ESCHERICHIA COLI AND *SALMONELLA* SP. IN DOMESTIC CATTLE AND WILD ROOSEVELT ELK: FECAL PATHOGENS AT THE WILDLIFE-DOMESTIC INTERFACE

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ABSTRACT

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Direct or indirect contact between domestic populations of animals and wildlife carries an inherent risk for transmission of pathogens that cause infectious disease. In Humboldt and Del Norte counties of northern California, ongoing conflict between ranchers and Roosevelt elk groups results from elk use of ranching pastures and pastures on private land. Fecal samples from elk in association with cattle, cattle, and from elk not in known association with cattle were assessed for the presence of bacteria *Salmonella enterica* and pathogenic *Escherichia coli* to assess whether association with cattle increases risk of infection for elk. Group identity (one of the elk groups or cattle group) was the leading parameter in infection likelihood models, and elk in association with cattle were over nine times more likely to have pathogenic non-O157:H7 *E. coli* isolated from their feces than elk that were not in association with cattle.

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INTRODUCTION

Direct or indirect contact between domestic populations of animals and wildlife carries an inherent risk for transmission of pathogens that cause infectious disease. For example, in California, tule elk (*Cervus canadensis nannodes*) can develop symptomatic or asymptomatic infections of *Mycobacterium avium paratuberculosis* (Manning et al. 2003). The associated disease, paratuberculosis or Johne's disease, causes a chronic granulomatous enteritis (inflammatory bowel disease) and can cause significant losses in domestic livestock as well as elk (Crawford et al. 2006, Forde et al. 2015). The transmission of *Brucella abortus* between wild Rocky Mountain elk (*Cervus elaphus nelsoni*), bison (*Bison bison*), and domestic cattle is of great concern in the greater Yellowstone area. This zoonotic disease agent causes brucellosis in ruminants and people and has been an important problem for reasons related to public health, agriculture, wildlife management and conservation (Bienen and Tabor 2006, Rhyan et al. 2013).

In Humboldt and Del Norte counties, California, USA, some Roosevelt elk (*Cervus canadensis roosevelti*) share pasturelands and meadows with domestic cattle (California Department of Fish and Wildlife 2018). This causes significant conflict requiring collaboration between landowners and wildlife managers (California Department of Fish and Wildlife 2018). In such situations shared cover, forage, mineral, and water resources are conducive to pathogen transmission (Dohna et al. 2014).

Humboldt and Del Norte counties are within the native geographic range of Roosevelt elk, with groups[a] living across public and private land, in rural and semi-rural areas, and throughout several types of habitat. Roosevelt elk historically occupied the upper third of coastal California, north throughout the coastal range to Vancouver Island in British Columbia, and eastward possibly as far as Mount Shasta (Harper et al. 1967, Quayle and Brunt 2003). As of the 1950s, the southern reaches of the population had been extirpated and Roosevelt elk ranged discontinuously from the Pacific coast forests and mountains of Humboldt County north through western Washington, with a population also on Vancouver Island, BC (Harper et al. 1967). Roosevelt elk have since recovered significantly in northern California, with an estimated population of 5700 in the state and ranging through much of Del Norte, Humboldt, Mendocino, Trinity and Siskiyou counties as well as western parts of Shasta County (California Department of Fish and Wildlife 2018).

Beef and dairy cattle are raised commercially and privately in northwestern California. There are no available data below county-level on population level or breakdown by production breed, and attempts to obtain more information through local associations were unsuccessful. Broadly, most beef ranchers in Humboldt and Del Norte counties utilize angus breeds including crosses with Herefords and Charolias while dairy cattle are largely Jersey hybrids with Holstein or other large producing breeds (J.W.

[a] 'Groups' refers to spatially discrete social sets of largely female and young male individuals that stay together the majority of the year. These might otherwise be referred to as herds, but these groups have not yet been classified as herds by California Department of Fish and Wildlife, with whose funds this project was conducted.

Stackhouse, UC Cooperative Extension, personal communication). Del Norte County is home to approximately 9700 cattle as of 2017 in 80 km^2 of land in farms. Humboldt has nearly 71,000 cattle on 2513 km^2 of farmland (United States Department of Agriculture 2019).

Elk and Cattle Disease

There have not been any extensive evaluations of infectious pathogens in Pacific northwest Roosevelt elk, though a number of small scale or anecdotal reports exist (Orr 1937, Bender et al. 1999, Wengert 2000) (Table 1). These pathogens generally can also infect cattle, though for those only identified to genus or family this may be dependent on species.

*Table 1. Summary of infectious and parasitic disease reports for Roosevelt elk (*Cervus canadensis roosevelti*) in the Pacific Northwest, USA. Cause of death (COD) is reported when animals died and were not euthanized for research.*

Pathogen	Impacted (examined)	Analysis	Notes	Reference	Location
Eimeria sp.	1 (20)	Fecal		Wengert 2000	Sinkyone Wilderness
Trichostrongylidae	15 (20)	Fecal		Wengert 2000	Sinkyone Wilderness
Other nematodes	14 (20)	Fecal		Wengert 2000	Sinkyone Wilderness
Dictyocaulus sp., *Trichuris* sp., *Capillaria* sp., *Strongylus* sp., coccidia	6 (6)	Fecal	Mostly in low numbers	Han and Mansfield 2014	SW Washington
Dictyocaulus viviparous	12 (16)	Fecal		Han 2019	SW Washington
Eimeria sp.	11 (16)	Fecal		Han 2019	SW Washington
Nematodes	16 (16)	Fecal		Han 2019	SW Washington
Treponema sp.	6 (14)	Hoof samples	Immunohistochemistry on select lesions	Han 2019	SW Washington
Clostridium haemolyticum	1(1)	Necropsy	COD bacillary hemoglobinuria	Bender 1999	Cathlamet, Washington
Lungworms, likely *Dictyocaulus viviparous*	3(3)	unknown	COD pneumonia	Orr 1937	Sequoia Park Zoo

None of these are known or expected to cause population-level disease in this area, with the exception of *Treponeme*-associated hoof disease (TAHD) which has recently appeared and is under investigation in Del Norte County (Han and Mansfield 2014, Han et al. 2019, California Department of Fish and Wildlife 2020).

In other areas of North America, diseases such as epizootic hemorrhagic disease (EHD), blue tongue, and Johne's disease affect cervid populations, but they have not been detected in Humboldt or Del Norte County elk serology and fecal testing (Carrington Hilson, California Department of Fish and Wildlife, unpublished data) (Cover et al. 2011, Rhyan et al. 2013). Of these, only Johne's is of concern to the cattle dairy or beef industries. Other significant cattle diseases with the potential to infect cervids include bovine viral diarrhea virus (BVDV, caused by *Pestivirus* spp. viruses) and bovine tuberculosis (caused by bacteria *Mycobacterium bovi*s) (Van Campen and Rhyan 2010, Passler et al. 2016). Bovine viral diarrhea virus has been detected serologically in Nebraska elk but not in Colorado, and there are no publicly available reports of elk disease or transmission to cattle (Duncan et al. 2008, Cover et al. 2011). *Mycobacterium bovis* can infect elk, though it has not been detected west of Montana (Miller and Sweeney 2013, Cattle Health Center 2022).

Cattle and wild ruminants are known reservoirs of pathogenic bacteria, including *Escherichia coli*, with the potential for transmission, colonization and re-colonization between populations of other species and beef cattle. Beef cattle alone were responsible for a third of all human outbreaks of pathogenic O157 *E. coli* between 2003 and 2012 in the United States (Heiman et al. 2015). In Washington the overall prevalence of toxin-

producing *E. coli* in cattle was 7% (Cobbold et al. 2004). In a review of beef cattle, pathogenic *E. coli* were recovered from feces at a weighted mean prevalence of 6.2% (range 0-57%) (Rhoades et al. 2009). Individuals can suffer classic disease signs but can also remain asymptomatic carriers (Alexander et al. 2009). Fecal bacteria are not well-studied in elk, but wildlife can contribute to fecal pollution in forested and fragmented habitats, and wild ruminants are potential pathogenic *E. coli* infection sources for humans and domestic ruminants (Sánchez et al. 2009, Franklin et al. 2013, Guber et al. 2015). Pathogenic *E. coli* have been found in a variety of wild cervids including red deer (*Cervus elaphus*), Rocky Mountain elk, white-tailed deer *(Odocoileus virginianus)*, roe deer (*Capreolus* spp.), and fallow deer (*Dama dama*) (Pagano et al. 1985, Renter et al. 2001, Sánchez et al. 2009, Franklin et al. 2013, Szczerba-Turek and Kordas 2020). In contrast to pigs, rabbits, and humans, cattle demonstrate limited distribution of the receptors for Shiga toxins including no expression in vasculature, which makes them resistant to pathology from this toxin (Pruimboom-Brees et al. 2000). Characterization of elk (identified only as *C. canadensis*) gut microbiota found similar bacterial taxonomic groups and diversity levels as in cattle suggesting similar biochemistry (Kim et al. 2019).

Cattle also harbor *Salmonella enterica*, and it has been implicated in deaths of captive elk, mostly calves (Foreyt et al. 2001, Hattel et al. 2007, Rhoades et al. 2009, Xie et al. 2016). Fecal prevalence estimates of *S. enterica* in the commercial cattle population are similar to those reported for pathogenic *E. coli*: 0.13% in rangeland cattle of a major produce region of California, 5.4% in Nebraska beef cattle, and 6.8% in Australian beef cattle (Fegan et al. 2004, Gorski et al. 2011, Schmidt et al. 2015). Cattle can be the source

of transmission to wildlife, as with wild boar in Catalonia (Mentaberre et al. 2013) *Salmonella enterica* was detected in 1% of white-tailed deer feces surveyed in Nebraska; the samples were hunter-submitted with unknown conditions prior to processing so this is a conservative percentage (Renter et al. 2006). Shared genetic profiles of pathogenic *E. coli* were found in isolates from deer and cattle in Michigan, indicating transmission although the direction was unknown (Singh et al. 2015). Sharing of pathogenic intestinal bacteria between elk and cattle has not been thoroughly explored.

Given the pathogen reservoir status of these species, a survey of infection and subsequently transmission risk is a useful exploration of disease ecology in this system. Here, I determined prevalence and variables correlated with infection of *E. coli* and *S. enterica* strains in selected groups of Roosevelt elk and associated cattle in northwestern California. I hypothesize that elk in proximity to cattle and possibly people will demonstrate higher prevalence of these pathogens.

The Pathogens

Salmonella enterica and *E. coli* are members of the family *Enterobacteriaceae* which are gram-negative, facultative anaerobic bacteria. Both are differentiated into a myriad of strains, some of which are pathogenic with specific virulence factors known to cause mild to serious disease across many host taxa. I will be using both the terms strains and types: strains are genetically differentiated. ‘Type’ will be used here to refer to groups of strains. These bacteria also have the potential to exchange genetic sequences among strains, and with other enteric bacteria species, some of which confer antibiotic

resistance (Schmidt et al. 2015). That potential, in addition to both infecting the gut, means they do not necessarily work in isolation of one another.

Escherichia coli

Escherichia coli lives in the intestinal tract and feces of warm-blooded animals and reptiles as well as environmental sources (Tenaillon et al. 2010). It is used by the Environmental Protection Agency (EPA) as an indicator organism for fecal contamination and bacterial encroachment of watersheds, as it can be transmitted through water and sediment (Tenaillon et al. 2010, Dirk van Elsas et al. 2011, Guber et al. 2015). Transmission usually occurs via a fecal-oral route though some species of flies act as mechanical vectors by contaminating food or water (VanderWaal et al. 2014). Pathogenic strains can cause diarrhea, enterocolitis, and hemolytic uremic syndrome (destruction of red blood cells and platelets leading to kidney failure) in humans (Bardiau et al. 2010).

These bacteria are frequently found in injured or diseased wild individuals though it is not always possible to tell whether it is the primary pathogen causing disease (Johnson et al. 1998, Hattel et al. 2007). It also exists at non-negligible levels in healthy individuals and populations, which can act as reservoirs, e.g. red deer in Spain and white-tailed deer in Nebraska (Renter et al. 2001, Díaz-Sánchez et al. 2013).

Escherichia coli has been broken up into eight phylogenetic groups, or phylogroups: A, B1, B2, C, D, E, F, and G. (Tenaillon et al. 2010, Koh et al. 2022). While these phylogroups do not have strict host associations, phylogroup A is generally associated with humans, while B1 and B2 are found more often in non-human mammals including wildlife (Tenaillon et al. 2010, Blyton et al. 2015, Mercat et al. 2016). Much of

the literature investigating *E. coli* in non-human animals focuses on strains classified by virulence property or antigen typing and are not differentiated phylogenetically, which may be one reason that host associations for the other phylogroups remain unclear (Sánchez et al. 2010, Tomino et al. 2020). In addition the C, E, F and phylogroups are rare relative to A, B1, B2 and D (Koh et al. 2022).

While there are several ways to characterize *E. coli* strains, this study used serogroups, based on the 'O' surface antigen: *E. coli* serogroup O157 possesses O antigen 157 versus non-O157 serogroups which do not. Serogroups are composed of serotypes, further distinguished using the H antigen. For example, serotype O157:H7 is a pathogen notorious for causing outbreaks of disease in humans and other animals (Lawn et al. 1977, Orskov et al. 1977, Capps et al. 2021). Serogroup O157 along with a selection of non-O157 serogroups are known for causing significant disease because of their *stx* genes which produce the Shiga toxin, making them shigatoxigenic *E. coli* (STEC) (Bardiau et al. 2010)[b]. Production of Shiga toxin (Stx, also called verotoxin) is one of the primary virulence characteristics of pathogenic *E. coli* along with the production of lesions that efface intestinal mucosa (Bardiau et al. 2010). The Shiga toxin can cause hemolytic uremic syndrome, edema, and hemorrhagic colitis (intestinal infection and toxin production leading to mucosal erosions and bloody diarrhea) (Sánchez et al. 2009).

The O157:H7 serotype has been the focus of many studies, to the neglect of non-O157:H7 STEC *E. coli* which also causes significant disease (Rounds et al. 2012, Fan et

[b] Shigatoxigenic *E. coli* are also considered enterohemorrhagic *E. coli* or EHEC.

al. 2019). Serotypes do not always reflect evolutionary relatedness due to antigenic shift and genetic exchange (Ju et al. 2012). This is also true of pathovars (STEC is a pathovar, characterization by pathology). While the previously described broad phylogeny of *E. coli* has been affirmed through multiple analyses, pathogenicity and serology, characteristics are not generally constrained to specific clades (Ishii et al. 2007).

Salmonella enterica

Salmonella enterica is an intracellular pathogen transmitted by ingestion of contaminated water or food (Coburn et al. 2007). Both wild and domestic animals can become infected and shed bacteria in feces with no clinical illness. Further, *S, enterica* survives well outside the host once shed (Bleasdale et al. 2009, Smith et al. 2002). This species is one of the primary causes of human bacterial gastroenteritis, causing 11% (approximately 1 million cases) of foodborne illnesses and 28% (approximately 378) of foodborne illness deaths annually in the United States (Scallan et al. 2011). Worldwide, *S. enterica* causes up to 1.3 billion human cases annually which take three major forms: enteric fever (typhoid fever), enterocolitis (bloody diarrhea), or bacteremia (bacteria in the blood) (Coburn et al. 2007). Non-human animals are one of the main sources of (non-typhoidal) *S. enterica* involved in human infections (Renter et al. 2006). In bovids and cervids, *S. enterica* are more likely to cause clinical disease and cause death in young animals, though this also depends on type (Foreyt et al. 2001, Mohler et al. 2009).

There were 2,557 documented *Salmonella* sp. serovars per the World Health Organization's most recent comprehensive report, and more are discovered every year (Grimont and Weill 2007, Bale et al. 2016). Serovars differ in antigenic presentation, and

are typified by host specificity or what sort of clinical disease they cause (Foreyt et al. 2001, Gal-Mor et al. 2014). These are divided into two types: typhoidal and non-typhoidal, which differ both in the mechanism and overall pathology of diseases. Typhoidal *S. enterica* serovars are specific to humans, and quite rare compared to the non-typhoidal serovars which cause the majority of non-human animal disease (Gal-Mor et al. 2014). A variety of serovars have been found in United States wildlife and cattle, none of which are typhoidal (

Table *2*).

Table 2. Salmonella enterica *serovars isolated from various species of wildlife and domestic cattle in the United States, including marine birds and mammals in rehabilitation centers, white-tailed deer (*Odocoileus virginianus*), Florida Key deer (*Odocoileus virginianus clavium*), Rocky Mountain elk (*Cervus canadensis nelsoni*), farmed red deer (*Cervus elaphus*) and domestic cattle (*Bos taurus*)*[a].

Serovar	Rehabilitated wildlife	WT Deer	FK Deer	RM Elk	Red Deer	Cattle
Agona	x					
Anatum						X
Dessau		x				
Enteriditis	x	x				
Give						X
Hartford			x			
Infantis		x				
Johannesberg	x					
Kentucky						x
Kralendyl			x			
Litchfield		x				
Montevideo	x					x
Newport	x				x	
Ohio	x					
Reading	x					
Serovars						
St. Paul	x					
Thompson					x	
Typhimurium				x		x
Weltevreden			x			

[a] (Foreyt et al. 2001, Sato et al. 2001, Nettles et al. 2002, Smith et al. 2002, Fegan et al. 2004, Adaska et al. 2006, Renter et al. 2006, Hattel et al. 2007, Mentaberre et al. 2013, Berardi et al. 2014, Xie et al. 2016)

Typhimurium is one of most important *Salmonella* serovars in terms of economic losses in livestock and severe human disease. A subtype of this known as *S. typhimurium* phage type 104 (DT104) is epidemic throughout North America and Europe and is

resistant to at least 5 antibiotics including ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline (Foreyt et al. 2001). In California sea lions (*Zalophus californianus*), 2% of feces and 5% of vomit samples contained *S. enterica* but it was cautioned that this estimate was likely low compared to actual prevalence (Berardi et al. 2014). Testing of birds and marine mammals at rehabilitation centers in California resulted in an overall prevalence of 4% and all isolates showed some level of resistance to antibiotics (Smith et al. 2002).

Predictions

My predictions are as follows, beginning with the central hypothesis and including related expectations based on epidemiological principles and review of the literature.

1. Increased proximity to people and cattle is expected to be correlated with higher prevalence of *S. enterica* as well as pathogenic *E. coli*.
2. *E. coli* prevalence likely will be high, nearly 100% as it is a common commensal gut microbe, but I expect *S. enterica* prevalence to be low, probably below 10%.
3. Larger groups of cattle or elk will have a higher prevalence of *S. enterica* as well as pathogenic *E. coli* strains. I do not expect prevalence to vary with age for either *E. coli* or *S. enterica* because age isn't a definitive factor in domestic cattle unless newborn calves are tested (Gow et al. 2008*a*, *b*, Rhoades et al. 2009).
4. Serotypes B1, B2 and D of *E. coli* are predicted to be found most frequently. In terms of *S. enterica* serovars, cattle will be most likely to be infected with

Typhimurium, Heidelberg, Anatum, Muenster, Dublin, Cerro, Montevideo, Give, Kentucky, and Muenchen which are the most common in the US including California (Sato et al. 2001, Fegan et al. 2004, Morningstar-Shaw et al. 2016, Xie et al. 2016). Serovars found in elk will likely be those found most commonly in California wildlife, including Typhimurium, Enteritidis, Infantis, Newport, Montevideo, Saint Paul, Reading and Agona (

5. Table *2*). I expect that the subtypes found in cattle or those in common between the two lists (Typhimurium, Newport, Montevideo) will be more likely found in the elk sharing grazing areas with cattle.

STUDY AREA

The elk groups under study are located in northern California counties of Del Norte and Humboldt, from 41° 59' 21" N (in Del Norte) to 40°23’24” N (in Humboldt) and from 124° 15' 47" W to 123° 32’ 32” W. Elevation in these counties ranges from sea level to over 2000m. This area is patchily inhabited by elk groups of varying size, and movement patterns (Carrington Hilson, California Department of Fish and Wildlife, personal communication). The elk utilize various habitats including coastal dunes, coastal and inland forested areas and grazed pastures (Weckerly and Ricca 2000, Weckerly 2005, Weckerly et al. 2013, Kolbe and Weckerly 2015). My sampling areas are named for landmarks or land ownership. From south to north the sampling areas are: Orick, Gold Bluffs, Crescent Beach, and Lincoln (Figure 1).

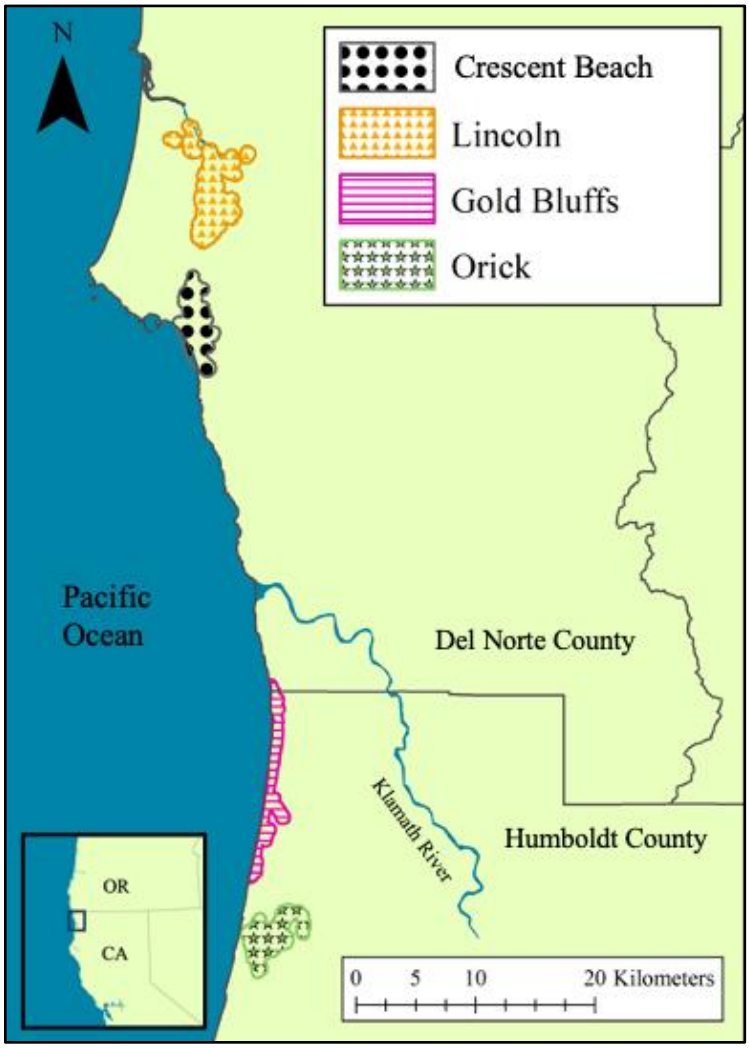


Figure 1. Study areas in Del Norte and Humboldt counties California, USA, 2018. Four target elk groups indicated by color and shapes. Orick and Lincoln are cattle-associated, cattle groups not indicated to ensure rancher privacy. (California Department of Forestry and Fire Protection 2009, GeoServer 2018)

The Orick sampling area is a privately-owned pasture-grazed cattle ranch bordered by National Parks forest and other private residences and ranches. Dominant species in the pasture include velvet grass (*Holcus lanatus*), slough sedge (*Carex obnupta*), silverweed (*Potentilla anserina*), clovers (*Trifolium* sp.), buttercup

(*Ranunculous* sp.), and tufted hairgrass (*Deschampsia cespitosa*). The surrounding forest includes Douglas fir (*Pseudotsuga menziesii*), red alder (*Alnus rubra*), Sitka spruce (*Picea sitchensis*), huckleberry (*Vaccinium ovatum*) and thimbleberry (*Rubus parviflorus*).

Gold Bluffs Beach (Gold Bluffs) is an area of coastal dunes, coastal swamps and abuts coniferous forest with occasional grassland meadows. The sampling location for this group was a coastal prairie, with dominant species including European beachgrass (*Ammophila arenaria*), slough sedge, soft rush (*Juncus effusus*), sweet vernal grass (*Anthoxanthum odoratum*), coyote brush (*Baccharis pilularis*), buttercup, velvet grass, silverweed, clovers, and giant vetch (*Vicia gigantea*). Dominating trees include Sitka spruce, red alder, and conifer forest. This area is within the Prairie Creek Redwoods State Park, with visitor levels that vary by season. Monthly precipitation for the Orick and Gold Bluffs Beach area between July and December ranged from zero to 37 cm between 2010 and 2015 (National Centers for Environmental Information (NCEI) 2019). Temperature historically ranges from 5 to 21.4° Celsius between July and December, the sampling period for this study (Western Regional Climate Center 2021).

Crescent Beach refers to the area surrounding the Crescent Beach Education Center: a recreational coastal beach at the southern end of Crescent City (population 7,643) (United States Census Bureau 2019). The beach is bordered by small marshes and prairies which include cow parsnip (*Heracleum maximum*), slough sedge, curled dock (*Rumex crispus*), umbrella sedge (*Cyperus* sp.), Pacific blackberry (*Rubus ursinus*), Himalayan blackberry (*Rubus armeniacus*), and buttercup. There are also thickets

composed of rhododendron (*Rhododendron maximum*), coastal willow (*Salex hookeriana*), Sitka spruce, pampas grass (*Cortaderia selloana*), cascara buckthorn (*Frangula purshiana*), California laurel (*Umbellularia californica*), and red alder. These are surrounded by the edge of coniferous forest: tan oak (*Notholithocarpus densiflorus*), sword fern (*Polystichum munitum*), elks clover (*Aralia californica*), skunk cabbage (*Symplocarpus foetidus*), salmonberry (*Rubus spectabilis*), red huckleberry, and thimbleberry. The sampling for these elk took place on a large lawn at the Education Center.

The Lincoln group is found primarily along Kings Valley Road, running parallel to highway 101 north of Crescent City and lined with residences and ranchland. These elk and cattle were sampled from a single large, private, cattle-grazed pasture in this area. Dominant species noted here include California fescue (Festuca californica), sweet vernal grass, slough sedge, Pacific and Himalayan blackberries, buttercup, sorrel (*Oxalis oregana*), dandelions (*Taraxacum* sp.), coyote brush, soft rush, big leaf maple (*Acer macrophyllum*), Douglas fir, and redwoods (*Sequoia sempervirens*). Monthly precipitation in these two sampling areas ranged from zero to 61.5 centimeters between July and December of 2014-2019 (National Centers for Environmental Information (NCEI) 2019). Crescent City temperature for the months July through December historically ranges from 4.5-19.7° Celsius (Western Regional Climate Center 2021).

MATERIALS AND METHODS

Elk and cattle groups were chosen based on property access and size of groups such that elk group size did not vary widely between experimental groups (Table 3). All animal observation and fecal collection was done in accordance with guidelines by the HSU (Humboldt State University) Institutional Animal Care and Use Committee (IACUC 17/18.W.45-A approved 1/26/2018). Land access was granted through verbal consent from private landowners and permits or written agreements with agencies. Prior to each sampling session, the appropriate landowner or agency was notified via phone or e-mail per previous agreement.

Table 3. Elk groups chosen for fecal sampling in Humboldt and Del Norte counties, California USA with approximate group sizes, locations, and land ownership. Group sizes are approximate based on recent surveys (Carrington Hilson, California Department of Fish and Wildlife, personal communication).

	Group	Size	Habitat	Location	Land Ownership
Cattle-Associated	Orick	60	Forest and ranchland	Orick	Private
	Lincoln	30	Pasture and forest	Smith River	Public and private
Non-Cattle-Associated	Gold Bluffs	30	Beach and forest	Gold Bluff Beach	California State Parks
	Crescent Beach	60	Pasture and forested backdunes	Crescent City	California State Parks, private, tribal

Fecal Sampling

Elk fecal sampling

Elk groups were located for sampling sessions using a combination of road surveys, radio telemetry and GPS (Global Positioning Systems) data from GPS-collared elk. One or more cow elk in each of the above groups had been captured and fitted with collars in a collaboration between HSU and CDFW (California Department of Fish and Wildlife) at least several months prior to this study. Once located, the elk group was observed, and available location data were used to determine whether the group had remained in one area for approximately two hours minimum. This was done to ensure that once fecal sampling began, sufficient fecal depositions would be found. Elk were observed from a minimum distance of 50 m. If the elk themselves moved closer, the observer would then move away if the elk approached within 30m or appeared anxious or aggressive. Elk were observed until they moved away from the area and the samples could be collected; sampling was abandoned when rain beyond a drizzle persisted.

Assessment of fecal depositions began with an infrared thermometer as soon as the elk moved away from an area (Raytek, Santa Cruz, California, USA). Elk defecate frequently and often simultaneously when they all start moving away from an area, thus if they spend at least a couple hours feeding or resting it is likely that most individuals have defecated in that area (personal observation). In the Uinta Mountains of Utah, elk were observed to defecate just over 23 times per day (Collins 1977). The infrared thermometer was used to find the warmest samples, assumed to be the freshest subset of

fecal samples to maximize the likelihood of obtaining samples from all individuals present. The area was walked in a serpentine fashion, searching for all fecal depositions. Each was measured by aiming the ray of the thermometer at the bulk of the deposition from about six inches away. The warmest feces were chosen, totaling the number of individuals counted during observation.

PELLETS WERE COLLECTED WITH A CLEAN GLOVE FROM EACH NEW SAMPLE IF PELLETS WERE PRESENT. IF THE SAMPLE WAS SOFT OR LIQUID, A STERILE DISPOSABLE SPOON (NASCO, FORT ATKINSON, WISCONSIN, USA) WAS USED TO PLACE FECES WITHIN A LABELLED WHIRL-PAK® (NASCO, FORT ATKINSON, WISCONSIN, USA) WHICH WAS THEN CLOSED. ONE GLOVE WAS REMOVED TO OPEN A RESEALABLE BAG, INTO WHICH THE STILL-GLOVED HAND PLACED THE SAMPLE. GLOVES WERE REMOVED AND THE RESEALABLE BAG WAS CLOSED AND PLACED IN A COOLER WITH AN ICE PACK. A GPS POINT WAS TAKEN FOR EACH SAMPLE AS WELL AS A PHOTO. FECAL TEXTURE, SIZE, APPEARANCE, AND ANY OTHER DISTINGUISHING CHARACTERISTICS WERE RECORDED ON THE DATASHEET (

Appendix A). Mucous was recorded as no mucous, some mucous, or much mucous, in addition to color (brown, yellow, or red). Elk pellets were measured in two dimensions if possible (some samples were too clumped or soft to determine pellet size) to facilitate possible age discrimination (Appendix C). Some samples were recorded as "medium" or "large" when direct measuring was not possible due to clumping. All elk sampling sessions consisted of one day total while cattle sampling for a given group of cattle could take multiple days as explained below.

Cattle fecal sampling

Cattle groups overlapping the cattle-associated elk at Orick and Lincoln sites were chosen based on the willingness of the landowner to participate in the study. The exact locations and rancher identifications are being withheld to respect requests for privacy. The Orick cattle group consisted of over 40 beef cattle, pasture-raised and observed to share forage with, and be in close association with, elk. These were largely cows of reproductive maturity, plus one bull and a few calves and yearlings. The Lincoln cattle group consisted of 35 dairy cows at reproductive maturity, observed during this study to share forage with elk.

All cattle in these groups were individually identifiable, either through ear tags applied by the ranchers or natural markings. Initially the cattle were observed, and their identifiers described as they defecated, but partway through the study a catalogue was created of tags or markings used to differentiate them. Sampling began with observation of the cattle group and defecation by identified individuals; thus the thermometer was not used to find the most recent samples. The observer's location was fixed and recorded both with coordinates and a landmark. Once defecation occurred, the cow's identifiers were recorded as well as a location based on distance using a rangefinder and bearing using a standard compass. This continued until either all cattle of interest had defecated, daylight was running out, or weather was deteriorating. Each fecal deposition was located using the distance and bearing recorded with reference to the original observer location. If a deposition was not located within five meters of the expected location, that sample was excluded.

Methods described above to ensure collection of only one fecal sample per individual were followed except for ten of the Lincoln cattle samples. These cattle were being packed up on a truck upon researcher arrival for the last day of their sampling, and ten samples were collected from the area where they were loaded. These ten samples were the freshest and farthest apart samples in that area and intended to capture some of the individuals who remained to be definitively sampled, though this cannot be known. Efforts to contact the rancher for continued access to the cattle were not returned.

An analysis of the bearing and distance values recorded for cattle fecal deposits was done to compare the forward and reverse bearing and distance measurements and provide some information about whether the correct fecal depositions were sampled. The overall average difference in degrees, or discrepancy, between the forward and reverse bearings was 9.15°, or 6.3° excluding two outliers. The Simmons rangefinder used has error of +/-0.91 meters up to about 9 meters distance, and error of +/- 46.6 meters at 550 meters. Average difference in distance between forward and reverse measurements was 3.23 meters. As accuracy is affected by distance, this was also done in distance categories, across which the average discrepancy in distance was not large while average bearing discrepancy varied (Appendix B). Not all samples had a recorded bearing or distance value as some were collected immediately after deposition. In addition, fecal deposition appearance aided collection: as cattle feces are usually very wet when excavated, the surface dryness was an indicator of freshness.

Fecal Processing

Immediately after collection, each fecal sample was split into aliquots. Approximately one gram was placed in a 15ml conical tube with 95% molecular biology-grade ethanol which was then frozen at a -20°C. Twenty-five grams were placed into one side of a Whirl-Pak® filter bag using a disposable sterile spoon. The remainder of the feces or however much would fit was placed into a fresh, labelled 4 oz. Whirl-Pak® bag and frozen at -20°C. If feces could not be processed immediately after sampling, fecal samples were stored at 4°C for a maximum of three days before processing, generally one day. Exceptions include one opportunistic mortality sample which was refrigerated (4°C) for several weeks before enrichment. Some of the fecal swabs were refrigerated for up to 6 weeks (with a range of 5 days to 6 weeks) before undergoing bacterial enrichment.

Approximately 100-120ml of tryptic soy broth (TSB) was added to the 25g of feces in each of the Whirl-Pak filter bags. The filter bags have a mesh filter down the center of the bag so as to allow liquid but no particles to pass through. This was then closed and placed in a second resealable bag and incubated with shaking at 100 RPM for two hours at 35°C, followed by 12 hours at 42°C. The samples were placed in a refrigerator at 4°C for a minimum of two hours. Five 2ml cryotubes were labelled for each sample, and 1ml of 50% glycerol placed in each. One ml of enriched solution from the liquid-only side of the filter bag was placed into each of the five cryotubes which were stored initially at -20°C then moved to -80°C. Samples were preserved in glycerol

between three and 11 days after enrichment was complete. In some instances, samples were re-enriched before being preserved if more than three days had passed since the first enrichment process.

Bacterial isolation and typing

These samples were shipped to the Rivadeneira Lab at the University of Arizona, where they underwent isolation for *E. coli* and *S. enterica*. The protocol used for *E. coli* O157 detection began with immunomagnetic separation of the TSB enrichment broths using an automated Dynal® BeadRetriever™ (Invitrogen, Carlsbad, California, USA). Resuspended beads are plated onto Rainbow Agar (Biolog, Hayward, California, USA) with novobiocin and tellurite as well as onto MacConkey II Agar with sorbitol and potassium tellurite and Cefixime (a cephalosporin antibiotic) then incubated for 24 hours at 37°C. For non-O157 STEC detection, the pre-enrichment broth was incubated in mEHEC® (MilliporeSigma, Burlington, Massachusetts, USA) selective media then plated on chromogenic STEC agar and positive colonies were confirmed by real-time PCR. The confirmed O157 and non-O157 STEC isolates were characterized using conventional PCR for virulence genes *stx1* and *stx 2*. *Salmonella enterica* was recovered by adding Rappaport-Vassiliadis broth, incubating and plating onto Xylose Lysine Tergitol agar plates then incubated (Dr. Paula Rivadeneira, University of Arizona, personal communication).

Positive *E. coli* samples were sent to Translational Genomics Research Institute (Tgen, Phoenix, Arizona) where they will undergo whole-genome sequencing. This arm of the project was delayed by the SARS-COV-2 pandemic and will be reported when its

analysis is completed. The overall and subtype prevalence of *S. enterica* and *E. coli* were estimated for each northwestern Roosevelt elk group and cattle.

Statistical Analysis

Cattle-elk association

Odds ratios were used to estimate the relative risk of infection in elk based on the presence or absence of association with cattle. Probability of pathogenic *E. coli* and *S. enterica* in elk groups was modeled using binomial generalized linear models, which were compared using Akaike's Information Criterion (AIC). Variables included group, date, average local human population density, and association of elk with cattle. The McKelvey-Zavoina pseudo-R^2 method was used to estimate of the amount of the original variance in infection status explained by the binomial model (McKelvey and Zavoina 1975). There are a number of methods for calculating a pseudo-R^2, and through testing in simulation this method performed best as a measure to correspond to R^2 in the ordinary least squares methods, making it more useful and easier to interpret than some other methods that rely on log-likelihood or ranking (Veall and Zimmermann 1994, Zheng and Agresti 2000).

Mapping and spatial analysis

ArcGIS Desktop (ESRI 2020) was used to create elk home ranges. Points from collar data for one cow from each elk group of interest were cleaned: collar-testing points were removed, time data was removed, and the points were restricted in time to February 2017 (start of current collaring efforts) through December 2018 so that they would be

relevant to the time of fecal collections and span at least one full year of movement. Two of the collars do not span that entire period: Lincoln collar data ends in 2017 due to harvest, while the Orick data starts later than the others in November 2017 when the first cow in the group was collared. Points were then imported and buffered to 500 meters and all points dissolved to make a polygon which was treated as the elk home range. A 2010 human population raster for the area was overlayed and clipped to the home range area, then the average human population for that region was used in modeling as an index of human population density (Falcone 2016). Other data sources used include base maps from ESRI (see map captions), and county outlines (County of Del Norte 2017, Humboldt County Community Development Services 2017).

RESULTS

Between June and December of 2018, 225 fecal samples were collected, 137 from elk (including one fecal sample collected opportunistically from a yearling Lincoln-area elk mortality) and 89 from cattle (Table 4). In addition, 35 rectal swabs were collected from elk calves between May and July 2018 (Table 5), and. This yields a total of 261 samples.

Table 4. Numbers of fecal samples collected from Roosevelt elk and domestic cattle in Humboldt and Del Norte counties, California, USA, 2018.

Species	Sampling Group	Collected	Included
Elk	Crescent Beach	40	40
	Gold Bluffs Beach	26	26
	Orick	40	40
	Lincoln	31	27
	Total elk samples	137	133
Cattle	Orick	59	41
	Lincoln	30	30
	Total cattle samples	89	71

Table 5. Rectal swabs collected from Roosevelt elk calves between May and July of 2018 in Humboldt and Del Norte counties California, USA: total 35.

Elk Group	Collected
Tollowa	6
Big Lagoon	3
Crescent Beach	1
Davison	5
Elk Prairie	1
Maneze/Lyons	9
Orick	5
Red Schoolhouse	4
Reservation Ranch	1

Cattle samples were collected throughout the sampling period, while elk sampling was clustered by elk group as required by the methodology (Figure 2).

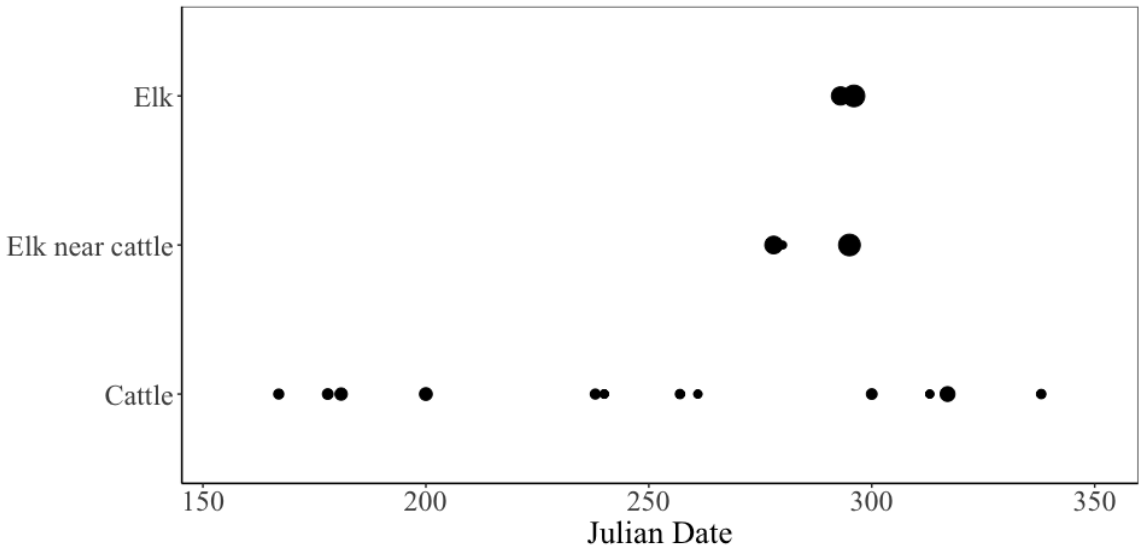


Figure 2. Elk and cattle fecal samples collected by Julian date and sampling category in Humboldt and Del Norte counties California, USA, 2018. Size of dot indicates relative number of samples taken from that group on that day (range 4-40).

A total of 205 fecal samples were cultured and typed by PCR for pathogens: non-O157 *E. coli* was isolated from at least one sample from each group, while O157 *E. coli* was found only in cattle-associated elk groups and *Salmonella* sp. was not found (Table 6, Figure 3). Pathogens were isolated from 21 of the 35 rectal swabs from calves, one of which tested positive for both *Salmonella* sp. and non-O157 *E. coli*. Ten were reported positive for non-O157 while no O157 E. coli was found. The only two *Salmonella* sp. positive samples in this study came from calves (Table 7).

Table 6. Pathogen isolate totals from fecal samples collected from cattle and Roosevelt elk in June-December 2018, Humboldt and Del Norte counties, California, USA.

Group (n)	O157 *E. coli*	Non-O157 *E. coli*	*Salmonella* sp.
Crescent Beach elk (40)	0	2 (5.0%)	0
Gold Bluffs elk (26)	0	2 (7.7%)	0
Lincoln cattle (30)	0	25 (83.3%)	0
Lincoln elk (26)	7 (27.0%)	9 (34.6%)	0
Orick cattle (41)	0	25 (61.0%)	0
Orick elk (40)	1 (2.5%)	17 (42.5%)	0
Lincoln elk mortality	0	1 (N/A)	0
Total (205)	8 (3.9%)	82 (40%)	0

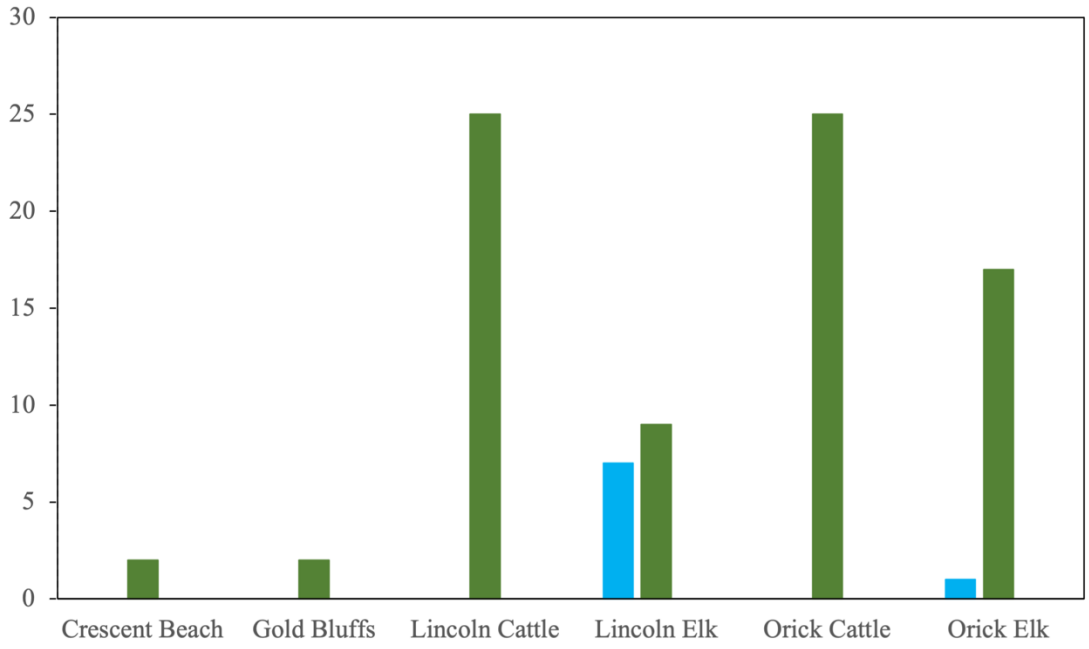


Figure 3. Counts of pathogen detection in fecal samples from selected Roosevelt elk and domestic cattle in Humboldt and Del Norte counties California, USA, 2018. Blue lines (lighter shading) represent counts of O157 E. coli*, and green (darker shading) represents non-O157* E. coli*.*

Table 7. Number of rectal swab samples from which pathogens were isolated, from Roosevelt elk calves in northwestern California, USA, May to July 2018, by elk group with n = total number of swabs tested.

Group (n)	O157 *E. coli*	Non-O157 *E. coli*	*Salmonella* sp.	Total
Tollowa (6)	0	2 (33.3%)	1 (16.7%)	3
Big Lagoon (3)	0	2 (66.7%)	0	2
Crescent Beach (1)	0	0	0	0
Davison[a] (5)	0	4 (80.0%)	1 (20.0%	5
Elk Prairie (1)	0	1 (100.0%)	0	1
Maneze/Lyons (9)	0	3 (33.3%)	0	3
Orick (5)	0	4 (80.0%)	0	4
Red Schoolhouse (4)	0	3 (75.0%)	0	3
Reservation Ranch(1)	0	1 (100.0%)	0	1
Total (35)	0	20 (54.3%)	2 (5.7%)	22 (62.9%)

[a] One sample tested positive for both *Salmonella* sp. and non-O157 *E. coli*.

Mucous was observed in 28 out of 203 samples. With mucous collapsed into a binary of presence vs absence, there was no significant effect on odds of infection based on observed mucous for O157 *E. coli* (p=1 by Fisher's Exact Test). I detected a lower prevalence of infection of non-O157 E. coli when mucous was observed: odds of detection were 86% lower in samples with mucous (CI 42.6%-99%, p = 0.0016 by Fisher's Exact Test).

Statistical Assessment of Infection Risk

When geographic groups of elk were are combined into those associated with cattle and those not associated with cattle (Table 8, Table 9), the elk in contact with cattle were approximately 9.6 times more likely to exhibit infection with non-O157 *E. coli* than elk that were not in contact with cattle (CI of odds ratio 3.41-35.43, $p = 3.27x10^{-6}$, Fisher's exact test). These cattle-associated elk also were also more likely to exhibit infection with O157 *E. coli*. An "enhanced" Haldane correction (which is a conservative method) adds one to the cell (and subtracts one from its row-mate) yielding an odds ratio of nearly 7.9 times more likely for cattle-associated elk to be infected (95% CI 1.36-203.3, p = 0.018, Table 10). No O157 *E. coli* was observed in the cattle tested in association with the O157-infected elk. *Salmonella* sp. did not occur in any adults or elk groups involved in the initial study design, so there were not enough samples for statistical analysis.

Table 8. Number of Roosevelt elk infected with O157 E. coli *by cattle-association in Humboldt and Del Norte counties California, USA, 2018.*

		Not Infected	Infected	Totals
Group	Elk	66	0	66
	Elk near cattle	58	8	66
	Totals	124	8	132

Table 9. Number of Roosevelt elk infected with Non-O157 E. coli *by cattle-association in Humboldt and Del Norte counties California, USA, 2018.*

		Not Infected	Infected	Totals
Group	Elk	62	4	66
	Elk near cattle	40	26	66
	Totals	102	30	132

Table 10. Odds ratios for infection with two serotypes of pathogenic E. coli *in elk associated with cattle (versus elk not associated with cattle) and 95% confidence limits, Humboldt and Del Norte counties California, USA, 2018.*

Pathogen	Odds Ratio	P-value	Lower Confidence Limit	Upper Confidence Limit
O157-E. coli	7.9	0.018	1.36	203.3
Non-O157 E. coli	9.6	$3.3x10^{-6}$	3.41	35.43

Binomial generalized linear models were used to evaluate the effect of the various parameters on the odds of infection with the pathogens (Table 11). The human population density indices included in these models are shown in Figure 4.

Table 11. Top eight candidate binomial generalized linear models for assessment of variation in number of non-O157 E. coli *positives from fecal cultures, ordered by AIC score. LRT is the likelihood ratio test for the model's overall fit versus the null model. Group here refers to membership in the cattle, cattle-associated elk, or elk groups and contributes two estimated parameters, one for each elk type relative to cattle.*

Model	Number of Parameters	AIC / Δ_{AIC}	Weight	LRT
E. coli ~ Group	3	210.91	0.47	$2.4 \cdot 10^{-15}$
E. coli ~ Group + Julian Date	4	1.46	0.23	$1.22 \cdot 10^{-14}$
E. coli ~ Group + Humans	4	1.6	0.21	$1.31 \cdot 10^{-14}$
E. coli ~ Group + Humans + Julian Date	5	3.41	0.09	$6.24 \cdot 10^{-14}$
E. coli ~ Humans + Julian Date	3	42.04	0	$3.22 \cdot 10^{-6}$
E. coli ~ Julian Date	2	47.53	0	$2.45 \cdot 10^{-5}$
E. coli ~ Humans	2	64.17	0	0.28

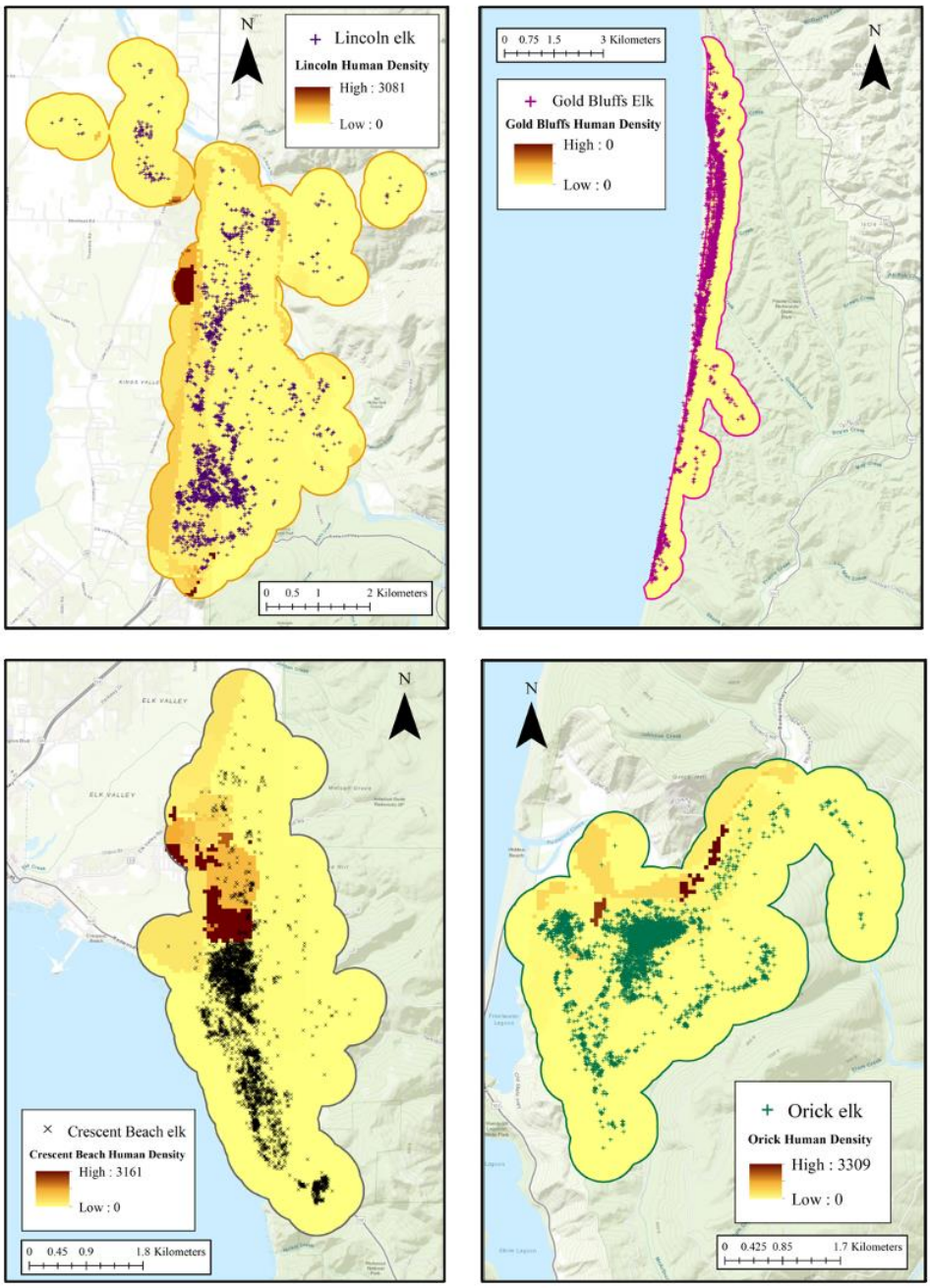


Figure 4. Human density within the range of each elk group (Humboldt on right, Del Norte on left, California, USA) shaded yellow (light) to brown (dark), low to high. (Esri Basemap World Topographic, credits: Esri, DeLorme, HERE, TomTom, Intermap, increment P. Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, GIS User Community.)

The top three models explaining risk of infection all included group (cattle, elk associated with cattle and elk not associated with cattle). The top model with group held more weight than the next two combined, emphasizing the importance of the group variable, which distinguishes elk based on proximity to cattle. Parameter estimates for variables shared between the top three models were in close agreement (Table 12). Based on the likelihood ratio tests comparing each model to its null, all models, except the last, represented a reasonable fit to the data. The McKelvey and Zavoina pseudo-R^2 values are all just over 40% (McKelvey and Zavoina 1975).

Table 12. Parameter estimates from the top three models (within 2 units of lowest AIC score) assessing variables relevant to non-O157 E. coli *infection in Roosevelt elk in Humboldt and Del Norte counties California, USA, in 2018, odds ratio for infection and its standard error (SE), the p-value for that parameter and the confidence interval around the point estimate. M-Z PR^2 = McKelvey-Zavoina pseudo-R^2.*

Model (M-Z PR^2)	Parameter	Odds Ratio	Standard Error	95% Confidence Interval	P-value
Group (0.405)	Elk	0.027	1.782	(0.009, 0.084)	4.21×10^{-10}
	Cattle Elk	0.273	1.436	(0.134, 0.555)	3.36×10^{-4}
Group + Julian Date (0.407)	Elk	0.031	1.836	(0.010, 0.103)	1.19×10^{-08}
	Cattle Elk	0.309	1.486	(0.142, 0.672)	3.03×10^{-3}
	Julian Date	0.997	1.004	(0.988, 1.005)	0.462
Group + Humans (0.408)	Elk	0.025	1.812	(0.008, 0.081)	5.79×10^{-10}
	Cattle Elk	0.274	1.437	(0.135, 0.557	3.51×10^{-4}
	Humans	1.004	1.006	(0.992, 1.015)	0.527

The parameter estimates from the top three models were used to calculate the reduction in risk of infection relative to cattle for the two elk groups, as well as change in risk related to date and human population density (Figure 5).

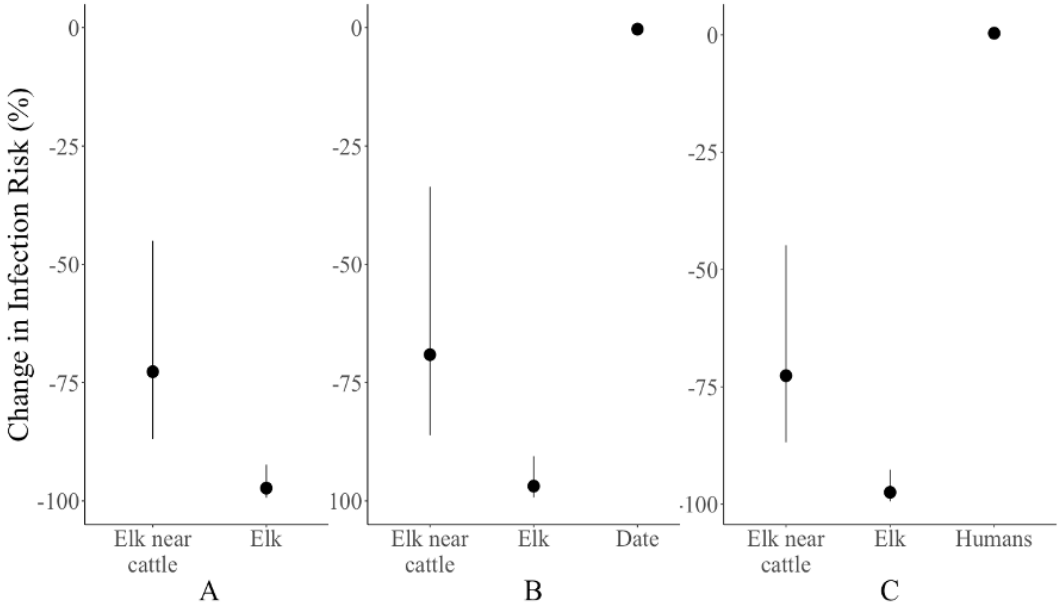


Figure 5. Percent change in infection risk for non-O157 E. coli *in Roosevelt elk of Humboldt and Del Norte counties California, USA 2018, relative to the risk to cattle, based on parameter estimates in top three models (panels A, B and C, respectively).*

DISCUSSION

Out of a total of 205 samples cultured and typed by PCR for pathogens, 8 samples were found positive for O157 *E. coli*, 102 for non-O157 *E. coli*, and 2 for *S. enterica*. The prevalence of non-O157 *E. coli* was high both in cattle (70.4%) and cattle-associated elk groups (39.4%), though no group was completely free of this pathogen. The distribution and prevalence of non-O157 *E. coli* in cattle and elk suggests transmission between these species when sharing space.

The majority of pathogenic *E. coli* isolated from the samples were non-O157 *E. coli* (91.9% of all positive samples including calf swabs). These data agree with findings that non-O157 *E. coli* is often a significant contributor to the pathogenic *E. coli* community at the wildlife-domestic interface (Rounds et al. 2012, Sánchez et al. 2009). It is of interest that very few cattle tested positive for O157 *E.* coli for which they are a known reservoir (Cobbold et al. 2004, Rhoades et al. 2009, Mentaberre et al. 2013).

Of the O157 *E. coli* isolates, seven of eight were found in elk in the Lincoln group. This was unexpected partly because no such isolates were found in the associated cattle group. However, these elk roam across a range that supports other livestock groups which were not sampled for this study (personal observation). Also, the Lincoln cattle sampling was interrupted by the removal of the cattle from that field; ten of those samples were collected after the cattle left and may not represent individuals distinct from those previously sampled. These are also dairy cattle, in contrast to the beef cattle of

Orick and were being grazed some distance from their home ranch. There may be a livestock or other anthropogenic reservoir of these pathogens elsewhere in the area, the elk may be exposed through a different sylvatic source, or the elk may themselves be a reservoir (Somarelli et al. 2007, Bardiau et al. 2010, Franklin et al. 2013, Blyton et al. 2015).

Salmonella sp. were only found in calf samples from non-focus elk groups (not included in the cattle-association study design). The complete lack of *Salmonella* sp. in adult cattle or elk follows from the tendency of this pathogen to sustain infection (and cause disease) in younger animals, which were underrepresented in this study (Coburn et al. 2007, Guizelini et al. 2020). No further statistical analysis was conducted on this pathogen.

The other calf positive for *Salmonella* was also positive for non-O157 *E. coli*, suggesting higher exposure, a random chance, or perhaps the susceptibility of that specific calf which was quite small when tagged (13.78kg vs calf average of around 25.5 kg for that age) and had diarrhea (personal observation). His rectal temperature was high (104.5 F) but not out of range for a hand-captured wild calf (Nigon 2020). Finally, as of 2018 this calf was still alive. There was evidence of disease in this calf, considering the diarrhea and high temperature, though information on his health was limited. The Davison group of elk, to which this calf belonged, are frequently near tourists as well as more recently making forays into the nearby town of Orick (where there are pastures with horses, personal observation).

Three additional calves from the Davison group were positive for non-O157 *E. coli*, though most groups from which calves were tested yielded positives for this pathogen. These groups live in areas that are regularly impacted by people or cattle (this includes feral cattle in some parts of the Bald Hills). Twenty of the non-O157 *E. coli* positives were from calves, four of which came from the Orick elk group, which exhibited a 42.5% prevalence of this pathogen. The other sixteen calf positives were from non-focus elk groups.

Association with cattle, as it has been assessed here, significantly increases the odds of infection with both serotypes of *E. coli* we measured, based on pathogen detection within elk groups. (Table 10). These odds are suggestive that some factor related to cattle exposure or the habitat around cattle ranching does increase the risk of infection in elk, though we cannot confirm that cattle exposure is causally responsible. Once genomic analysis is complete for these samples, we will be able to assess the likelihood of direct links. As the positive sample numbers are much smaller for O157 *E. coli*, as well as the issues with Lincoln cattle sampling previously discussed, this result is not as convincing as that for non-O157 *E. coli* and risk modeling was not performed.

Binomial Generalized Linear Models

The most heavily weighted model in the candidate set uses animal ‘group’ alone to predict infection with non-O157 *E. coli* (Table 11). In all three top models, belonging to one of the two elk groups negatively impacts the odds of infection (Table 12).

Non-cattle associated elk were about 97% less likely to be infected with non-O157 *E. coli* than were cattle, and cattle-associated elk were about 75% less likely than were cattle, including when human population density and date were considered (Figure 5). In further support, the McKelvey-Zavoina pseudo-R^2 values for all three were approximately 40%, suggesting that any one of these models explains about 40% of the variation in the status (infected or not) of the elk. If our models do indeed explain 40% of the variation, with few explanatory variables, this is further indication that association between elk and cattle is an infection risk factor.

Human population density and presence of livestock or cattle were correlated with infection by pathogenic *E. coli,* but since the paired sample groups (cattle and the elk they share forage with) occurred in the same spot the correlation with human density could not be thoroughly assessed here. The elk-only groups were in the lowest (Gold Bluffs) and the highest (Crescent Beach) human density areas in the models. There is almost no change in infection risk based on the human population parameter (odds ratio of approximately one, Figure 6, Panel C) indicating that the group parameter explains more of the observed variation between groups. This is likely therefore an uninformative parameter, or pretending variable in the context of these data. This study was not designed to investigate the impact of local human population density further. Fine-scale livestock maps would be of great use for this as they would enable models to distinguish between human and livestock presence more precisely.

There is some degree of collinearity between Julian date and group due partly to the sampling timeline. Cattle samples were collected throughout the sampling period, while elk samples were collected mostly between Julian dates 275-300, corresponding to the month of October. Each elk group was sampled on a single day in that period. Julian date was retained as a variable because seasonality impacts shedding intensity and infection prevalence for both *E. coli* and *Salmonella* sp. pathogens (Sato et al. 2001, Menrath et al. 2010). This includes a relationship with precipitation as well as higher rates of fecal shedding in the warmer months of fall, and seasonality likely changes between systems depending on ranching methods (Cobbold et al. 2004, Rhoades et al. 2009, Gorski 2012, Schmidt et al. 2015). Without serial fecal sampling, it is difficult to assess the standalone significance of season, but there was very little change in risk associated with seasons (Figure 5, Panel B). Date is also therefore an uninformative parameter, or pretending variable, but only in the specific context of these data.

Mucous was grossly observed in 14% of all fecal samples collected. For O157 *E. coli*, there was no significant effect on the odds of infection from observed mucous. For non-O157 *E. coli*, there was a decreased risk of infection, by 47% ($p = 0.0016$). The reason for this is unclear but could be due to the way this pathogen impacts elk and their fecal deposition. It could also be an artifact of small sample size and lack of rigor in mucous assessment.

Excess mucous in manure is not diagnostic for these infections and there is no published literature on fecal mucous in elk. Thus it is not too surprising that mucous

observed grossly does not aide in infection diagnosis. It is also worth noting that some sample collections extended into evening hours, when lighting was not good for observation. Mucous assessments were not thorough, and more careful examination including microscopic fecal smear assessment would likely be necessary to improve detection.

Overall prevalence of STEC *E. coli* in this study (both O157 and non-O157) was 28.8%. In elk the overall prevalence of non-O157 *E. coli* is 22.7%, among the higher published prevalence values in wild cervids and possibly the highest published prevalence in elk (Espinosa et al. 2018, Tomino et al. 2020) (

Table *13*). It is worth noting that the studies referenced below vary in how narrowly they define the pathovars they are searching for and which genes they target in PCR. In addition, the method of detection used may bias results, and feces collected directly from rectums may give different returns than feces that sit on the ground before collection (Sánchez et al. 2009).

Table 13. Previously reported prevalence of pathogenic E. coli *in wild cervids including bacterial isolation and identification methods (F = fecal culture, S = serology, B = biochemistry, MS = mass spectrometry).*

Species	Prevalence	*E. coli* type	Methods	Region	Study	Notes
Wild cervids	15%	EPEC, STEC	F, PCR	Belgium	Bardiau 2010	
Red deer (206)	24.7%	STEC	F, PCR, S	Spain	Sanchez 2009	Direct from rectum
WT deer (1608)	0.25%	O157:H7	F, S	Nebraska, USA	Renter 2001	Hunter-submitted
Mule Deer (15)	20%	STEC	F, PCR, S	Colorado, USA	Franklin 2013	
Ruminants (33)	4.7	STEC	Varied	Global	Espinosa 2018	Review
Red deer (295)	34.0	Non-O157 STEC	F, PCR, B	South-central Spain	Díaz-Sánchez 2013	1.5% O157:H7
Roe deer (179)	52.5	STEC	F, PCR, S	NW Spain	Mora 2012	
Sika deer (305)	16.7	STEC	F, MS, PCR	Japan	Tomino 2020	2.0% of isolates O157
RM Elk (468)	7.1%	STEC	F, PCR, S	Colorado, USA	Franklin 2013	

The lowest prevalence in this table, 0.25%, is from a study of O157:H7 *E. coli* specifically, and this finding is corroborated by several other O157:H7 studies in wild ungulates (Renter et al. 2001). A study that surveyed 206 red deer in addition to several

other species found only a 1.5% O157:H7 prevalence in the roe deer (García-Sánchez et al. 2007). Another study that surveyed 212 fecal samples from white-tailed deer found a prevalence of 2.4% O157:H7 (Sargeant et al. 1999). This suggests that our finding in elk (low O157 prevalence and high non-O157 prevalence) is not uncommon, though it is unclear why. Perhaps there is a biochemical reason (testing may require wild ungulate tissue bacterial cultures), or that infection of these animals with this specific type of *E. coli* requires particular sources and transmission conditions. The finding here of a 27% O157:H7 prevalence for Lincoln elk in this study suggests that such conditions may occur, though it cannot be said what they are without further study. I suspect it is a combination of biochemical interaction between pathogen antigens and host cells and bacterial serotype fitness in wild soil. There is evidence for differential survival of O157 and non-O157 STEC based on soil properties (Ma et al. 2014). The possibility also exists of contamination to the Lincoln elk samples that occurred in no other group, though this seems unlikely as protocols did not vary between elk groups. It is also worth noting that some deer do not congregate as much as elk do and can be less likely to share space with cattle when the option is present (Dohna et al. 2014).

The high prevalence of non-O157 *E. coli* is likely due in part to targeting elk near cattle (and people), as many of the studies in the table look at animals that live more remotely than about half of the elk in this study. The 2013 Colorado study found higher prevalence of STEC E. coli in urban elk (11%) than in elk associated with livestock (0.8%), though the livestock in their study were described only as “free-ranging”

(Franklin et al. 2013). If these livestock range over large open areas and that is where they are encountered by elk, this is a different scenario than the livestock in this study which are in fenced fields that elk enter. That being said, this may indicate that proximity to human population density is a factor of similar or more importance than livestock proximity for some types of pathogenic *E. coli*.

In a study characterizing pathogens in a mixed land use watershed, the presence of microbial source trackers associated with ruminants more than doubled the odds of Shiga toxin presence (odds ratio 2.29, CI [1.60-3.61]) (Bradshaw et al. 2016). They also looked for cattle source trackers but those were not as important for Shiga toxin as ruminants, suggesting that wild ruminants play a significant role in contaminating that watershed. Testing feces from other livestock, deer, and water sources in Humboldt and Del Norte counties will be important in better understanding the dynamics at play.

In cattle the prevalence of non-O157 STEC *E. coli* was 70%, which is also high in comparison with related studies, though prevalence in cattle ranges widely (Jeon et al. 2013, Islam et al. 2014) (Table 14).

Table 14. Selected studies of pathogenic E. coli *in domestic cattle with reported prevalence (%), type of* E. coli *targeted by the study, location, and relevant methods. Possible methods include immunomagnetic separation (IMS), fecal culture and isolation (F), PCR, biochemistry (B), and serology (S).*

Cattle (n)	%	*E. coli*	Methods	Region	Study
All (40[a])	7.4	O157	Various	NA	Islam 2014
Beef (91)	40.7	O157	F, PCR	FL, USA	Jeon 2013
Beef ([d])	8.2-53.7	STEC	F, PCR	MI, USA	Vasco 2021
Dairy ([d])	8.7-28.0	STEC	F, PCR	MI, USA	Vasco 2021
Beef (44[a])	7.9	O157	IMS	USA	Ekong 2015
Beef (44[a])	4.2	O157	IMS	USA	Ekong 2015
Dairy (44[a])	2.3	O157	IMS	USA	Ekong 2015
Dairy (44[a])	0.40	O157	IMS	USA	Ekong 2015
Dairy (100)	8.0	STEC	F, PCR, B	WA, USA	Cobbold 2004
Feedlot (50)	3.0	STEC	F, PCR, B	WA, USA	Cobbold 2004
Range (50)	11.0	STEC	F, PCR, B	WA, USA	Cobbold 2004
Beef (7519)	6.0	O157:H7	IMS, F, PCR	Canada	Cernicchiaro 2009
Beef (≤1000)	2.7	O157:H7	IMS, PCR	Mexico	N-Bravo 2013
Beef (43[a])	6.2[b]	VTEC	Various	Global	Rhoades 2009

[a] Meta analysis, n refers to the number of studies included
[b] Weighted mean prevalence, range 0%-57%.
[c] Prevalence calculated at farm level, not individual animal.
[d] Various herds ranging in size

Prevalence estimates in cattle suffer method-related shortcomings including bias of culture techniques, a narrow focus on a particular *E. coli* type or strain, and inconsistent reporting of disease (Adaska et al. 2006, Cernicchiaro et al. 2009, Rhoades et al. 2009, Gorski 2012). These issues are presumably relevant to collections from wild populations as well. Studies including all pathogenic types, though financially and logistically more difficult, would likely return higher and more comparable prevalence values.

It is difficult to know if ranching practices contribute to the high prevalence of non-O157 *E. coli* (70%) found in this study, particularly with zero prevalence of O157 *E. coli* for which cattle are a known reservoir. Ranchers were not surveyed about their practices, and the research on production risk factors has not uncovered many broadly applicable management recommendations (Venegas-Vargas et al. 2016) (Table 15). There is some evidence for higher rates of pathogenic *E. coli* in feedlot versus dairy cattle though the wide variety in production practices makes these factors difficult to untangle (Rhoades et al. 2009). Shiga-toxigenic *Escherichia coli* shedding appears to rise in the warmer fall months, which is when most of the collection for this study was done (Cobbold et al. 2004, Rhoades et al. 2009, Menrath et al. 2010). Factors that reflect condition and stress of the animals also appear to be relevant, which is not surprising as stress often impacts immune responses (Kuhnert et al. 2005, Menrath et al. 2010). There is ample speculation in the literature about feed type and feed contamination as well as pasture use (Rhoades et al. 2009, Vasco et al. 2021). For instance, contradictory evidence

have been found for the impact of access to pastures spread with manure, and feeding barley versus corn (Rhoades et al. 2009). In an investigation of cattle microbiota from farms with high and low STEC prevalence, higher prevalence farms fed a diet composed largely of forage, though these farms also did not use antihelminthics, while the low prevalence farms did (Vasco et al. 2021). The forage-based cattle had highly diverse microbiota profiles, associated with high-forage diets such as those of the cattle in this study. It is likely that a complex interaction of factors such as housing, feed, cattle type, cattle movement, and antiparasitics or antibiotics impact prevalence in cattle.

Table 15. Risk factors for domestic cattle infection with pathogenic Escherichia coli *from selected studies.*

Cattle	*E. coli*	Risk Factors	Country	Study
Various	STEC	Production system[a], season	USA	Cobbold 2004
Beef	O157:H7	Pig presence, corn silage, percentage of cows, cows to shows	Canada	Cernicchiaro 2009
Various	O157	Production type[a], age	Belgium	Cobbaut 2009
Dairy	STEC	Month, lactation duration and contents, nutritional condition, presence of super-shedder, somatic cell count	Germany	Menrath 2010
Various	O157	Cattle type, region, detection method[b]	North America	Islam 2014
Dairy	STEC	Lactation, temperature, no antihelminthic treatment, no pasture access	Michigan	Venegas-Vargas 2016
Dairy	STEC	Age, milk contents, pasture use, unifeed trailer[b]	Switzerland	Kuhnert 2005
Beef	O157	Region, detection method[c]	Global	Ekong 2015

[a] Production system refers to dairy vs feedlot vs range, while production type refers to the product: beef, veal, dairy.
[b] Unifeed trailers are mobile feed-mixing machines
[c] Detection method is not a risk factor per se but was implicated in heterogeneity between studies in the review.

The prevalence of *S. enterica* in cattle is often under 10% (Gutema et al. 2019) (Table 16). The fact that we found none in cattle may relate to our isolation and culture methods or local ecology and production practices. Additionally, our cattle sample size of 71 was small for a pathogen that exists in other populations at relatively low rates. There are few available surveys on *Salmonella* in wild cervids, and those that do report prevalence under or equal to one percent, often zero (Appendix E).

Table 16. Prevalence of Salmonella enterica *in cattle, from selected studies, including relevant methods and region. Methods include biochemistry (B), intestinal fecal culture (IF), fecal culture (F), rectal swab culture (R), immunomagnetic separation (IMS), and pulsed-field gel electrophoresis (PFGE).*

Cattle (n)	%	Methods	Region	Study
All (26[a])	16.0	Various	North America	Gutema 2019
All (71[a])	9.0	Various	Global	Gutema 2019
Beef feedlot (184)	5.4	R, PCR	Nebraska, USA	Schmidt 2015
Beef overall (310)	6.8	IF, IMS, serology	Australia	Fegan 2004
Beef feedlot (155)	9.0	IF, IMS, serology	Australia	Fegan 2004
Beef grassfed (155)	4.5	IF, IMS, serology	Australia	Fegan 2004
Beef feedlot (27)	7.4	F, serology, B	Texas, USA	Xie 2016
Beef grassfed (795)	0.13	F, microarray, IMS, PCR	California, USA	Gorski 2011

[a] Meta-analysis, n refers to number of studies included

No *Salmonella* was isolated from adult elk in this study. Aside from the positive calf already discussed, the other from which *Salmonella* sp. was isolated lives in an area that also supports several ranches, including a large dairy ranch operation. These elk groups were not part of the design for this study so we do not have representative cattle

samples and cannot make any definitive statements about where this one calf may have contracted the pathogen.

Bearing and distance measurements appear to have been fairly consistent forward and backward, suggesting that observed fecal depositions were likely those collected. As bearings were read on a standard compass, observed average discrepancies between. 2.5 and 9 degrees are within a reasonable margin of error. An average distance discrepancy between 2.4 and 6.6 meters is also reasonable when considering that the forward measurement target was often the soiled rump of a black cow (low reflective potential), and sometimes in motion. Backwards distance measurements were sometimes difficult to obtain. However considering the error of the rangefinder, a discrepancy of under 2 meters for piles under 25 meters away, and up to about 6 meters for piles over 100 meters away, does not strike us as concerning. The aide of the golden dung fly (*Scathophaga stercoraria*)—alighting preferentially on recently deposited feces—was useful in effectively navigating to target feces among a group of older deposits (Parker 1970).

Sample processing variables such as timeline and sterility can alter results in microbial surveys. The bacterial enrichment timeline was not always met as the required incubation equipment was located in a classroom, limiting its availability. This could have introduced some variation in bacterial survival or growth which impacted pathogen detection. Some potential problems such as contamination of samples (either cross-contamination or introduction of outside pathogens) might falsely increase rates of pathogen detection. The natures of these impacts are difficult to know without further

experimentation and knowledge of the entire fecal microbial profile plus microbes introduced by the ground they fell on. However, the data shows no evidence of obvious contamination, so these rates of detection reflect at least an index of infection rates. In addition, *S. enterica* is resistant to cold storage, and fecal microbiota profiles in general were found robust to storage even at room temperature for several days (Takano et al. 1979, Dominianni et al. 2014).

Sample Characteristics

Time between defecation and collection varied widely as stated previously: from close to zero to 332 minutes for cattle (not measurable for elk). Nearly 6 hours is a long time for bacteria, but this did not seem to impact the rate of positive detections of non-O157 *E. coli* (Appendix D). The figure does suggest some clustering around lower times, but this is also the case for negatives (it was the goal) and was not apparent statistically.

Temperature of the feces at collection did not appear to impact pathogen detection results, as positive and negative results are distributed across the observed range of fecal temperatures. Other changes within that period are possible (or likely given the sudden change in environment) including microbial competition between fecal flora, impacts from environmental microbes or macrobes, and abiotic factors. Efforts were made to collect feces from the middle of the cow piles but this is more difficult with elk pellet depositions that often scatter upon impact with the ground. Comparison of positive versus

negative samples and the time between deposition and sampling showed no apparent effect of elapsed time on positive detections.

Though it was not tested in this study, antibiotic resistance is a concern at the livestock-wildlife interface and deserves mention (Sulzner et al. 2014, Ballash et al. 2021). In wildlife, antibiotic resistance of enteric bacterial communities is affected by proximity to domestic animals. In Zimbabwe, buffalo with no cattle contact had a significantly lower antibiotic resistance score than cattle or the buffalo with cattle contact ($P < 0.01$) and there was no significant difference between the buffalo with contact and the cattle with which they were in contact (Mercat et al. 2016). All antibiotic resistance (ABR) found in the buffalo was also found in the cattle. Antibiotic resistance in *E. coli* and *S. enterica* strains occur widely in cattle. As of yet, this occurrence has not been conclusively linked to antibiotic use in many cases, and there are host age and seasonal effects that are not well understood further indicating the importance of continued research on the role of cattle in transmission of these pathogens (Cernicchiaro et al. 2009, Gow et al. 2008*b*, Rhoades et al. 2009).

While the effects of ABR in enteric bacterial communities of wildlife are unclear, the mounting evidence for spreading antibiotic resistance attests to the potential impact of contact with domestic animals on the bacterial profiles of wildlife (Mercat et al. 2016). For example, high levels of tetracycline resistance were found in the *E. coli* isolates from starlings associated with a cattle feedlot (Gaukler et al. 2009). Future genomic analysis may allow for genetic resistance assessment in this study's samples.

Revisiting Predictions

1. I expected *E. coli* prevalence would be high, nearly 100% as it is a common commensal gut microbe, but expected *S. enterica* prevalence would be low, probably below 10%.

The testing in this study did not include all kinds of *E. coli* but prevalence of non-O157:H7 *E. coli* was high in some groups: ranging from 5% to 83%. *Salmonella enterica* prevalence was even lower than expected, with 0% of cattle and 5.7% of elk calves only, from a limited sample. This prediction is supported by the data, though not fully tested.

2. I expected that larger groups would have a higher prevalence of *S. enterica* and pathogenic *E. coli* strains as in larger groups, each individual is surrounded by more individuals that all encounter various transmission risks, thus likely more opportunities for transmission between these social animals. I did not expect any age stratification for either *E. coli* or *S. enterica* as the evidence in domestic cows for age stratification is not definitive unless newborn calves are being tested (Gow et al. 2008*a*, *b*, Rhoades et al. 2009).

There were not enough replicates to examine group size as a factor, and too few calves were tested to evaluate age stratification in cattle. Some evidence was found for age stratification of *S. enterica* in elk; only calf rectal swabs tested positive. As previously stated, Salmonellosis is mostly a disease of the very young, and serious elk disease has only been convincingly documented in calves (Foreyt et al. 2001, Mohler

et al. 2009). Of the two calves that tested positive, one remained alive at the end of monitoring and the fate of the other remains unknown due to tag loss (Nigon 2020). Considering our small sample size and limited coverage of different groups of calves, it is difficult to say much about the role of this pathogen in the population. However, managers should be aware that some elk calves are infected with *S. enterica*.

3. I also expected that increased proximity to people and cattle would be correlated with higher prevalence of *S. enterica* and higher prevalence of pathogenic *E. coli*. It also seemed likely that this difference would overwhelm differences in prevalence based on the specific habitats of the groups.

This prediction was supported by the results. Cattle-associated elk were far more likely to harbor non-O157:H7 *E. coli* than non-cattle associated elk, and a model including average nearby human population density ranked third. The parameter itself did not appear to be significant, but most of our elk were near low-population centers so there was not enough variation in this parameter and the human population data itself was too coarse. Fine-scale spatial habitat use may have a significant effect on the relationship between human population and infection risk: for example, more contaminated areas would be riskier if they occur around forage than if they cover a movement corridor. The variety of habitat used by the different elk groups, for instance beach and dune swale versus pastures, may also impact environmental reservoir and bacterial persistence factors.

4. In terms of *E. coli* serotypes, I suspected to find B1, B2 and D to be among the highest in prevalence as these are commonly found in non-human animals. In terms of *S. enterica* serovars, I expected cattle to be infected with those most common in the US and specifically Typhimurium, Enteritidis, Newport, Heidelberg, Javiana, Anatum, Muenster, Montevideo, Give, Kentucky and Muenchen as they are commonly found in California. My predictions for the serovars found in elk was based on the serovars found in California wildlife, serovars found in species taxonomically close to elk, and those known to have wide host ranges: specifically Typhimurium, Enteritidis, Infantis, Newport, Montevideo, Saint Paul, Reading and Agona. I expected the subtypes found in cattle or those in common between the two lists (Typhimurium, Enteritidis, Newport, Montevideo) would be more likely found in the elk sharing grazing areas with cattle.

Genome sequencing for samples was delayed by the SARS-CoV-2 pandemic. Thus, I am unable to comment on either *E. coli* clades or *S. enterica* serovars in this thesis. These data will be analyzed to allow more confident comments on these transmission dynamics and presented in a future publication.

MANAGEMENT IMPLICATIONS

Wildlife have previously been implicated as sources of contamination that have caused outbreaks of illness in humans, and the presence of pathogenic bacteria in feces presents a risk (Rabatsky-Ehr et al. 2002, Soare et al. 2021). Total counts of *Enterobacteriaciae* in hunted wild ruminant samples can be higher than what is reported for domestically slaughtered ruminants (Obwegeser et al. 2012). Commercial beef operations undertake extensive processing interventions to reduce pathogens in final meat products and such procedures are far from standardized in a field setting (Narvaez-Bravo et al. 2013, Schmidt et al. 2015, Soare et al. 2021). The prevalence of pathogenic bacteria found here reinforce the importance of carefully cleaning carcasses intended for consumption.

There is no evidence to suggest that *E. coli* is a threat to northwestern California elk or cattle at this time. Mortality in northern California elk groups was recently evaluated with a focus on calf mortality and survival. Calf mortality was due primarily to predation, followed by nutritional insufficiency while yearling and adult mortality was caused largely by humans (harvests, roadkill, fence entanglement, poaching). Two of 32 adults appear to have succumbed to pneumonia of unknown etiology, but there was no evidence of widespread infectious disease (Nigon 2020). The main disease currently impacting local Roosevelt elk is treponeme-associated hoof disease (TAHD) (California Department of Fish and Wildlife 2020).

On a broader scale, human association can impact communities of *E. coli* in wildlife via direct transmission, horizontal gene transfer among strains, and changing selection pressures, which may be altered through human hygienic regimens including antimicrobial use (Gaukler et al. 2009, Blyton et al. 2015, Mercat et al. 2016). We do not know what the long-term impacts of these kinds of changes could be. High throughput sequencing comparisons of cervid microbiota have found differences in diversity and relative abundance of bacterial taxa in the feces related to captivity, supplementary feeding, and chronic wasting disease (CWD) status (Delgado et al. 2017, Hu et al. 2018, Menke et al. 2019, Minich et al. 2021). While many differences are linked to season and diet (with certain taxa required to extract energy from various materials), many of the causal pathways are currently unknown (Delgado et al. 2017, Minich et al. 2021).

E. coli is a useful indicator for studying transmission dynamics because it is ubiquitous, well-studied, and shares a niche with many enteric pathogens (VanderWaal et al. 2014, Mercat et al. 2016). Elk and cattle exhibit similar feeding modes and have similar digestive tracts, thus an investigation into the population structure of enteric pathogens along with an analysis of their contact provides managers with useful information about enteric pathogen occurrence and transmission among these populations. It may be useful in future possible situations of disease threat or other assessments.

CONCLUSIONS

In conclusion, there is evidence for a link between elk interaction with cattle or human activity and infection of elk in Humboldt and Del Norte counties with non-O157 *E. coli*. These elk and cattle harbor non-O157:H7 *E. coli* more often than O157 *E. coli*, and few adults are infected with *S. enterica*. Use of areas with higher human or livestock densities increases the likelihood of infection for elk, though the immediate source of transmission is unknown. There is no evidence that these pathogens threaten either domestic cattle or Roosevelt elk populations in these areas at this time, but knowledge of pathogen transmission between populations may be useful as new disease risks arise. Future analysis of the genomic sequences from the isolated pathogens may provide details on the transmission pathways between these groups.

REFERENCES

Adaska, J. M., A. J. Silva, A. C. B. Berge, and W. M. Sischo. 2006. Genetic and phenotypic variability among *Salmonella enterica* serovar Typhimurium isolates from California dairy cattle and humans. Applied and Environmental Microbiology 72:6632–6637.

Alexander, K. A., L. D. Warnick, and M. Wiedmann. 2009. Antimicrobial resistant *Salmonella* in dairy cattle in the United States. Veterinary Research Communications 33:191–209.

Bale, J., D. Meunier, F.-X. Weill, E. dePinna, T. Peters, and S. Nair. 2016. Characterization of new *Salmonella* serovars by whole-genome sequencing and traditional typing techniques. Journal of Medical Microbiology 65:1074–1078.

Ballash, G. A., L. Munoz-Vargas, A. Albers, P. M. Dennis, J. T. LeJeune, D. F. Mollenkopf, and T. E. Wittum. 2021. Temporal trends in antimicrobial resistance of fecal *Escherichia coli* from deer. EcoHealth 18:288–296.

Bardiau, M., F. Grégoire, A. Muylaert, A. Nahayo, J.-N. Duprez, J. Mainil, and A. Linden. 2010. Enteropathogenic (EPEC), enterohaemorragic (EHEC) and verotoxigenic (VTEC) *Escherichia coli* in wild cervids. Journal of Applied Microbiology 109:2214–2222.

Bender, L. C., P. B. Hall, M. M. Garner, and J. L. Oaks. 1999. Bacillary hemoglobinuria in a free-ranging elk calf. Journal of Zoo and Wildlife Medicine 30:293–296.

Berardi, T., K. Shapiro, B. A. Byrne, and W. Miller. 2014. Prevalence and characterization of *Salmonella* shed by captive and free-range California sea lions (*Zalophus californianus*) from a rehabilitation center and three state reserves along the California coast. Journal of Zoo and Wildlife Medicine 45:527–533.

Bienen, L., and G. Tabor. 2006. Applying an ecosystem approach to brucellosis control: can an old conflict between wildlife and agriculture be successfully managed? Frontiers in Ecology and the Environment 4:319–327.

Bleasdale, B., P. J. Lott, A. Jagannathan, M. P. Stevens, R. J. Birtles, and P. Wigley. 2009. The *Salmonella* pathogenicity island 2-encoded type III secretion system is

essential for the survival of *Salmonella enterica* serovar Typhimurium in free-living amoebae. Applied and Environmental Microbiology 75:1793–1795.

Blyton, M. D. J., H. Pi, B. Vangchhia, S. Abraham, D. J. Trott, J. R. Johnson, and D. M. Gordon. 2015. Genetic structure and antimicrobial resistance of *Escherichia coli* and cryptic clades in birds with diverse human association. M. W. Griffiths, editor. Applied and Environmental Microbiology 81:5123–5133.

Bradshaw, J. K., B. J. Snyder, A. Oladeinde, D. Spidle, M. E. Berrang, R. J. Meinersmann, B. Oakley, R. C. Sidle, K. Sullivan, and M. Molina. 2016. Characterizing relationships among fecal indicator bacteria, microbial source tracking markers, and associated waterborne pathogen occurrence in stream water and sediments in a mixed land use watershed. Water Research 101:498–509.

California Department of Fish and Wildlife. 2018. Elk conservation and management plan. California Department of Fish and Wildlife, California, USA.

California Department of Fish and Wildlife. 2020. Elk hoof disease detected in Del Norte County herd. CDFW News. <https://cdfgnews.wordpress.com/2020/05/07/elk-hoof-disease-detected-in-del-norte-county-herd/>. Accessed 24 Nov 2021.

California Department of Forestry and Fire Protection. 2009. 1:24,000 county boundaries. California Department of Forestry and Fire Protection, California Department of Fish and Wildlife, California, USA.

Capps, K. M., J. B. Ludwig, P. B. Shridhar, X. Shi, E. Roberts, C. DebRoy, N. Cernicchiaro, R. K. Phebus, J. Bai, and T. G. Nagaraja. 2021. Identification, Shiga toxin subtypes and prevalence of minor serogroups of Shiga toxin-producing *Escherichia coli* in feedlot cattle feces. Nature Scientific Reports 11. <http://www.nature.com/articles/s41598-021-87544-w>. Accessed 1 Apr 2022.

Cattle Health Center. 2022. National bovine brucellosis and tuberculosis updates. Q1 Report Fiscal Year 2022, Ruminant Health Center, Strategy and Policy, Animal and Plant Health Inspection Service, Riverdale, Maryland, USA.

Cernicchiaro, N., D. L. Pearl, S. Ghimire, C. L. Gyles, R. P. Johnson, J. T. LeJeune, K. Ziebell, and S. A. McEwen. 2009. Risk factors associated with *Escherichia coli* O157:H7 in Ontario beef cow–calf operations. Preventive Veterinary Medicine 92:106–115.

Cobbold, R. N., D. H. Rice, M. Szymanski, D. R. Call, and D. D. Hancock. 2004. Comparison of Shiga-toxigenic *Escherichia coli* prevalences among dairy,

feedlot, and cow-calf herds in Washington State. Applied and Environmental Microbiology 70:4375–4378.

Coburn, B., G. A. Grassl, and B. B. Finlay. 2007. *Salmonella*, the host and disease: a brief review. Immunology and Cell Biology 85:112–118.

Collins, W. B. 1977. Diet composition and activities of elk on different habitat segments in the lodgepole pine yype, Uinta Mountains, Utah. Utah State University, Logan, Utah, USA.

County of Del Norte. 2017. DN_parcels. <co.del-norte.ca.us/GIS>. Accessed 30 Jan 2019.

Cover, M. A., S. E. Hygnstrom, and S. R. Groepper. 2011. Surveillance of selected diseases in free-ranging elk (*Cervus elaphus nelsoni*) in Nebraska, 1995—2009. Great Plains Research 21:145–151.

Crawford, G. C., M. H. Ziccardi, B. J. Gonzales, L. M. Woods, J. K. Fischer, E. J. B. Manning, and J. A. K. Mazet. 2006. *Mycobacterium avium* subspecies *paratuberculosis* and *Mycobacterium avium* subsp. *avium* infections in a tule elk (*Cervus elaphus nannodes*) herd. Journal of Wildlife Diseases 42:715–723.

Delgado, M. L., P. Singh, J. A. Funk, J. A. Moore, E. M. Cannell, J. Kanesfsky, S. D. Manning, and K. T. Scribner. 2017. Intestinal microbial community dynamics of white-tailed deer (*Odocoileus virginianus*) in an agroecosystem. Microbial Ecology 74:496–506.

Díaz-Sánchez, S., S. Sánchez, S. Herrera-León, C. Porrero, J. Blanco, G. Dahbi, J. E. Blanco, A. Mora, R. Mateo, I. Hanning, and D. Vidal. 2013. Prevalence of Shiga toxin-producing *Escherichia coli*, *Salmonella* spp. and *Campylobacter* spp. in large game animals intended for consumption: Relationship with management practices and livestock influence. Veterinary Microbiology 163:274–281.

Dirk van Elsas, J., A. V. Semenov, R. Costa, and J. T. Trevors. 2011. Survival of *Escherichia coli* in the environment: fundamental and public health aspects. The International Society for Microbial Ecology Journal 5:173–183.

Dohna, H. zu, D. E. Peck, B. K. Johnson, A. Reeves, and B. A. Schumaker. 2014. Wildlife–livestock interactions in a western rangeland setting: Quantifying disease-relevant contacts. Preventive Veterinary Medicine 113:447–456.

Dominianni, C., J. Wu, R. B. Hayes, and J. Ahn. 2014. Comparison of methods for fecal microbiome biospecimen collection. BMC Microbiology 14:103.

Duncan, C., H. Van Campen, S. Soto, I. K. LeVan, L. A. Baeten, and M. W. Miller. 2008. Persistent *Bovine Viral Diarrhea virus* infection in wild cervids of Colorado. Journal of Veterinary Diagnostic Investigation 20:650–653.

Espinosa, L., A. Gray, G. Duffy, S. Fanning, and B. J. McMahon. 2018. A scoping review on the prevalence of Shiga-toxigenic *Escherichia coli* in wild animal species. Zoonoses and Public Health 65:911–920.

Falcone, J. A. 2016. US block-level population density raster for 2010. USGS ScienceBase. <http://dx.doi.org/10.5066/F74J0C6M>. Accessed 21 Jan 2021.

Fan, R., K. Shao, X. Yang, X. Bai, S. Fu, H. Sun, Y. Xu, H. Wang, Q. Li, B. Hu, J. Zhang, and Y. Xiong. 2019. High prevalence of non-O157 Shiga toxin-producing *Escherichia coli* in beef cattle detected by combining four selective agars. BMC Microbiology 19. <https://bmcmicrobiol.biomedcentral.com/articles/10.1186/s12866-019-1582-8>. Accessed 1 Apr 2022.

Fegan, N., P. Vanderlinde, G. Higgs, and P. Desmarchelier. 2004. Quantification and prevalence of *Salmonella* in beef cattle presenting at slaughter. Journal of Applied Microbiology 97:892–898.

Forde, T., M. Pruvot, J. D. Buck, and K. Orsel. 2015. A high-morbidity outbreak of Johne's disease in game-ranched elk. Canadian Veterinary Journal 56:479–483.

Foreyt, W. J., T. E. Besser, and S. M. Lonning. 2001. Mortality in captive elk from salmonellosis. Journal of Wildlife Diseases 37:399–402.

Franklin, A. B., K. C. VerCauteren, H. Maguire, M. K. Cichon, J. W. Fischer, M. J. Lavelle, A. Powell, J. J. Root, and E. Scallan. 2013. Wild ungulates as disseminators of Shiga toxin-producing *Escherichia coli* in urban areas. M. M. Heimesaat, editor. PLoS ONE 8:e81512.

Gal-Mor, O., E. C. Boyle, and G. A. Grassl. 2014. Same species, different diseases: how and why typhoidal and non-typhoidal *Salmonella enterica* serovars differ. Frontiers in Microbiology 5. <http://journal.frontiersin.org/article/10.3389/fmicb.2014.00391/abstract>. Accessed 18 Dec 2016.

García-Sánchez, A., S. Sánchez, R. Rubio, G. Pereira, J. M. Alonso, J. Hermoso de Mendoza, and J. Rey. 2007. Presence of Shiga toxin-producing *E. coli* O157:H7 in a survey of wild artiodactyls. Veterinary Microbiology 121:373–377.

Gaukler, S. M., G. M. Linz, J. S. Sherwood, N. W. Dyer, W. J. Bleier, Y. M. Wannemuehler, L. K. Nolan, and C. M. Logue. 2009. *Escherichia coli*, *Salmonella*, and *Mycobacterium avium* subsp. *paratuberculosis* in wild European starlings at a Kansas cattle feedlot. Avian Diseases 53:544–551.

GeoServer. 2018. IHO Sea Areas. V3 edition. <<geo.vlilx.be/geoserver>>. Accessed 20 Apr 2022.

Gorski, L. 2012. Selective enrichment media bias the types of *Salmonella enterica* strains isolated from mixed strain cultures and complex enrichment broths. S. Bereswill, editor. PLoS ONE 7:e34722.

Gorski, L., C. T. Parker, A. Liang, M. B. Cooley, M. T. Jay-Russell, A. G. Gordus, E. R. Atwill, and R. E. Mandrell. 2011. Prevalence, distribution, and diversity of *Salmonella enterica* in a major produce region of California. Applied and Environmental Microbiology 77:2734–2748.

Gow, S. P., C. L. Waldner, A. Rajić, M. E. McFall, and R. Reid-Smith. 2008*a*. Prevalence of antimicrobial resistance in fecal generic *Escherichia coli* isolated in western Canadian cow-calf herds. Part I—Beef calves. Canadian Journal of Veterinary Research 72:82.

Gow, S. P., C. L. Waldner, A. Rajić, M. E. McFall, and R. Reid-Smith. 2008*b*. Prevalence of antimicrobial resistance in fecal generic *Escherichia coli* isolated in western Canadian beef herds. Part II-Cows and cow-calf pairs. The Canadian Journal of Veterinary Research 72:91–100.

Grimont, P. A., and F.-X. Weill. 2007. Antigenic formulae of the *Salmonella* serovars. Antigenic formulae of the *Salmonella* serovars, World Health Organization Collaborating Centre for Reference and Research on *Salmonella*, Paris, France.

Guber, A. K., J. Fry, R. L. Ives, and J. B. Rose. 2015. *Escherichia coli* survival in, and release from, white-tailed deer feces. M. W. Griffiths, editor. Applied and Environmental Microbiology 81:1168–1176.

Guizelini, C. C., J. F. Tutija, D. R. Morais, F. B. Bacha, C. A. N. Ramos, C. R. B. Leal, M. E. Zaquetti, and R. A. A. Lemos. 2020. Outbreak investigation of septicemic

salmonellosis in calves. The Journal of Infection in Developing Countries 14:104–108.

Gutema, F. D., G. E. Agga, R. D. Abdi, L. De Zutter, L. Duchateau, and S. Gabriël. 2019. Prevalence and serotype diversity of *Salmonella* in apparently healthy cattle: systematic review and meta-analysis of published studies, 2000–2017. Frontiers in Veterinary Science 6:102.

Han, S., and K. G. Mansfield. 2014. Severe hoof disease in free-ranging Roosevelt elk (*Cervus Elaphus Roosevelti*) in southwestern Washington, USA. Journal of Wildlife Diseases 50:259–270.

Han, S., K. G. Mansfield, D. S. Bradway, T. E. Besser, D. H. Read, G. J. Haldorson, D. P. Alt, and J. H. Wilson-Welder. 2019. Treponeme-associated hoof disease of free-ranging elk (*Cervus elaphus*) in Southwestern Washington State, USA. Veterinary Pathology 56:118–132.

Harper, J. A., J. H. Harn, W. W. Bentley, and C. F. Yocom. 1967. The status and ecology of the Roosevelt elk in California. Wildlife Monographs 3–49.

Hattel, A. L., D. P. Shaw, J. S. Fisher, J. W. Brooks, B. C. Love, T. R. Drake, and D. C. Wagner. 2007. Mortality in Pennsylvania captive elk (*Cervus elaphus*): 1998–2006. Journal of veterinary diagnostic investigation 19:334–337.

Heiman, K. E., R. K. Mody, S. D. Johnson, P. M. Griffin, and L. H. Gould. 2015. *Escherichia coli* O157 outbreaks in the United States, 2003–2012. Emerging Infectious Diseases 21. <http://wwwnc.cdc.gov/eid/article/21/8/14-1364_article.htm>. Accessed 14 Apr 2022.

Hu, X., G. Liu, Y. Li, Y. Wei, S. Lin, S. Liu, Y. Zheng, and D. Hu. 2018. High-throughput analysis reveals seasonal variation of the gut microbiota composition within forest musk deer (*Moschus berezovskii*). Frontiers in Microbiology 9:1674.

Humboldt County Community Development Services. 2017. County boundary. <humboldtgov.org/276/GIS-Data-Download>. Accessed 12 Dec 2017.

Ishii, S., K. P. Meyer, and M. J. Sadowsky. 2007. Relationship between phylogenetic groups, genotypic clusters, and virulence gene profiles of *Escherichia coli* strains from diverse human and animal sources. Applied and Environmental Microbiology 73:5703–5710.

Islam, Md. Z., A. Musekiwa, K. Islam, S. Ahmed, S. Chowdhury, A. Ahad, and P. K. Biswas. 2014. Regional variation in the prevalence of *E. coli* O157 in cattle: a meta-analysis and meta-regression. Q. Zhang, editor. PLoS ONE 9:e93299.

Jeon, S. J., M. Elzo, N. DiLorenzo, G. C. Lamb, and K. C. Jeong. 2013. Evaluation of animal genetic and physiological factors that affect the prevalence of *Escherichia coli* O157 in cattle. A. M. Ibekwe, editor. PLoS ONE 8. <https://dx.plos.org/10.1371/journal.pone.0055728>. Accessed 1 Apr 2022.

Johnson, S. P., S. Nolan, and F. M. Gulland. 1998. Antimicrobial susceptibility of bacteria isolated from pinnipeds stranded in central and northern California. Journal of Zoo and Wildlife Medicine 288–294.

Ju, W., G. Cao, L. Rump, E. Strain, Y. Luo, R. Timme, M. Allard, S. Zhao, E. Brown, and J. Meng. 2012. Phylogenetic analysis of non-O157 Shiga toxin-producing *Escherichia coli* strains by whole-genome sequencing. Journal of Clinical Microbiology 50:5.

Khoshbakht, R., M. Tabatabaei, H. Shirzad Aski, and H. Shayegh. 2015. Distribution of *Salmonella*, *Arcobacter*, and thermophilic *Campylobacter* spp. among Persian fallow deer (*Dama mesopotamica*) population in Dasht-e-Arzhan Wildlife refuge, southern Iran. Comparative Clinical Pathology 24:777–781.

Kim, J.-H., S. W. Hong, B.-Y. Park, J. G. Yoo, and M.-H. Oh. 2019. Characterisation of the bacterial community in the gastrointestinal tracts of elk (*Cervus canadensis*). Antonie van Leeuwenhoek 112:225–235.

Koh, X. P., Z. Shen, C. F. Woo, Y. Yu, H. I. Lun, S. W. Cheung, J. K. C. Kwan, and S. C. K. Lau. 2022. Genetic and ecological diversity of *Escherichia coli* and cryptic *Escherichia* clades in subtropical aquatic environments. Frontiers in Microbiology 13:811755.

Kolbe, N. R., and F. W. Weckerly. 2015. Home-range overlap of Roosevelt elk herds in the Bald Hills of Redwood National Park. California Fish and Game 101:208–217.

Kuhnert, P., C. R. Dubosson, M. Roesch, E. Homfeld, M. G. Doherr, and J. W. Blum. 2005. Prevalence and risk-factor analysis of Shiga toxigenic *Escherichia coli* in faecal samples of organically and conventionally farmed dairy cattle. Veterinary Microbiology 109:37–45.

Lawn, A. M., I. Orskov, and F. Orskov. 1977. Morphological distinction between different H serotypes of *Escherichia coli*. Journal of General Microbiology 101:111–119.

Lillehaug, A., B. Bergsjø, J. Schau, T. Bruheim, T. Vikøren, and K. Handeland. 2005. *Campylobacter* spp., *Salmonella* spp., verocytotoxic *Escherichia coli*, and antibiotic resistance in indicator organisms in wild cervids. Acta Veterinaria Scandinavica 46:1.

Ma, J., A. Mark Ibekwe, D. E. Crowley, and C.-H. Yang. 2014. Persistence of *Escherichia coli* O157 and non-O157 strains in agricultural soils. Science of The Total Environment 490:822–829.

Manning, E. J. B., T. E. Kucera, N. B. Gates, L. M. Woods, and M. Fallon-McKnight. 2003. Testing for *Mycobacterium avium* subsp. *paratuberculosis* infection in asymptomatic free-ranging tule elk from an infected herd. Journal of Wildlife Diseases 39:323–328.

McKelvey, R., and W. Zavoina. 1975. A statistical model for the analysis of ordinal level dependent variables. Journal of Mathematical Sociology 4:103–120.

Menke, S., M. Heurich, M. Henrich, K. Wilhelm, and S. Sommer. 2019. Impact of winter enclosures on the gut bacterial microbiota of red deer in the Bavarian Forest National Park. Wildlife Biology 2019. <https://bioone.org/journals/wildlife-biology/volume-2019/issue-1/wlb.00503/Impact-of-winter-enclosures-on-the-gut-bacterial-microbiota-of/10.2981/wlb.00503.full>. Accessed 13 Apr 2022.

Menrath, A., L. H. Wieler, K. Heidemanns, T. Semmler, A. Fruth, and N. Kemper. 2010. Shiga toxin producing *Escherichia coli*: identification of non-O157:H7-super-shedding cows and related risk factors. Gut Pathogens 2:7.

Mentaberre, G., M. C. Porrero, N. Navarro-Gonzalez, E. Serrano, L. Domínguez, and S. Lavín. 2013. Cattle drive *Salmonella* infection in the wildlife-livestock interface: *Salmonella* infection in the wildlife-livestock interface. Zoonoses and Public Health 60:510–518.

Mercat, M., O. Clermont, M. Massot, E. Ruppe, M. de Garine-Wichatitsky, E. Miguel, H. Valls Fox, D. Cornelis, A. Andremont, E. Denamur, and A. Caron. 2016. *Escherichia coli* population structure and antibiotic resistance at a buffalo/cattle interface in Southern Africa. C. A. Elkins, editor. Applied and Environmental Microbiology 82:1459–1467.

Miller, R. S., and S. J. Sweeney. 2013. *Mycobacterium bovis* (bovine tuberculosis) infection in North American wildlife: current status and opportunities for mitigation of risks of further infection in wildlife populations. Epidemiology and Infection 141:1357–1370.

Minich, D., C. Madden, M. V. Evans, G. A. Ballash, D. J. Barr, K. P. Poulsen, P. M. Dennis, and V. L. Hale. 2021. Alterations in gut microbiota linked to provenance, sex, and chronic wasting disease in white-tailed deer (*Odocoileus virginianus*). Scientific Reports 11:13218.

Mohler, V. L., M. M. Izzo, and J. K. House. 2009. Salmonella in calves. Veterinary Clinics of North America: Food Animal Practice 25:37–54.

Morden, C.-J. C., R. B. Weladji, E. Ropstad, E. Dahl, and Ø. Holand. 2011. Use of faecal pellet size to differentiate age classes in female Svalbard reindeer *Rangifer tarandus platyrhynchus*. Wildlife Biology 17:441–448.

Morningstar-Shaw, B. R., T. A. Mackie, D. K. Barker, and E. A. Palmer. 2016. *Salmonella* serotypes isolated from animals and related sources. United States Department of Agriculture, Diagnostic Bacteriology Laboratory, National Veterinary Services Laboratories.

Narvaez-Bravo, C., M. F. Miller, T. Jackson, S. Jackson, A. Rodas-Gonzalez, K. Pond, A. Echeverry, and M. M. Brashears. 2013. *Salmonella* and *Escherichia coli* O157:H7 prevalence in cattle and on carcasses in a vertically integrated feedlot and harvest plant in Mexico. Journal of Food Protection 76:786–795.

National Centers for Environmental Information (NCEI). 2019. Land-based station data. National Oceanic and Atmospheric Administration (NOAA). <https://www.ncdc.noaa.gov/data-access/land-based-station-data>. Accessed 28 Mar 2019.

Nettles, V. F., C. F. Quist, R. R. Lopez, T. J. Wilmers, P. Frank, W. Roberts, S. Chitwood, and W. R. Davidson. 2002. Morbidity and mortality factors in Key deer (*Odocoileus virginianus clavium*). Journal of Wildlife Diseases 38:685–692.

Nigon, E. M. 2020. Estimates of calf survival and factors influencing Roosevelt elk mortality in northwestern California. Dissertation, Humboldt State University, California, USA.

Obwegeser, T., R. Stephan, E. Hofer, and C. Zweifel. 2012. Shedding of foodborne pathogens and microbial carcass contamination of hunted wild ruminants. Veterinary Microbiology 159:149–154.

Orr, R. T. 1937. Notes on the life history of the Roosevelt elk in California. Journal of Mammalogy 18:62.

Orskov, I., F. Orskov, B. Jann, and K. Jann. 1977. Serology, chemistry, and genetics of O and K antigens of *Escherichia coli*. Bacteriological Reviews 41:667–710.

Pagano, A., G. Nardi, C. Bonaccorso, V. Falbo, C. Passi, V. Sanguinetti, and A. Mantovani. 1985. Faecal bacteria of wild ruminants and the alpine marmot. Veterinary research communications 9:227–232.

Parker, G. A. 1970. The reproductive behaviour and the nature of sexual selection in *Scatophaga stercoraria* L. (Diptera: Scatophagidae). V. The female's behaviour at the oviposition site. Behavior 37:140–168.

Passler, T., S. S. Ditchkoff, and P. H. Walz. 2016. Bovine viral diarrhea virus (BVDV) in white-tailed deer (*Odocoileus virginianus*). Frontiers in Microbiology 7. <http://journal.frontiersin.org/Article/10.3389/fmicb.2016.00945/abstract>. Accessed 15 Dec 2016.

Pruimboom-Brees, I. M., T. W. Morgan, M. R. Ackermann, E. D. Nystrom, J. E. Samuel, N. A. Cornick, and H. W. Moon. 2000. Cattle lack vascular receptors for *Escherichia coli* O157:H7 Shiga toxins. Proceedings of the National Academy of Sciences 97:10325–10329.

Quayle, J. F., and K. R. Brunt. 2003. Status of Roosevelt elk (*Cervus elaphus roosevelti*) in British Columbia. Wildlife Bulletin, British Columbia Ministry of Water, Land, and Air Protection and Ministry of Sustainable Resource Management, Victoria, BC. <http://www.env.gov.bc.ca/wld/documents/statusrpts/b106.pdf>. Accessed 10 Jan 2017.

Rabatsky-Ehr, T., D. Dingman, R. Marcus, R. Howard, A. Kinney, and P. Mshar. 2002. Deer meat as the source for a sporadic case of *Escherichia coli* O157: H7 infection. Emerging Infectious Diseases 8:525.

Renter, D. G., D. P. Gnad, J. M. Sargeant, and S. E. Hygnstrom. 2006. Prevalence and serovars of *Salmonella* in the feces of free-ranging white-tailed deer (*Odocoileus virginianus*) in Nebraska. Journal of Wildlife Diseases 42:699–703.

Renter, D. G., J. M. Sargeant, S. E. Hygnstorm, J. D. Hoffman, and J. R. Gillespie. 2001. *Escherichia coli* O157:H7 in free-ranging deer in Nebraska. Journal of Wildlife Diseases 37:755–760.

Rhoades, J. R., G. Duffy, and K. Koutsoumanis. 2009. Prevalence and concentration of verocytotoxigenic *Escherichia coli*, *Salmonella enterica* and *Listeria monocytogenes* in the beef production chain: a review. Food Microbiology 26:357–376.

Rhyan, J. C., P. Nol, C. Quance, A. Gertonson, J. Belfrage, L. Harris, K. Straka, and S. Robbe-Austerman. 2013. Transmission of brucellosis from elk to cattle and bison, Greater Yellowstone Area, USA, 2002–2012. Emerging Infectious Diseases 19:1992–1995.

Rounds, J. M., C. E. Rigdon, L. J. Muhl, M. Forstner, G. T. Danzeisen, B. S. Koziol, C. Taylor, B. T. Shaw, G. L. Short, and K. E. Smith. 2012. Non-O157 Shiga toxin–producing *Escherichia coli* associated with venison. Emerging Infectious Diseases 18:279–282.

Sánchez, S., A. García-Sánchez, R. Martínez, J. Blanco, J. E. Blanco, M. Blanco, G. Dahbi, A. Mora, J. Hermoso de Mendoza, J. M. Alonso, and J. Rey. 2009. Detection and characterisation of Shiga toxin-producing *Escherichia coli* other than *Escherichia coli* O157:H7 in wild ruminants. The Veterinary Journal 180:384–388.

Sánchez, S., R. Martínez, A. García, D. Vidal, J. Blanco, M. Blanco, J. E. Blanco, A. Mora, S. Herrera-León, A. Echeita, J. M. Alonso, and J. Rey. 2010. Detection and characterisation of O157:H7 and non-O157 Shiga toxin-producing Escherichia coli in wild boars. Veterinary Microbiology 143:420–423.

Sargeant, J. M., D. J. Hafer, J. R. Gillespie, R. D. Oberst, and S. J. Flood. 1999. Prevalence of *Escherichia coli* O157:H7 in white-tailed deer sharing rangeland with cattle. Journal of the American Veterinary Medical Association 215:792–794.

Sato, K., T. E. Carpenter, J. T. Case, and R. L. Walker. 2001. Spatial and temporal clustering of *Salmonella* serotypes isolated from adult diarrheic dairy cattle in California. Journal of Veterinary Diagnostic Investigation 13:206–212.

Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M.-A. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin. 2011. Foodborne illness acquired in the United States—major pathogens. Emerging Infectious Diseases 17:7–15.

Schmidt, J. W., G. E. Agga, J. M. Bosilevac, D. M. Brichta-Harhay, S. D. Shackelford, R. Wang, T. L. Wheeler, and T. M. Arthur. 2015. Occurrence of antimicrobial-resistant *Escherichia coli* and *Salmonella enterica* in the beef cattle production and processing continuum. M. W. Griffiths, editor. Applied and Environmental Microbiology 81:713–725.

Singh, P., Q. Sha, D. W. Lacher, J. Del Valle, R. E. Mosci, J. A. Moore, K. T. Scribner, and S. D. Manning. 2015. Characterization of enteropathogenic and Shiga toxin-producing *Escherichia coli* in cattle and deer in a shared agroecosystem. Frontiers in Cellular and Infection Microbiology 5. <http://www.frontiersin.org/Cellular_and_Infection_Microbiology/10.3389/fcimb.2015.00029/abstract>. Accessed 13 Apr 2022.

Smith, W. A., J. A. K. Mazet, and D. C. Hirsh. 2002. *Salmonella* in California wildlife species: prevalence in rehabilitation centers and characterization of isolates. Journal of Zoo and Wildlife Medicine 33:228–235.

Soare, C., T. N. McNeilly, and A. Seguino. 2021. A review of potential risk factors linked to shiga toxin-producing *Escherichia coli* (STEC) in wild deer populations and the practices affecting the microbial contamination of wild deer carcasses with enteric bacteria. Food Control 127:108128.

Somarelli, J. A., J. C. Makarewicz, R. Sia, and R. Simon. 2007. Wildlife identified as major source of *Escherichia coli* in agriculturally dominated watersheds by BOX A1R-derived genetic fingerprints. Journal of Environmental Management 82:60–65.

Sulzner, K., T. Kelly, W. Smith, and C. K. Johnson. 2014. Enteric pathogens and antimicrobial resistance in turkey vultures (*Cathartes aura*) feeding at the wildlife-livestock interface. Journal of Zoo and Wildlife Medicine 45:931–934.

Szczerba-Turek, A., and B. Kordas. 2020. Fallow deer (*Dama dama*) as a reservoir of Shiga toxin-producing *Escherichia coli* (STEC). Animals 10:881.

Takano, M., A. B. Simbol, M. Yasin, and I. Shibasaki. 1979. Bactericidal effect of freezing with chemical agents. Journal of Food Science 44:112–115.

Tenaillon, O., D. Skurnik, B. Picard, and E. Denamur. 2010. The population genetics of commensal *Escherichia coli*. Nature Reviews Microbiology 8:207–217.

Tomino, Y., M. Andoh, Y. Horiuchi, J. Shin, R. Ai, T. Nakamura, M. Toda, K. Yonemitsu, A. Takano, H. Shimoda, K. Maeda, Y. Kodera, I. Oshima, K. Takayama, T. Inadome, K. Shioya, M. Fukazawa, K. Ishihara, and T. Chuma. 2020. Surveillance of Shiga toxin-producing *Escherichia coli* and *Campylobacter* spp. in wild Japanese deer (*Cervus nippon*) and boar (*Sus scrofa*). Journal of Veterinary Medical Science 82:1287–1294.

United States Census Bureau. 2019. American FactFinder. <https://factfinder.census.gov/faces/nav/jsf/pages/index.xhtml>. Accessed 28 Mar 2019.

United States Department of Agriculture. 2019. 2017 census of agriculture. Geographic Area Series, National Agricultural Statistics Service, United States.

Van Campen, H., and J. Rhyan. 2010. The role of wildlife in diseases of cattle. Veterinary Clinics of North America: Food Animal Practice 26:147–161.

VanderWaal, K. L., E. R. Atwill, Lynne. A. Isbell, and B. McCowan. 2014. Linking social and pathogen transmission networks using microbial genetics in giraffe (*Giraffa camelopardalis*). S. Altizer, editor. Journal of Animal Ecology 83:406–414.

Vasco, K., B. Nohomovich, P. Singh, C. Venegas-Vargas, R. E. Mosci, S. Rust, P. Bartlett, B. Norby, D. Grooms, L. Zhang, and S. D. Manning. 2021. Characterizing the cattle gut microbiome in farms with a high and low prevalence of Shiga toxin producing *Escherichia coli*. Microorganisms 9. <https://www.mdpi.com/2076-2607/9/8/1737>. Accessed 1 Apr 2022.

Veall, M. R., and K. F. Zimmermann. 1994. Evaluating Pseudo-R2's for binary probit models. Quality & Quantity 28:151–164.

Venegas-Vargas, C., S. Henderson, A. Khare, R. E. Mosci, J. D. Lehnert, P. Singh, L. M. Ouellette, B. Norby, J. A. Funk, S. Rust, P. C. Bartlett, D. Grooms, and S. D. Manning. 2016. Factors associated with Shiga toxin-producing *Escherichia coli* shedding by dairy and beef cattle. J. Björkroth, editor. Applied and Environmental Microbiology 82:5049–5056.

Weckerly, F. W. 2005. Grass and supplemental patch selection by a population of Roosevelt elk. Journal of Mammalogy 86:630–638.

Weckerly, F. W., A. E. Julian, and K. Schmidt. 2013. Group size dynamics of female Roosevelt elk in Redwood National and State Parks, California. <https://digital.library.txstate.edu/handle/10877/5981>. Accessed 5 Jan 2017.

Weckerly, F. W., and M. A. Ricca. 2000. Using presence of sign to measure habitats used by Roosevelt elk. Wildlife Society Bulletin 146–153.

Wengert, G. 2000. Demography, range, habitat, condition and parasites of the Roosevelt elk (*cervus elaphus roosevelti*) of Sinkyone wilderness state park, Mendocino county, California. California Ste Parks.

Western Regional Climate Center. 2021. Western Regional Climate Center. <https://wrcc.dri.edu>. Accessed 16 Jul 2021.

Xie, Y., J. W. Savell, A. N. Arnold, K. B. Gehring, J. J. Gill, and T. M. Taylor. 2016. Prevalence and characterization of *Salmonella enterica* and *Salmonella* bacteriophages recovered from beef cattle feedlots in south Texas. Journal of Food Protection 79:1332–1340.

Zheng, B., and A. Agresti. 2000. Summarizing the predictive power of a generalized linear model. Statistics in Medicine 19:1771–1781.

APPENDICES

Appendix A: Datasheet used for elk and cattle fecal collection in Humboldt and Del Norte Counties 2018. Second page of datasheet follows on the next page.

Fecal Sampling Datasheet (Roosevelt elk in Humboldt & Del Norte Counties Summer 2018, Emily Armstrong Buck)

Date	Start Time	End time	Group/Session
			Observer

Sample # = Group + Obs# + Date
Texture = surface texture, overall consistency *Shape = pellets / pile / clumps* *Surface = wet vs. dry, shiny vs. dull, etc.*
Behavior Notes

Group Composition:

Temperature	Precip	Wind	Cloud Cover

Fecal Samples

Sample #	Time	Temp	Nugget Size	Shape/Texture	Surface/shine	Easting	Northing
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							
19							
20							

Continued from: Date Time Group

Fecal Sampling Continued

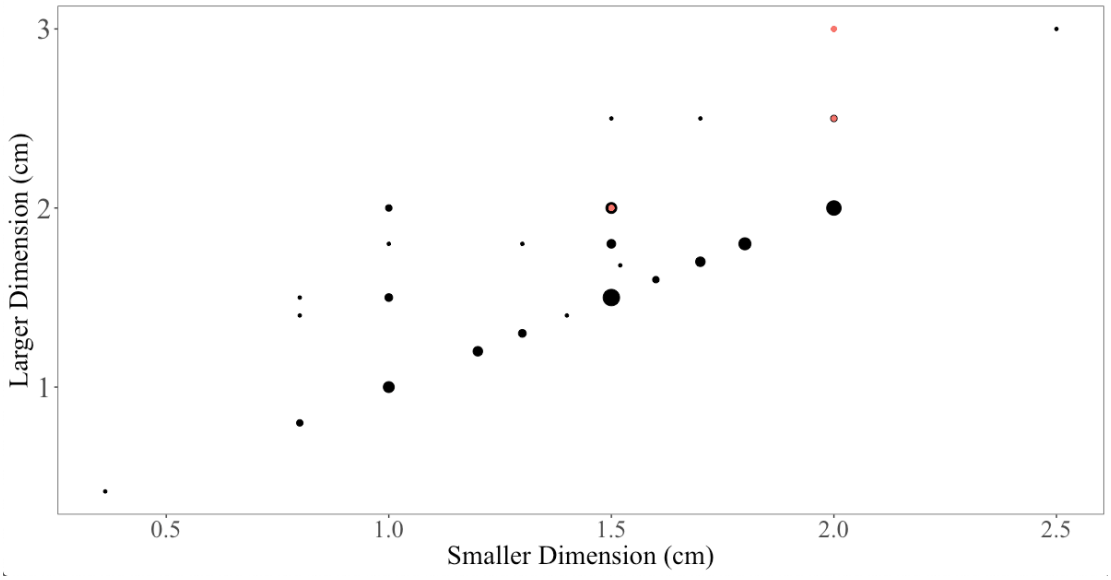
Sample #	Time	Temp	Nugget Size	Texture/Shape	Surface	Easting	Northing
21							
22							
23							
24							
25							
26							
27							
28							
29							
30							
31							
32							
33							
34							
35							
36							
37							
38							
39							
40							

Appendix B: Average difference in forward and reverse bearings (degrees) and distance (meters) from cattle fecal sampling.

Distance Category (m)	Number of Samples	Average Bearing Discrepancy (°)	Average Distance Discrepancy (m)
0-25	8	9[a]	2.62
26-50	23	8.3[a]	2.39
51-75	14	2.33	2.86
76-100	10	7.88	4.4
>100	5	2.5	6.6

[a] Average after one outlier removed from each of these groups.

Appendix C: Length and width (cm) of collected Roosevelt elk pellets from Humboldt and Del Norte counties California, USA, 2018. Different sized points indicate relative number of samples with those dimensions. Pellet sizes estimated instead of measured are shown in red (lighter shade).



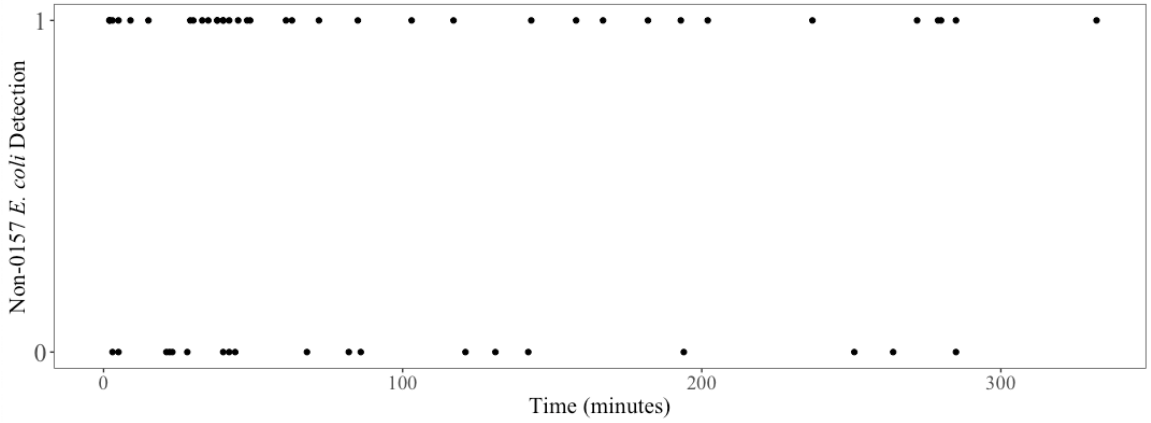
Pellet dimensions ranged from 0.8cm to 3cm, with an average small dimension of 1.52 cm (sd = 0.36, n=95) and average large dimension of 1.68 cm (sd = 0.42, n=95). With dimensions added for estimated medium or large pellets (medium = 1.5x2cm, large = 2x3cm), lower dimension average changed to 1.57cm (sd = 0.36, n = 120) and higher dimension average changed to 1.86cm (sd = 0.55, n = 120). A pellet with any dimension at or below 1cm was labeled as calf feces based on anecdotal observation, giving 17 calf samples, 101 adult elk samples, and 18 uncategorized samples. Elk pellet measurements in two dimensions provided a rough grouping for sizes, though due to shape variation a dry-weight or volume measurement may have been more precise. Three-dimension

measurement was most accurate in classifying age for Svalbard reindeer (*Rangifer tarandus platyrhynchnus*)(Morden et al. 2011). In these reindeer, they found distinctions largely between adults and calves, less so between yearlings and adults. As here we are not seeking to distinguish yearlings, a two-dimension measurement is likely sufficient. Regardless, as assessed here, we do not have sufficient sampling coverage across age classes to estimate the impact of age on infection.

Appendix D: Sample Handling Assessment

Time to collection

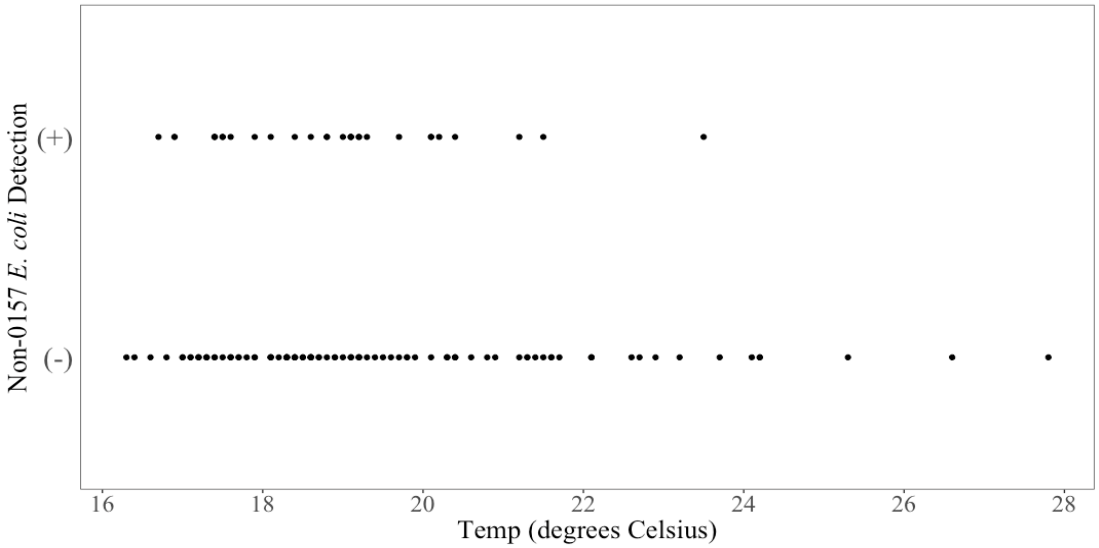
One potential confounding factor is the amount of time feces were exposed to the environment between deposition and collection. While it is difficult to know this for the elk samples (individual defecations were not observed), the possible impact of that elapsed time was assessed for cattle samples. Time between deposition and collection for cow feces ranged from zero to 332 minutes, with a mean of 48 and median of 89 minutes (Table A). A binomial GLM comparing pathogen detection (non-O157 *E. coli*) and time elapsed before collection indicated no significant relationship between the two ($p = 0.895$, $df = 55$).



Appendix D Table A. Relationship between time elapsed (minutes) from cattle defecation and feces collection, and the detection of non-O157 *E. coli* (1 is detected, 0 is not detected) from samples collected in Humboldt and Del Norte counties California, USA, 2018.

Fecal temperature

We assessed the potential effect of fecal temperature on the rate of positive cultures. A binomial glm (generalized linear model) comparing only temperature and presence or absence of positive culture found no effect of temperature on presence of a positive culture (p=0.18, with no significant drop in deviance: p = 0.17). A graph comparing temperature with positives does not suggest correlation (Table B).



Appendix D Table B. Positive (+) and negative (-) results for culture and isolation of non-O157 *E. coli* by the temperature of the fecal samples at time of collection, from Roosevelt elk of Humboldt and Del Norte counties California, USA, 2018.

Appendix E: Published prevalence estimates of *Salmonella enterica* in wild cervids including methods of bacterial isolation and identification (F = fecal culture, B = biochemistry, S = serology).

Species	Prevalence	Methods	Region	Study
Red deer (295)	0.3	F, B	South-central Spain	Diaz-Sanchez 2013
Red deer, roe deer, moose, reindeer (50 each)	0	F, B, S	Norway	Lillehaug 2005
Red deer, roe deer, chamois, and ibex (239 total)	0	F, B	Switzerland	Obwegeser 2012
Persian fallow deer (63)	0	F, PCR	Southern Iran	Khoshbakht 2015
Iberian ibex (313)	1.0[a]	F, B, S	NE Spain	Navarro-Gonzalez 2014
WT deer (500)	1.0	F, S	Nebraska	Renter 2006

[a] Including one fatal case of septicemic salmonellosis