percentage) from a coyote exposed to tissues with high prion loads was ~23%. Notably, coyotes do not have the bite force required to open the skulls of adult ungulates, and observational studies have not detected coyotes consuming CNS tissue from cervid carcasses (Wiersma 2001, Bye et al. in review). Thus, CNS tissue consumption by coyotes *in situ* is unlikely relative to other, more accessible tissue types.

Variability in Prion Shedding

We observed several notable patterns during the controlled experiments. Firstly, two coyotes never produced fecal samples above the 35% threshold during the collection windows, pointing to potential individual variations in prion sequestration potential. These individuals were older and of different sexes: a 7-year-old female (1604) and a 9-year-old male (1421). One coyote (1408) had detectable seeding activity in the sample collected between 108-120 HPI, which was significantly different from the negative control, but prior fecal samples were below the 35% threshold or showed no evidence of prion seeding activity via RT-QuIC. Given this unlikely result, sample contamination may be the cause of this result, as we did not disinfect caging or den boxes during trials. Lastly, we saw a curious lack of prion seeding activity from 24-36 HPI for all eight coyotes during the high exposure trial despite seeding activity being relatively high during the prior and subsequent collection windows. Safar et al. (2008) observed a similar phenomenon in Syrian hamsters orally challenged with Scrapie prions; they saw elevated prion shedding at 2 and 8 DPI. This apparent retention and later release of prions may be due to absorption by alimentary lymphoid cells and/or epithelial cells which are later sloughed off into the gastrointestinal tract, where they are then excreted (Fernando and McCraw 1973, Krüger et al. 2008). Previously, this phenomenon was only observed in animal models for prion diseases (i.e., Syrian hamsters, transgenic mice), where the exposed host contracted the disease.

We and others assumed that prions would be detected in feces for the average intestinal transit time of the focal species and collected feces accordingly (VerCauteren et al. 2012, Nichols et al. 2015, Baune et al. 2021, Davis 2024). However, we discovered that intestinal transit time may not be a reliable indicator for the duration that scavengers and predators excrete ingested prions. RT-QuIC is an extremely sensitive assay that can detect minute amounts of prions (≥ 1

femtogram [fg, 10-15 g] PrP^{Sc}) in a sample (Atarashi et al. 2011, Dong and Satoh 2021). It is probable that the assay can detect prions outside of species' intestinal transit time. We suggest future studies take this into account when determining their sampling period.

Our results are supported by existing literature, and we expanded upon seminal work by Nichols et al. (2015). Our work and theirs suggest that the main window of coyote prion excretion is from 0-60 HPI or 0-2.5 DPI for tissues with prion loads at or less than 8 $LogLD_{50}/g$, or CNS tissue. We tested lower doses of CWD-positive inoculum and more tissues post-mortem than their original study and allowed for a longer period between the initial exposure and euthanasia of study individuals. Nichols et al. (2015) did not detect prions via immunohistochemistry in the brains or mesenteric lymph nodes of their experimental coyotes five days after oral inoculation of 10% CWD positive elk brain homogenates. Separately, Jennelle et al. (2009b) did not detect evidence of prions in the brains of 200 roadkill or hunter-trapped coyotes collected from the epicenter of CWD in Wisconsin, USA via IDEXX HerdChek Assay (Joly et al. 2013). Together with our necropsy results, this suggests that prions ingested by coyotes are likely not sequestered in their tissues. Instead, their digestive process appears to be a highly effective mechanism for neutralizing prions from ingested tissues. Additionally, canids exhibit a notable resistance to prion diseases, further suggesting that they serve as dead-end reservoirs for CWD (Lysek et al. 2005, Fernández-Borges et al. 2017).

Conclusions

Spatial and temporal factors likely influence coyotes' ability to influence CWD epidemiology. Coyotes' home range sizes vary due to several factors, including habitat composition, season, and whether they are resident, transient, or dispersing. Resident coyotes can have home ranges of 11.3 ± 5.8 km^2 (S. Colorado; Gese et al. 1988) to 13.5 ± 7.0 km^2 (Alabama,

Georgia, and South Carolina; Ward et al. 2018). Transient coyotes travel much farther but tend to represent ~30% of the population (Gese et al. 1988, Ward et al. 2018). Food availability and predictability also influence how coyotes utilize their home ranges. In central Colorado, Hein and Andelt (1996) documented coyotes investigating and spending time in less frequented parts of their home range when there was ungulate carrion on the landscape, though their monitored coyotes did not visit carcasses outside their home ranges. This may not apply during dispersal periods or for transient coyotes and has implications for the spatial extent of coyotes' prion sequestration potential. Seasonal conditions further affect coyote foraging behavior. In Yellowstone National Park, Wyoming, USA, Gese et al. (1996) reported that adult coyotes travel less and scavenged more from ungulate carrion during winter, particularly during periods of deep snowpack. These patterns suggest that coyotes may play a disproportionate role in CWD ecology during winter months when scavenging behavior intensifies.

Coyotes, along with bobcats and mountain lions, play a significant role in controlling the environmental spread of CWD by ingesting CWD-laden tissues (Baune et al. 2021, Davis 2024). Future research should expand quantitative, experimental feeding studies to mammals with large home range sizes, such as bears and wolves, and to common avian and invertebrate scavengers. Furthermore, introducing lower, more realistic exposure levels to feeding trials is necessary for appropriate model parameterization (VerCauteren et al. 2012, Nichols et al. 2015, Baune et al. 2021). Wildlife managers can take steps to reduce CWD transmission risks where there are existing epidemics and slow transmission in recently exposed populations by leveraging the ecosystem services these species provide. Additionally, incorporating the activities of carcass consumers into the epidemiological modeling of CWD is important for elucidating the transmission dynamics of this complex disease.

ETHICS STATEMENT

The Institutional Animal Care and Use Committee of the United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, NWRC approved all procedures used in this study (QA-3553).

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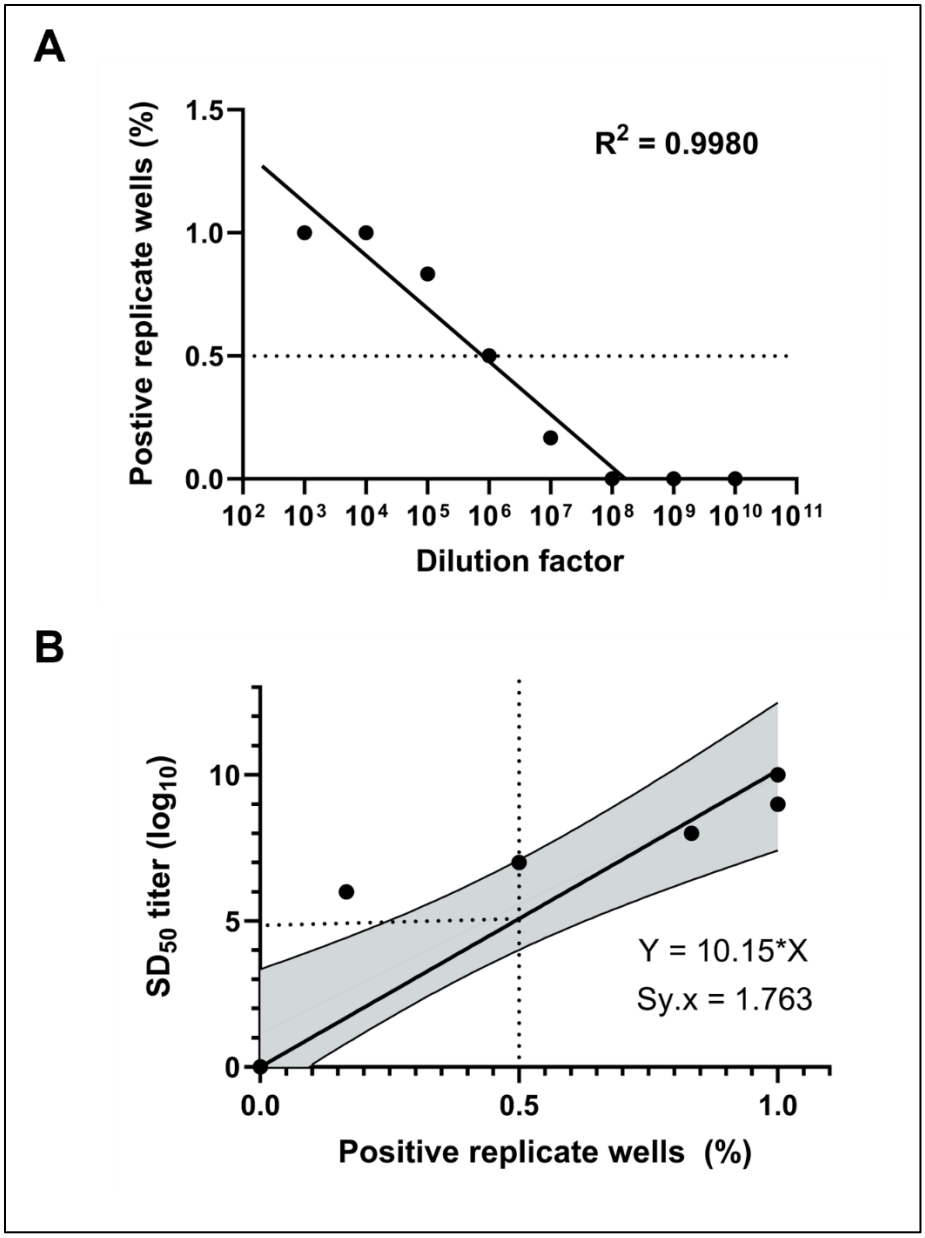

Figure 2.1. A) Seeding $Dose_{50}$ (SD_{50}) standard curve for our E2 isolate. This was generated using the Reed-Muench calculator. **B)** SD_{50} titration curve for fecal samples. The SD_{50} titer (log_{10}) for coyote fecal samples is calculated by inputting the proportion of positive replicate wells to total replicate wells for the RT-QuIC assay into the equation.

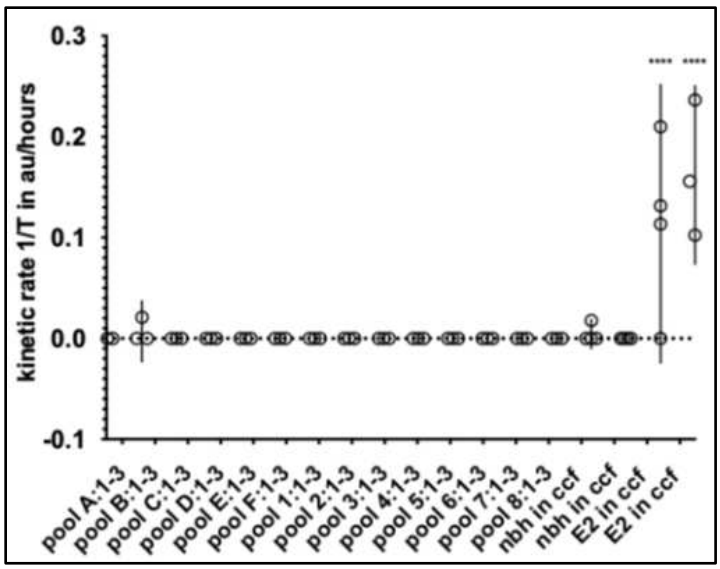

Figure 2.2 Real-time quaking induced conversion (RT-QuIC) average kinetic rates for low exposure trial (0.1% E2 brain homogenate) coyote fecal samples. This trial approximated the prion load in muscle and liver tissues of infected deer. The RT-QuIC plate was pooled according to the strategy developed by Wagner et al. (2023). Pool B:1-3 contained fecal homogenates from 8/8 coyotes who produced a sample by 24 hours post-ingestion (HPI). The control wells are on the right side of the figure; negative brain homogenate in coyote control feces (nbh in ccf), and E2 positive brain homogenate in coyote control feces (E2 in ccf).

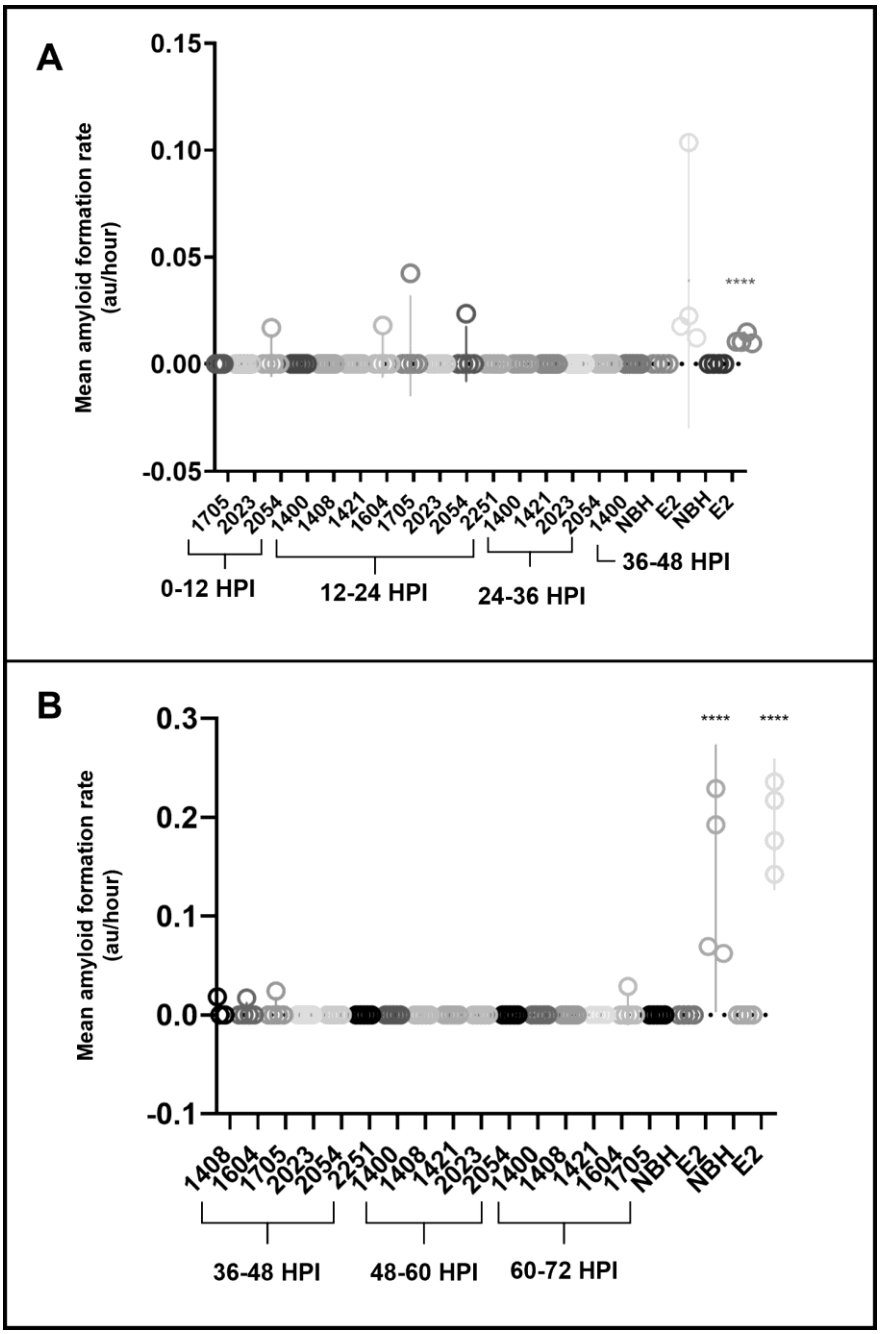

Figure 2.3. Real-time quaking induced conversion (RT-QuIC) average kinetic rate for moderate exposure trial (1% E2 brain homogenate) coyote fecal samples. This trial approximated the prion load in extraneural tissues of infected deer. The control wells are on the right side of each figure: negative brain homogenate (NBH) and E2 positive brain homogenate (E2). **A)** This RT-QuIC plate contained coyote fecal samples from 0-48 hours post-ingestion (HPI). None of these samples was statistically different than the negative control. **B)** This RT-QuIC plate contained coyote fecal samples from 24-72 HPI. None of these samples were statistically different than the negative control.

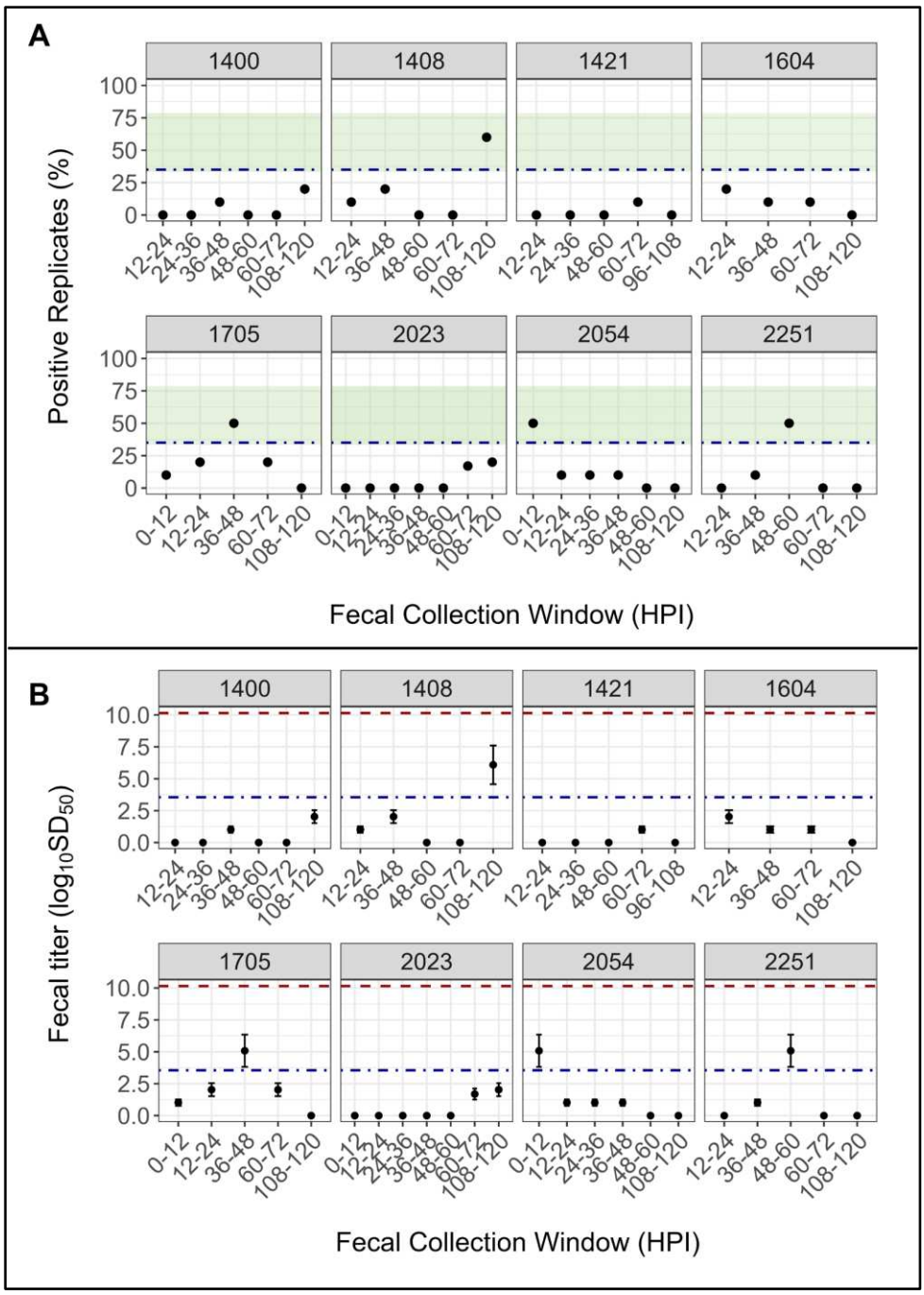

Figure 2.4. The moderate exposure trial (1% E2 brain homogenate) approximated the prion load in extraneural tissues of infected deer. HPI stands for hours post-ingestion and the blue dotted line represents our 35% threshold in both figure panels. Coyotes did not always defecate between 12-hour collection windows. The reported collection windows reflect the period in which they produced fecal samples. **A)** Percentage of positive real-time quaking induced conversion (RT-QuIC) wells for coyote fecal samples. **B)** Fecal titers (log_{10} SD_{50}) and their 95% confidence intervals for coyote fecal samples. We calculated fecal titers for each sample (y) by plotting the proportion of RT- QuIC positive replicate wells to total replicate wells (x) on the SD_{50} curve using the equation y = 10.15x. The red dotted line represents the inoculum's SD_{50} titer (log_{10}).

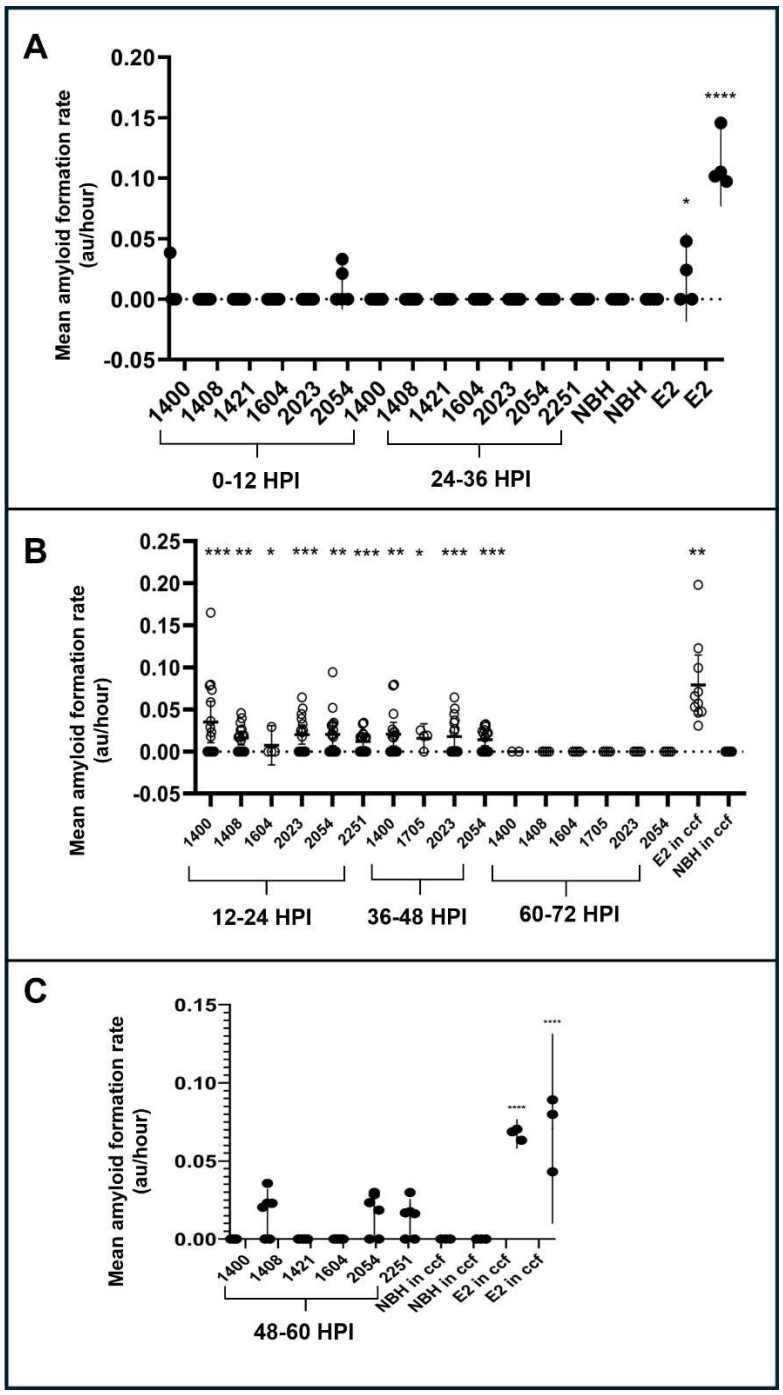

Figure 2.5. Real-time quaking induced conversion (RT-QuIC) average kinetic rate for high exposure trial (10% E2 brain homogenate) coyote fecal samples. This trial approximated the prion load in the central nervous system tissues of infected deer. The control wells are on the right side of each figure: negative brain homogenate in coyote control feces (NBH in ccf) and E2 positive brain homogenate in coyote control feces (E2 in ccf). A, B and C are the different RT-QuIC plates run for this trial. The window of prion shedding appears to be from 0-60 hours post-ingestion (HPI), with a cessation in shedding from 24-36 HPI. Peak shedding appears to occur by 24 HPI and between 36-48 HPI.

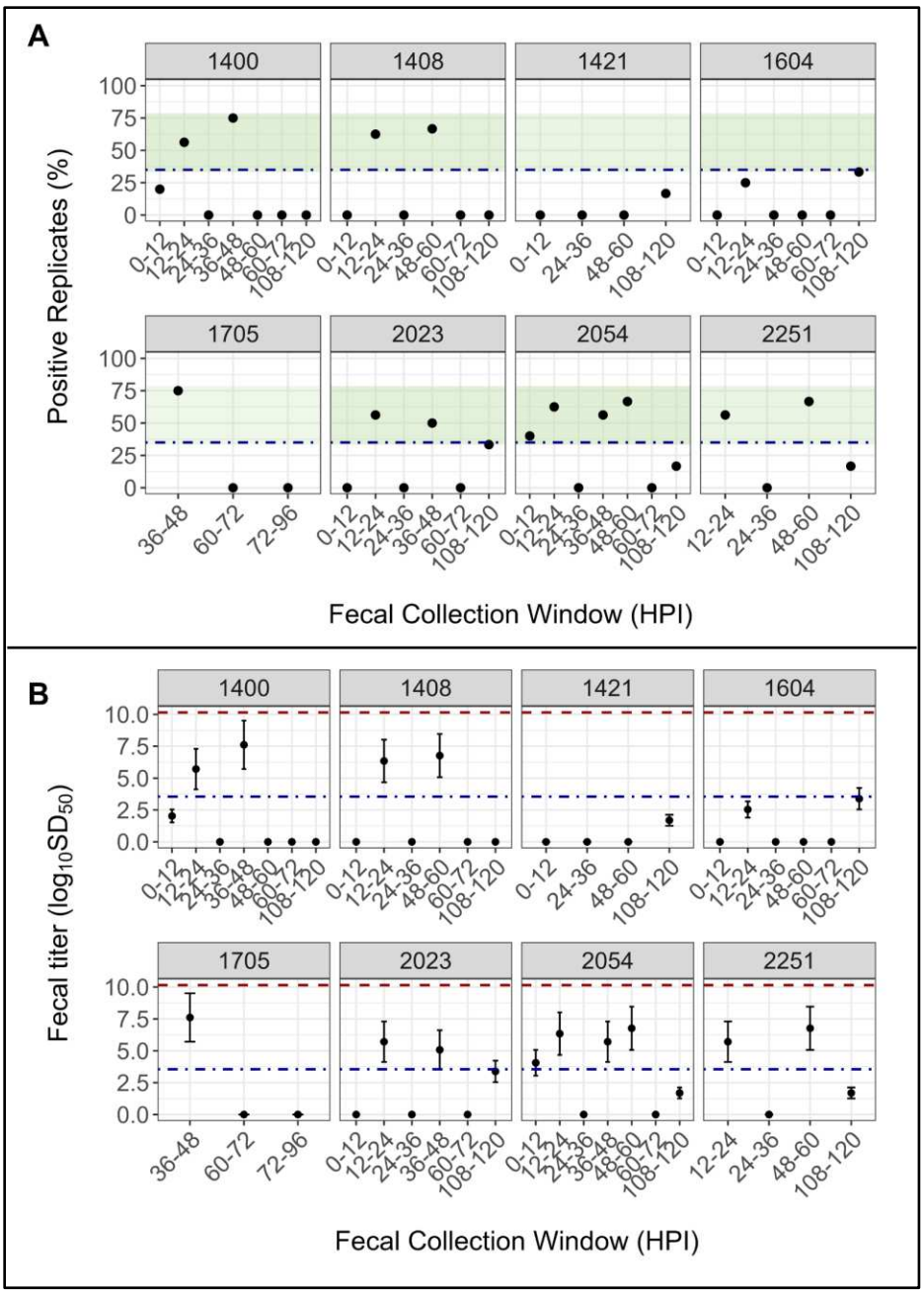

Figure 2.6. The high exposure trial (10% E2 brain homogenate) approximated the prion load in the central nervous system tissues of infected deer. HPI stands for hours post-ingestion and the blue dotted line represents our 35% threshold in both figure panels. Coyotes did not always defecate between 12-hour collection windows. The reported collection windows reflects the period in which they produced fecal samples. **A)** Percentage of positive real-time quaking induced conversion (RT-QuIC) wells for coyote fecal samples. **B)** Fecal titers (log_{10} SD_{50}) and their 95% confidence intervals for coyote fecal samples. We calculated fecal titers for each sample (y) by plotting the proportion of RT- QuIC positive replicate wells to total replicate wells (x) on the SD_{50} curve using the equation y = 10.15x. The red dotted line represents the inoculum's SD_{50} titer (log_{10}).

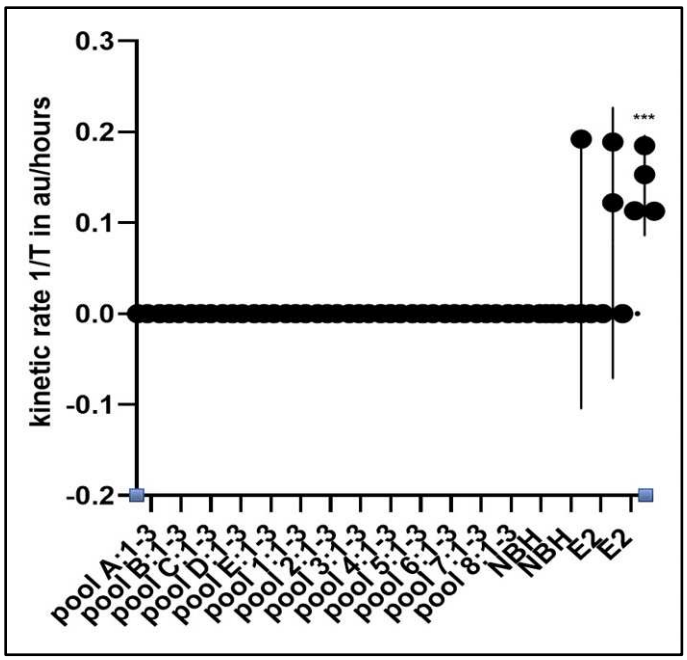

Figure 2.7. Real-time quaking induced conversion (RT-QuIC) average kinetic rate for necropsy tissue samples: spleen, whole brain, obex, mesenteric lymph nodes, and retropharyngeal lymph nodes. The RT-QuIC plate was pooled according to the strategy developed by Wagner et al. (2023). The control wells are on the right side of the figure: negative brain homogenate (NBH) and E2 positive brain homogenate (E2). None of the pooled replicate wells showed any evidence of prion seeding activity. Therefore, we did not detect evidence of prions in the tissues of our study coyotes.

Table 2.1. Dilutions of E2 positive brain homogenate (BH) in phosphate-buffered saline (PBS) for each coyote trial.

Trial	Total Aliquot (mL)	Dilution
Low exposure (.1%)	50	0.5 mL 10% E2 BH + 49.95 mL PBS
Moderate exposure (1%)	50	5 mL 10% E2 BH + 45 mL PBS
High exposure (10%)	50	50 mL10% E2 BH

Table 2.2. Pooled real-time quaking induced conversion results for low exposure trial (0.1% E2 brain homogenate) coyote fecal samples. This trial approximated the prion load in muscle and liver tissues of infected deer. HPI stands for hours post ingestion.

Collection Window (HPI)	Proportion of Fecal Samples Produced	Positive Replicate Wells	
0-12	2/8	0/6	0%
12-24	8/8	1/6	17%
24-36	8/8	0/6	0%
36-48	8/8	0/6	0%
48-60	7/8	0/6	0%
60-72	8/8	0/6	0%

Table 2.3. Real-time quaking induced conversion (RT-QuIC) results for moderate exposure trial (1% E2 brain homogenate) prion positive coyote fecal samples. This trial approximated the prion load in extraneural tissues of infected deer. HPI stands for hours post ingestion. We calculated the seeding dose$_{50}$ (SD_{50}) titer (log_{10}) by inputting the proportion of positive replicate wells to total replicate wells into the SD_{50} curve for the RT-QuIC assay (Figure 2.1). By comparing the fecal samples' titer to the inoculum's titer (10.15 log_{10}), we could calculate the percent prions recovered from each fecal sample.

Coyote ID	Collection Window (HPI)	Rep. wells	Pos. wells	Mean fecal SD_{50} (log_{10})	95% CI fecal SD_{50} (log_{10})	Mean fecal SD_{50} reduction (log_{10})	Prion recovery	95% CI Prion recovery
2054	0-12	10	50%	5.01	3.53-6.62	5.08	0.00084%	0.00000243 - 0.02917427%
1705[a]	36-48	10	50%	5.01	3.53-6.62	5.08	0.00084%	0.00000243 - 0.02917427%
2251	48-60	6	50%	5.01	3.53-6.62	5.08	0.00084%	0.00000243 - 0.02917427%
1408[*]	108-120	5	60%	6.09	4.57-7.61	4.06	0.00870%	0.00026301 - 0.28840315%

[*] Possible sample contamination.
[a] Did not produce a sample during the previous 12-hour collection window.

Table 2.4. Real-time quaking induced conversion (RT-QuIC) results for high exposure trial (10% E2 brain homogenate) prion positive coyote fecal samples. This trial approximated the prion load in the central nervous system tissues of infected deer. HPI stands for hours post ingestion. We calculated the seeding dose$_{50}$ (SD_{50}) titer (log_{10}) by inputting the proportion of positive replicate wells to total replicate wells into the SD_{50} curve for the RT-QuIC assay (Figure 2.1). By comparing the fecal samples' titer to the inoculum's titer (10.15 log_{10}), we could calculate the percent prion reduction, and the percent prions recovered from each fecal sample.

Coyote ID	Collection Window (HPI)	Rep. wells	Pos. wells	Mean fecal SD_{50} (log_{10})	95% CI fecal SD_{50} (log_{10})	Mean fecal SD_{50} reduction (log_{10})	Prion recovery	95% CI Prion recovery
2054	0-12	5	40%	4.06	3.05-5.07	6.09	0.00008%	0.00000791 - 0.00083560%
1400	12-24	16	56%	5.71	4.12-7.30	4.44	0.00363%	0.00000932 - 0.14105224%
1408	12-24	16	63%	6.34	4.67-8.01	3.81	0.01562%	0.00033400 - 0.73071833%
2023	12-24	16	56%	5.71	4.12-7.30	4.44	0.00363%	0.00000932 - 0.14105224%
2054	12-24	16	63%	6.34	4.67-8.01	3.81	0.01562%	0.00033400 - 0.73071833%
2251[a]	12-24	16	56%	5.71	4.12-7.30	4.44	0.00363%	0.00000932 - 0.14105224%
1400	36-48	4	75%	7.61	5.71-9.51	2.54	0.29007%	0.00366860 - 22.9350663%
1705[a]	36-48	4	75%	7.61	5.71-9.51	2.55	0.29007%	0.00366860 - 22.9350663%
2023	36-48	16	50%	5.01	3.53-6.62	5.08	0.00084%	0.00000243 - 0.02917427%
2054	36-48	16	56%	5.71	4.12-7.30	4.44	0.00363%	0.00000932 - 0.14105224%
1408[+]	48-60	6	67%	6.77	5.07-8.46	3.38	0.04137%	0.00083497 - 2.04960420%
2054	48-60	6	67%	6.77	5.07-8.46	3.38	0.04137%	0.00083497 - 2.04960420%
2251[+]	48-60	6	67%	6.77	5.07-8.46	3.38	0.04137%	0.00083497 - 2.04960420%

[+] Prior fecal sample missing.
[a] Did not produce a sample during the previous 12-hour collection window.

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APPENDIX S1

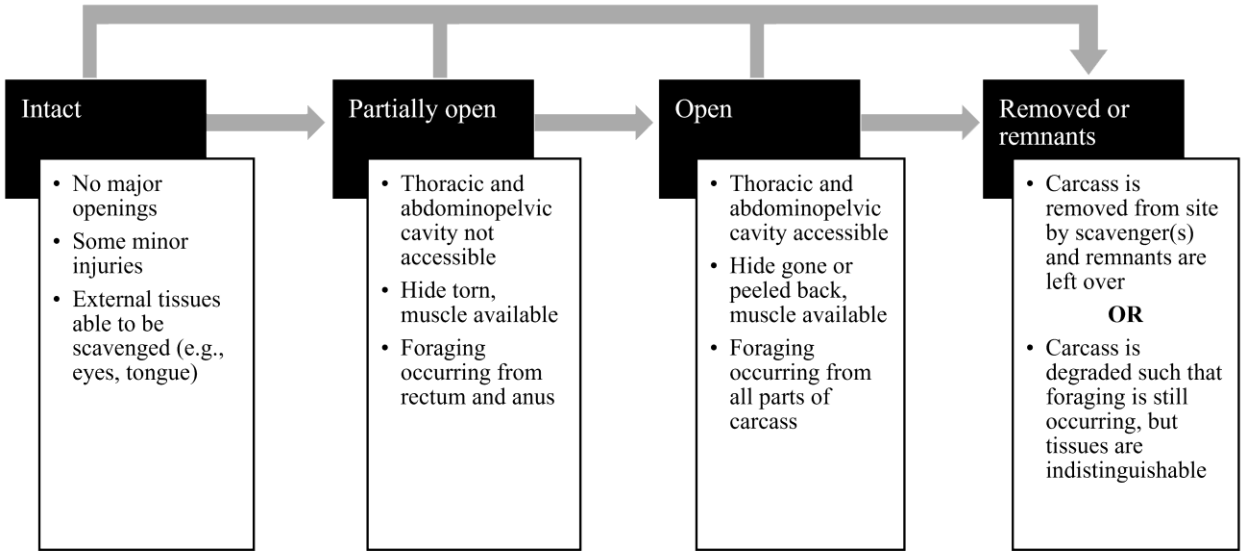

Figure S1.1. Our methodology for classifying carcass decomposition stage from studied carcasses in northern Colorado, USA, from January 2022 to May 2023.

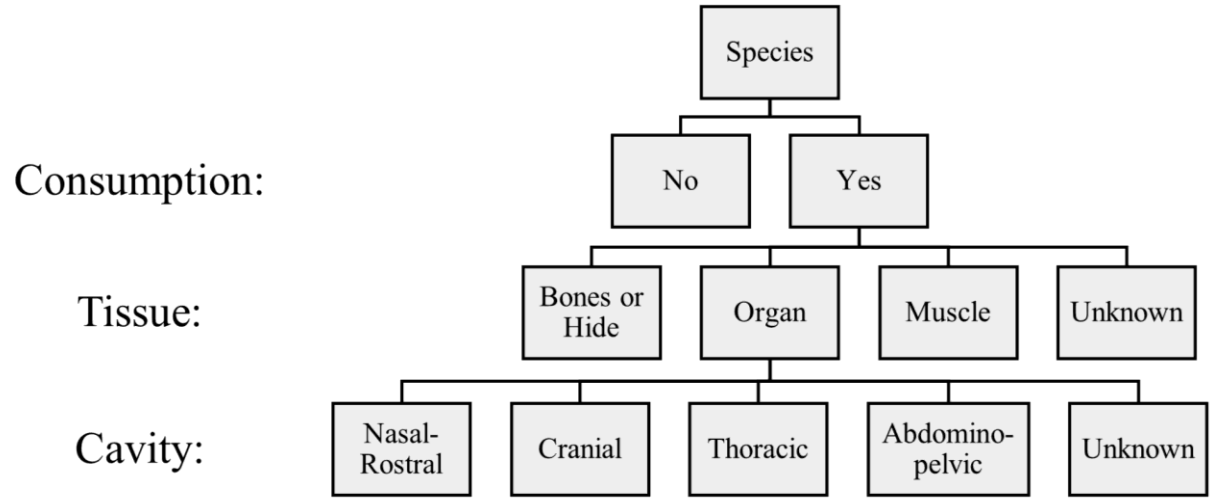

Figure S1.2. Our methodology for recording species consumptive activities at carcass sites in northern Colorado, USA, from January 2022 to May 2023. Unknown body cavity refers to when a carcass is degraded to the point that there are no longer body cavities able to be identified, but organ consumption is still occurring.

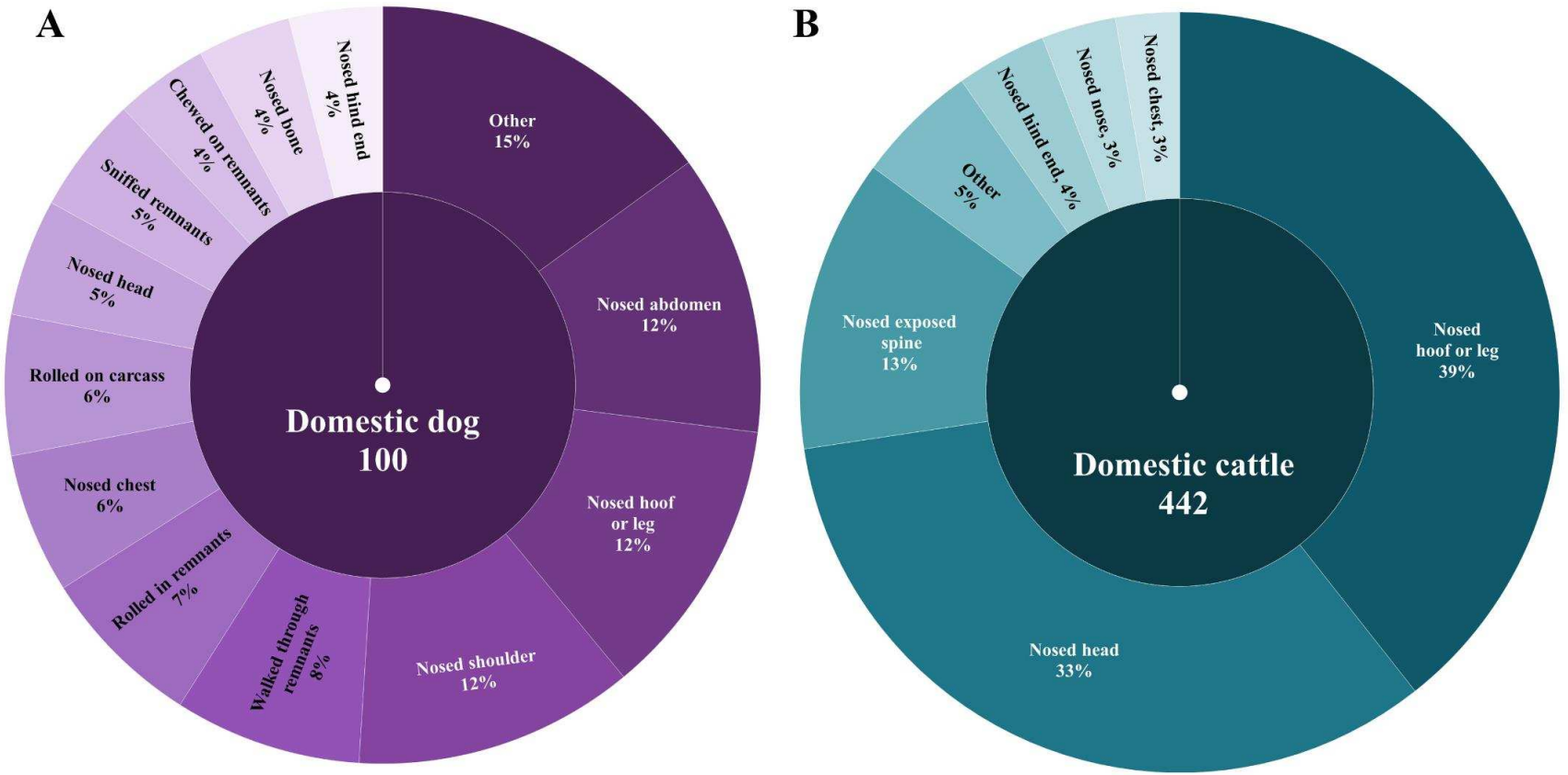

Figure S1.3. Detailed observed interactions with disease transmission potential for domestic cattle and domestic dogs at cervid carcasses (n = 20) in northern Colorado, USA, from January 2022 to May 2023. **A)** We observed a total of 100 interactions with disease transmission potential for domestic dogs. Other is composed of interaction categories with two or fewer observations. These interactions for domestic dogs are: chewed on spine (n=2), licked remnants (n=2), rolled near carcass (n=2), nosed carcass remnants (n=2), consumption from abdominopelvic cavity (n=1), consumption near hind end (n=1), consumed intestines (n=1), licked near hind end (n=1), licked organs (n=1), nosed nose (n=1), and picked up spine (n=1). **B)** We observed a total of 442 interactions with disease transmission potential for domestic cattle. Other is composed of categories with ten or fewer observations. These interactions for domestic cattle are nosed abdomen (n = 8), nosed hindquarter (n = 7), nosed antler (n = 4), nosed side (n = 2), licked head (n = 1), and nosed back (n=1).

Table S1.1. Landscape covariates for daily species diversity generalized linear mixed models and the sources for the data.

Landscape attribute	GLMM covariate	Source
Riparian	Distance from carcass to water (m)	U.S. Fish and Wildlife Service National Wetlands Inventory 2024
Roads	a. Distance from carcass to highways (m) b. Distance from carcass to non-highway roads (m)	Colorado Department of Transportation 2024a Colorado Department of Transportation 2024b Colorado Department of Transportation 2024c
Terrain	a. Aspect (°) b. Roughness (m)	Hollister et al. 2023 Hollister et al. 2023
Canopy cover	No canopy (0) Canopy (1)	At carcass site
Habitat	2019 National Landcover Database classifications	Dewitz and U.S. Geological Survey 2024
Human impact	Global Human Modification Index (GHM)	Kennedy et al. 2019 available through Google Earth Engine
Temperature	a. Average temperature (°C) at each carcass b. Coefficient of variation of temperature (°C) at each carcass	NLDAS project 2021, Xia et al. 2012
Season	Winter, Spring, Summer, and Fall	Based on the 1st day the carcass was active

Table S1.2. Detailed detections per species from January 2022 to May 2023 at cervid carcass sites (n = 20) in northern Colorado, USA, from Figure 1.2. Minimum group sizes across all species were 1.

Species	Num. of detections	Percent detections
Black-billed magpie	688	45.68
Coyote	275	18.26
Turkey vulture	148	9.83
Red fox	73	4.85
Domestic cattle	38	2.52
Black bear	36	2.39
Yellow-breasted chat	35	2.32
Common raven	33	2.19
Golden eagle	31	2.06
Striped skunk	25	1.66
Domestic dog	19	1.26
Prairie dog	18	1.2
Human	12	0.8
Mouse (*Peromyscus* sp.)	12	0.8
Mule deer	12	0.8
Rabbit (*Sylvilagus* sp.)	6	0.40
Raccoon	6	0.40
Bald eagle	5	0.33
Unknown species	5	0.33
Mountain lion	3	0.20
Unknown bird	3	0.20
Domestic horse	2	0.13
Elk	2	0.13
Domestic cat	1	0.07
Grackle	1	0.07
Red-tailed hawk	1	0.07
Spotted towhee	1	0.07
Unknown canine	1	0.07
Unknown ungulate	1	0.07
Wild turkey	1	0.07

Table S1.3. Species-specific discovery and first feeding frequencies at cervid carcasses (n = 20) in northern Colorado, USA, from January 2022 to May 2023, along with the average time to discovery and first forage for species with more than one occurrence. The average time to discovery and first forage are reported in days, indicating how long after placement a carcass was discovered or was first fed from.

Species	Discovery Frequency	Avg. Time to Discovery (days)	First Feed Frequency	Avg. Time to First Forage (days)
Black-billed magpie	7	.39	9	1.37
Coyote	4	3.81	4	12.06
Turkey vulture	2	.89	4	1.92
Species	**Discovery Frequency**	**Time to Discovery (days)**	**First Feed Frequency**	**Time to First Forage (days)**
Domestic cattle	1	.24	0	—
Domestic dog	1	1.91	1	1.91
Human	1	3.34	0	—
Mouse (*Peromycus* sp.)	1	.51	1	1.41
Mule deer	1	10.16	0	—
Raccoon	1	.67	0	—
Red fox	1	.29	0	—
Golden eagle	0	—	1	2.66

Table S1.4. Akaike Information Criteria (AIC_c) selection table for generalized linear models (GLM) of site-level species diversity, which we grouped into various sub-models: anthropogenic, abiotic, habitat, and landscape. The global human modification index (GHM) model was the top model; all other candidate models did not perform better than the null model. NLCD refers to the 2019 National Land Cover Database habitat classifications from Dewitz and U.S. Geological Survey (2024).

Sub-model: anthropogenic	K	AIC_c	ΔAIC_c	AIC_c Weight	Log-Likelihood
GHM	3	28.39	0.00	0.57	-10.45
Null	2	30.43	2.04	0.21	-12.86
GHM + dist. to road (m)	4	31.52	3.13	0.12	-10.43
Dist. to road (m)	3	31.76	3.37	0.11	-12.13
Sub-model: abiotic	K	AIC_c	ΔAIC_c	AIC_c Weight	Log-Likelihood
Null	2	30.43	0.00	0.68	-12.86
Coef. var. temp (°C)	3	32.11	1.67	0.29	-12.30
Season	5	37.27	6.84	0.02	-11.49
Coef. var. temp (°C) + season	6	41.43	10.99	0.00	-11.48
Sub-model: habitat	K	AIC_c	ΔAIC_c	AIC_c Weight	Log-Likelihood
Null	2	30.43	0.00	0.79	-12.86
Canopy cover	3	33.05	2.62	0.21	-12.78
NLCD	7	42.29	11.86	0.00	-9.48
Canopy cover + NLCD	8	47.51	17.08	0.00	-9.21
Sub-model: landscape	K	AIC_c	ΔAIC_c	AIC_c Weight	Log-Likelihood
Null	2	30.43	0.00	0.44	-12.86
Aspect (°)	3	31.48	1.04	0.26	-11.99
Dist. to water (m)	3	31.82	1.39	0.22	-12.16
Aspect (°) + dist. to water (m)	4	33.98	3.55	0.07	-11.66

Table S1.5. Frequency of organ consumption observations by species and body cavity from studied carcasses (n = 20) in northern Colorado, USA, from January 2022 to May 2023.

	Number of observations				Percentage of observations			
Species	Abdominopelvic	Thoracic	Cranial	Unknown	Abdominopelvic	Thoracic	Cranial	Unknown
Turkey vulture	554	24	23	311	60.75	2.63	2.52	34.10
Black-billed magpie	296	6	—	93	74.94	1.52	—	23.54
Black bear	7	28	—	51	8.14	32.56	—	59.30
Common raven	23	—	2	16	56.10	—	4.88	39.02
Coyote	15	5	—	14	44.12	14.71	—	41.18
Red fox	8	5	—	—	61.54	38.46	—	—
Domestic dog	—	9	—	—	—	100.00	—	—
Bald eagle	5	—	—	—	100.00	—	—	—
Golden eagle	3	—	—	—	100.00	—	—	—

Table S1.6. AICc model selection for all possible combinations of covariates for the foraging duration GLMM. Site is included as a random intercept for each model.

Model	K	AIC_c	ΔAIC_c	AIC_c Weight	Log-Likelihood
Days since placement + species + avg. temp. (°C)	15	41514.73	0.00	1.00	-20742.32
Species + avg. temp. (°C)	14	41530.63	15.90	0.00	-20751.27
Days since placement + species	14	41547.26	32.54	0.00	-20759.59
Species	13	41561.65	46.93	0.00	-20767.79
Days since placement + avg. temp. (°C)	5	42022.24	507.51	0.00	-21006.11
Avg. temp. (°C)	4	42027.12	512.40	0.00	-21009.56
Days since placement	4	42069.90	555.18	0.00	-21030.95
(1 | Site)	3	42072.06	557.34	0.00	-21033.03

Table S1.7. Fixed effect summary for the top-performing foraging duration model. Bald eagle was the reference category for the Species covariate. Pairwise contrasts for the categorical predictor is detailed in Table S1.8

Coefficient	Chi-square	p-value	Exponentiated Estimate	Exponentiated Std. Error
Intercept	155.07	< 0.001	14.58	1.24
Days Since Placement	18.43	< 0.001	0.90	1.03
Avg. Temp. (°C)	34.87	< 0.001	0.78	1.05
Species	558.57	< 0.001	—	—
Black bear	—	< 0.001	2.67	1.19
Coyote	—	0.049	1.48	1.22
Domestic dog	—	0.140	0.51	1.58
Golden eagle	—	0.684	0.95	1.15
Black-billed magpie	—	< 0.001	0.48	1.14
Mountain lion	—	0.371	1.26	1.30
Common raven	—	0.277	1.16	1.14
Red fox	—	0.730	0.94	1.18
Striped skunk	—	0.610	1.30	1.68
Turkey vulture	—	0.109	1.26	1.15

Table S1.8. Pairwise contrasts (Tukey method) for the categorical predictor – species – for the top foraging duration model. The ratio column represents the estimated ratio of foraging bout duration means for the two species being compared. Values greater than 1 indicate that the first species in the contrast has a higher estimated response than the second.

Contrast	Ratio	Standard Error	z-ratio	p-value
Black bear / Magpie	5.53	0.67	14.06	< 0.001
Magpie / Common raven	0.42	0.03	-11.62	< 0.001
Magpie / Turkey vulture	0.38	0.03	-14.96	< 0.001
Golden eagle / Magpie	1.96	0.15	8.63	< 0.001
Coyote / Magpie	3.07	0.46	7.45	< 0.001
Black bear / Golden eagle	2.82	0.41	7.24	< 0.001
Black bear / Red fox	2.83	0.45	6.50	< 0.001
Magpie / Red fox	0.51	0.05	-6.38	< 0.001
Black bear / Turkey vulture	2.12	0.26	6.18	< 0.001
Black bear / Common raven	2.31	0.33	5.85	< 0.001
Bald eagle / Magpie	2.07	0.27	5.69	< 0.001
Bald eagle / Black bear	0.38	0.07	-5.57	< 0.001
Magpie / Mountain lion	0.38	0.09	-4.29	< 0.001
Black bear / Domestic dog	5.24	2.38	3.65	0.012
Black bear / Coyote	1.81	0.33	3.19	0.054
Black bear / Mountain lion	2.12	0.50	3.19	0.055
Golden eagle / Turkey vulture	0.75	0.08	-2.85	0.140
Coyote / Golden eagle	1.57	0.26	2.66	0.217
Coyote / Red fox	1.57	0.27	2.62	0.239
Coyote / Domestic dog	2.90	1.22	2.53	0.286
Red fox / Turkey vulture	0.75	0.09	-2.36	0.393
Golden eagle / Common raven	0.82	0.08	-2.12	0.566
Domestic dog / Turkey vulture	0.41	0.18	-2.05	0.616
Bald eagle / Coyote	0.68	0.13	-1.97	0.668
Magpie / Striped skunk	0.37	0.19	-1.97	0.672
Domestic dog / Mountain lion	0.40	0.20	-1.86	0.746
Domestic dog / Common raven	0.44	0.120	-1.84	0.757
Bald eagle / Turkey vulture	0.80	0.11	-1.61	0.881
Common raven / Red fox	1.23	0.16	1.59	0.887
Bald eagle / Domestic dog	1.96	0.90	1.47	0.929
Coyote / Common raven	1.28	0.22	1.46	0.932

Contrast	Ratio	Standard Error	z-ratio	p-value
Domestic dog / Striped skunk	0.39	0.26	-1.42	0.944
Domestic dog / Golden eagle	0.54	0.24	-1.39	0.952
Black bear / Striped skunk	2.05	1.06	1.38	0.953
Domestic dog / Red fox	0.54	0.24	-1.38	0.954
Golden eagle / Mountain lion	0.75	0.18	-1.21	0.981
Mountain lion / Red fox	1.33	0.33	1.18	0.985
Bald eagle / Common raven	0.86	0.12	-1.09	0.992
Coyote / Turkey vulture	1.18	0.18	1.04	0.994
Bald eagle / Mountain lion	0.79	0.21	-0.89	0.998
Common raven / Turkey vulture	0.92	0.09	-0.85	0.999
Golden eagle / Striped skunk	0.73	0.37	-0.63	1.000
Coyote / Mountain lion	1.17	0.30	0.63	1.000
Red fox / Striped skunk	0.72	0.37	-0.63	1.000
Bald eagle / Striped skunk	0.77	0.40	-0.51	1.000
Bald eagle / Golden eagle	1.06	0.15	0.41	1.000
Mountain lion / Common raven	1.09	0.26	0.36	1.000
Bald eagle / Red fox	1.06	0.18	0.35	1.000
Coyote / Striped skunk	1.13	0.58	0.24	1.000
Domestic dog / Magpie	1.06	0.47	0.12	1.000
Golden eagle / Red fox	1.00	0.13	0.01	1.000
Mountain lion / Striped skunk	0.97	0.53	-0.06	1.000
Mountain lion / Turkey vulture	1.00	0.23	0.01	1.000
Common raven / Striped skunk	0.89	0.45	-0.23	1.000
Striped skunk / Turkey vulture	1.04	0.53	0.07	1.000