PNEUMONIA DYNAMICS IN BIGHORN SHEEP

(OVIS CANADENSIS)

A Dissertation in
Biology
by
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Abstract

Wildlife disease modeling operates in an information-poor domain, and better methods for understanding and aggregating available data are crucial for efficient response to emerging wildlife disease events. Here, I use a variety of datasets to characterize demographic consequences and drivers of persistent pneumonia in bighorn sheep (*Ovis canadensis*).

First, I use a long-term dataset to explore the persistent demographic consequences of pneumonia, and find that disease persistence poses a greater threat to bighorn population recovery than the startling all-age die-off events surrounding pathogen introduction.

Second, I pair social network data and information on lamb survival to examine the scale of transmission during summer lamb disease events. I find that populations restructure into groups of consistent sizes, even as population size declines. Furthermore, transmission appears to localize into particular ewe groups. Together, these observations suggest that pneumonia may operate via an approximately frequency-dependent transmission process, with the consequence that populations have no critical community size below which we would expect the pathogens to fade-out.

Third, I use high-resolution summer association and contact networks, paired with lamb outcomes and individual-level infection status to evaluate whether yearlings and dry ewes pose the same probability of transmission given contact with lambs that dams pose. I find that yearlings and dry ewes pose a much lower risk to lambs than do infected dams, suggesting that efforts to halt transmission by reducing prevalence should perhaps focus primarily on dams, and less-intensively on yearlings and dry ewes.

Bighorn sheep spatial and social ecology is not consistently territorial, nor do bighorns consistently live in fission-fusion groups, nor do they consistently form matrilineal groups. Yet predicting local spatial and social ecology would be extremely useful for forecasting pneumonia epidemic progression. In the fourth chapter, I develop a general framework relating spatial and social network metrics
to underlying behavioral drivers like social affinity and responsiveness to habitat heterogeneity, and to readily measurable metrics like group sizes and individual home range structures, in an effort to understand how these various metrics relate and to provide the beginnings of a predictive framework for pathogen transmission risk.

Finally, I examine social research patterns, in an effort to understand the disjunct nature of wildlife disease research. I find that the research separates into clear veterinary, ecological, and mathematical biology domains, with limited cross-domain collaboration.
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2.1 Empirical patterns of pneumonia in bighorn sheep. (a) Disease status of twelve focal Hells Canyon bighorn populations since 1995. Open squares are years with no documented pneumonia. Grey circles are years with documented pneumonia in adults. Black circles are years with documented or suspected [1] pneumonia in lambs. Population-years with no data are left blank. (b) Lambda and recruitment (March lamb:ewe ratio) for every population-year in the Hells Canyon study. Colors represent different disease classes, and point size scales with population size. Dashed lines are at lambda = 1 (i.e., static population size) and recruitment = 0.2, (this equates to 20 lambs per hundred ewes, a current Western Association of Fish and Wildlife Agencies guideline for a healthy population). Boxplots show the marginal distributions of recruitment and estimated lambda for healthy years (blue) as compared to years with any documented disease (red). Boxes contain the 25th to 75th quantiles, and lines extend to 2.5th and 97.5th quantiles.
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E.6 Journal community attributes associated with the 1551 papers in journals from the three major journal communities. “Directed” networks are those in which edges need not be symmetric in weight (i.e., one paper can cite another without the reciprocal occurring, so the paper network is directed; co-authorship roles, however, are reciprocal, so the author network is undirected). Lead author affiliation was designated based on author institution as listed in the paper bank metadata. Total and percent citations from papers in each community to papers in the other communities are shown in the final three columns. Within-community citations are bolded.

E.7 Publication growth rate model output.

E.8 Model output for parametric terms from the within-paper author diversity GAM.
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Dedication

This work is dedicated to my parents, Margaret Saunders and Steve Manlove, who taught me not only to ask questions, but also to pursue answers; to Michael Lerch, my partner in crime, without whom this endeavor would have been truly impossible; and finally, to the ewes and lambs at my Hells Canyon field sites, whose lives and deaths form the basis for all the material presented here.
Chapter 1
Introduction

1.1 Big data/no data: The modern dilemma of wildlife disease ecology

Even as biological sciences are increasingly enamored of big data [2,3], information access continues to limit how we manage and understand infectious diseases in wildlife. Field datasets used to study wildlife diseases are dominated by the survival and relocation datastreams that form the basis for standard conservation management. As technology allows us to look much more closely at some aspects of animals’ lives, scientific progress in wildlife biology will now be hindered by our ability to map those data onto questions that address our fundamental theoretically-driven hypotheses.

Tricking the inferences necessary for managing infection disease out of these datasets is not trivial (e.g., [4–6]). Both survival and relocation data contain little information about disease transmission per se, yet they are all that is available for natural systems that defy reproduction in captivity. As a consequence, wildlife disease investigators must leverage every element of information in those datastreams, and bolster them with results from captive studies [7]. Knowing which datasets provide the most value, and developing a clear framework for how to map those datasets onto high-priority disease questions would facilitate economically and scientifically efficient responses to emerging wildlife diseases.

Infectious disease modeling approaches draw heavily from four major human diseases: cholera, malaria, measles, and HIV/AIDS. John Snow’s work mapping cholera [8] and rickets [9] in London in the 1850s and applying those maps to
identify sources of infection demonstrated the value of detailed epidemiological investigation and spatial proximity. At the end of the 19\textsuperscript{th} century, Ronald Ross brought us the insight that vectors and their dynamics might heavily shape disease transmission, and he later put forward an early probabilistic approach to epidemic progressions \cite{?,10}. From measles researchers (Bartlett \cite{11}, to Anderson and May \cite{12}, through to practicing disease modelers today \cite{13,14}), epidemiologists learned to mathematically characterize systems in great detail using population-level incidence data, while leveraging major perturbations like the onset of measles vaccination in the 1960s. The emergence of HIV in the late 1970s and early 1980s pushed disease modelers to refine modeling techniques for diseases with frequency-dependent transmission \cite{15,16}, and motivated the first studies linking within-host processes to transmission and population-level patterns of disease incidence \cite{17,18}.

These ground-breaking studies of human pathogens pushed disease ecologists working on disease in many other systems. Research response to the bovine spongiform encephalopathy and foot-and-mouth disease and bovine spongiform encephalopathy outbreaks in the United Kingdom in the late 1980s to early 1990s, and in 2001, respectively, raised expectations about the speed and accuracy of public health response to emerging disease in livestock. Results from funding pulses to understand emerging corona, henipa, filo, and hantaviruses, all of which are apparently driven by underlying sylvatic dynamics, raised expectations about what is possible in studies of wildlife disease as well. In turn, progress on sylvatic dynamics of relevant human spillover systems has motivated wildlife disease ecologists working on conservation problems to study the underlying non-linear dynamics of their systems. However, at each stage of separation from human disease, available information and funding to pursue underlying disease dynamics declines. As a consequence, wildlife disease research in non-human, non-agricultural systems is regularly confounded by two factors: 1) a lack (real or perceived) of the information necessary to address our questions and parameterize our models; and 2) a desire (motivated in part by increasingly complex mathematical analyses) to capture every aspect of biological reality within our systems, increasing the risk of chronic model overparameterization.

With an eye toward those two factors, I embarked on an intensive study of \textit{Mycoplasma ovipneumoniae} in bighorn sheep. Most of my chapters attempt to speak to two issues at once: first, I try to provide rigorous research on pneumonia in
bighorn sheep that has real management implications; and second, I use the bighorn system as a jump-off point to demonstrate some approaches for drawing stronger inferences in wildlife disease systems in general. The common question across all chapters is, “How can we access and leverage every bit of available information to understand and manage an ongoing wildlife disease event?”. Several chapters depart from the bighorn sheep system to directly address general information mapping challenges for wildlife (in Chapter 5), and multidisciplinary collaboration (in Chapter 6). These chapters explicitly acknowledge the wider challenges associated with acquiring and utilizing data during on-going wildlife disease events.

1.2 Bighorn sheep and their pneumonia

Bighorn sheep in the Rocky Mountain west have suffered an extended period of population decline since the advent of European settlers, and their domestic sheep and goats. This decline is generally attributed to infectious agents carried asymptomatically by domestic ovids that wreak havoc on bighorn sheep. Pathogen introduction often results in highly visible all-age outbreaks, which have been extensively – albeit opportunistically – documented throughout the twentieth century. While populations could potentially rebound from die-offs if the causal agents faded out locally (as in the cases of Phocine [19] and canine distemper viruses [20], and plague [21]), perhaps the more insidious aspect of bighorn sheep pneumonia is that adult mortalities are followed by years of poor recruitment [1]. Population growth rates during this persistence phase are highly variable: in some years, very few (or occasionally, no) animals die of disease, whereas in other years, disease kills all or nearly all juveniles. The underlying factors leading to these differential outcomes remain unclear, impeding agency response and policy development alike.

I set out to characterize ecological aspects of pneumonia in bighorn sheep, particularly the heterogeneous outcomes of pathogen persistence, relying primarily on population- and individual-level data from the wild.

Pneumonic lesions in bighorn lungs are diverse in composition even within a single epidemic, a problem which stymied research efforts for decades. While lung worm, pasteurella spp., and Selenium deficiencies have all been implicated in bighorn sheep pneumonia in recent decades (Figure ??), recent work demonstrated
a critical role for *Mycoplasma ovipneumoniae* ("M.ovi") in the bighorn pneumonia complex. *M.ovi* colonizes the upper respiratory tract of Caprinae. In doing so, *M.ovi* impedes motion of the ciliary escalator through a combination of direct adhesion and hydrogen peroxide secretion. Impeded motion of the ciliary escalator provides an opportunity for all resident microflora of the upper respiratory tract to migrate downward into the lung. The switch from an aerobic upper respiratory tract environment to the anaerobic lower respiratory tract, coupled with a pathological immune response in the lung, leads to acute, and apparently diverse, disease in the lower respiratory tract.

Like other ovids, bighorn sheep exhibit no prenatal transfer of maternal antibodies; the preponderance of maternal antibody transfer occurs via the colostrum [22].

### 1.2.1 Contrasting definitions of “wildlife health”

One challenge that continues to befuddle the bighorn research community is what we mean when we say a population is “healthy”. Population “health” is characterized in three distinct ways, with each invoking a different line of research (Table 1.1). Without some consensus of what we mean by “healthy”, studies are not directly comparable, and apparently disparate results cloud research progress.

A primary goal of state wildlife agencies is to maintain strong wildlife herds. Thus, the first possible definition of “healthy” is whether populations meet management goals. This definition is easy to use, since it rests soley on readily available population count data, but it suffers from some important caveats. First, this definition relies on aggregate herd-level data, and may overlook fine-scale spatial disease dynamics. It can also potentially miss establishment of chronic pathogens, which take years to accumulate sufficient prevalences to be detectable at the population level (this, for example, may be the case for chronic wasting disease in North American cervids, [23]). Finally, without knowledge about causes of mortality, this definition cannot distinguish between a disease event and any other mortality source, allowing observers an easy means of attributing disease effects to changing predation, climate patterns, or other drivers. Despite these weaknesses, the management-objectives definition is regularly applied to some bighorn herds, including the Eastern Absaroka and Whiskey Basin herds in Wyoming, and Montana’s Missouri Breaks herd, all of which could be classified as diseased under a
Figure 1.1: **Within-host dynamics of bighorn sheep pneumonia.** Pathogens invade and colonize the upper respiratory tract, where production of hydrogen peroxide leads to cilia sloughing, which in turn creates an opportunity for pathogens to invade downward into the lower respiratory tract, which is the site of the most severe pathology. Most animals who survive disease are thought to either clear the lower respiratory of disease entirely, or to sequester pathogen particles locally and halt their proliferation. Clearance of the lower respiratory tract is often followed by clearance of the upper respiratory tract, although a subset of animals continue to carry pathogens in the upper respiratory tract, without severe downward invasion.
different definition.

A second definition of population health hinges on the presence of clinical disease, with herds deemed “healthy” unless individuals exhibit signs of disease. Since clinical diagnoses are difficult to determine using strictly observational data, this definition is more frequently applied to individuals necropsied post-mortem. While this definition produces very few false positives (populations identified as diseased under this definition almost certainly do have some disease present), it lacks specificity for several reasons. First, carcasses degrade rapidly, and in scavenger-rich regions, few carcasses may last long enough for appropriate post-mortem assessments. Second, this definition requires animals to die of disease in order for diagnosis. Therefore, diseases with high metabolic costs that might hamper reproductive fitness without immediately effecting survival may be overlooked. Third, this definition can only be applied in real time; without symptomatic animals or carcasses available, no health determination can be made.

The final definition of wildlife population health hinges on the presence of particular causative agents. Under this definition, populations are deemed “healthy” if live animals show no evidence of current (or sometimes past) infection. Unlike the first definition, this definition is mechanistically specific: it targets the pathogen directly and is not subject to observer detection biases or scavenger clean-up rates. Unlike the second definition, it does not require managers to find carcasses rapidly for diagnosis. It can also detect pathogens prior to the manifestation of their full demographic consequences. On the other hand, it requires knowledge of particular agents of concern, and the ability to test for these agents without destroying the animals. Furthermore, since pathogen presence does not necessarily imply disease presence, this definition may on occasion classify a population as “diseased” even when the causal agent is not currently harming the fitness of any single host.

1.2.2 Potential sources of heterogeneity

Three different processes govern disease effects on host population dynamics, and heterogeneity in either parameter could produce heterogeneous patterns of disease-induced mortality. The first is the disease-induced mortality rate, the rate at which infected animals die from disease. Some authors have argued that wildlife health is almost entirely emergent from ecosystem health (e.g., [24]). This is analogous to
assuming that pathogens are always present in wildlife populations, but that hosts keep those pathogens in check, except under inopportune environmental conditions that lead to degraded immune fitness and increased disease-induced mortality rates. While this hypothesis might hold for mortalities from macroparasite infections, like the ensemble of macroparasites currently wreaking havoc on the southerly end of the moose population [25–27], in other cases it clearly falls short: some extreme examples include morbillivirus events in wildlife species have dramatically reduced carnivore and seal populations [28,29]. If disease-induced mortality rate is the key driver of disease effects, we would expect no relationship between prevalence and health or growth rate.

A second process that could produce heterogeneous patterns of disease-induced mortality is transmission. Transmission is a particularly important driver of heterogeneity when within-host processes and outcomes are relatively consistent. For example, in the case of measles, individual disease progressions are highly predictable, and have relatively low variation. Therefore, most the variation that we see in measles epidemic dynamics arises due to heterogeneity in transmission. If transmission is crucial, then heterogeneity in outcomes arise simply because only a subset of hosts are exposed to the pathogen at all. In the bighorn system, earlier analyses failed to reveal consistent environmental themes associated with disease events [30,31]. Therefore, here we take the view that transmission is key to determining herd-level outcomes.

A third potential source of heterogeneity in this system comes from variation in the rate at which infected animals shed. While heterogeneous shedding rates are not universally understood, they are key contributors to disease dynamics in a few well-studied systems. In HIV, for example, infection is (or has historically been) an absorbing state: individuals generally do not recover. However, the infectiousness of HIV-positive individuals varies dramatically as a function of time since infection [17,18,32]. In other systems, infectiousness is known to vary over host-specific factors, as occurs with host pregnancy status and malaria [33]. In still other systems, like *Salmonella enterica* serovar *typhi* and *Staphylococcus aureus* [34,35], hosts clearly vary in shedding rate, but the mechanisms underlying this variation remain unclear.

In this system, all three processes may play a role, and part of the challenge of this system is in differentiating between the processes using the available data.
Fortunately, my work was coupled with an intensive animal handling push. Since we had infection status information on many of the animals studied here, we were able to being parsing all three sources of heterogeneity in tandem.
<table>
<thead>
<tr>
<th>Definition of “Diseased”</th>
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<th>Factors leading to discrepancies with other definitions</th>
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<th>Data requirements</th>
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<tr>
<td>Herd does not meet management goals</td>
<td>Pathogen-driven, disease-induced mortalities constrain population growth</td>
<td>Disease burden is too small to detect; other drivers that actually produce changes randomly co-occur with disease, but changes are attributed to disease status</td>
<td>Predation, density effects, disease-induced mortality; definition of management objective</td>
<td>Population-level counts (plus individual-level survival/ reproduction data if available)</td>
<td>All drivers of mortality are aggregated together</td>
<td>Shanthalingam et al. 2014</td>
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<tr>
<td>Disease present</td>
<td>Pathogen causes disease, and disease-induced mortality constrain population growth</td>
<td>Pathogen may be present but commensal; morbidity/mortality may be too low to effect herd management objectives; other agents may cause identical clinical disease</td>
<td>Host/Pathogen genetic diversity, environmental condition (esp. nutrition)</td>
<td>Individual-level health sampling data from dead/morbid animals</td>
<td>Difficult to get complete census of mortalities due to scavengers/predators</td>
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</tr>
<tr>
<td>Pathogen present</td>
<td>Pathogen predisposes herd toward having disease, which constrains population growth</td>
<td>Pathogen may be present but not virulent / not transmitting; pathogen may be absent, but presence of another agent may produce clinically similar disease</td>
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1.3 Dissertation objectives

The goals of this dissertation were threefold. First, I characterized the long-term population dynamics of bighorn sheep populations in the aftermath of pathogen introduction events (Chapter 2). Second, I examined the role that spatial and social contact patterns play in driving heterogeneity in lamb disease events (Chapters 3 and 4). Third, I addressed two general questions motivated by the bighorn work. First, how do we theoretically relate spatial and social contact networks to group size and animal space use patterns (Chapter 5), and second, is interdisciplinarity a growing trend in One Health modeling collaborations (Chapter 6)?

1.4 Collaborative work

My work is embedded in the work of a broader research consortium, whose major contributors are Tom Besser, Frances Cassirer, Paul Cross, Peter Hudson, Andy Dobson, Raina Plowright, and myself. As the primary analyst in this group, I worked closely on three published co-authored manuscripts: Cassirer et al., 2013 (Appendix G), Plowright et al. 2013 (Appendix H), and Cassirer et al. 2016 (Appendix K). In Cassirer et al. 2013, I designed and constructed Figures 2, 3, and 5, and Table 2, in addition to contributing the corresponding methods and results text, as well as revisions to the rest of the manuscript. I designed and conducted the preponderance of the analysis in Plowright et al. 2013, including construction of all figures except Figure 1, and all tables. I wrote much of the methods and results text, and provided revisions on the remainder of the manuscript. I collected the field data underlying Cassirer et al. 2016, conducted statistical analysis of field data, constructed Figure 1, and provided revisions to all text. In addition to these published projects, I am currently collaborating on preparation for another four bighorn manuscripts: two led by Dr. Plowright, one led by Emily Almberg (Montana Fish, Wildlife, and Parks), and one led by Dr. Cassirer. I am the second author on all of these projects.

I also made smaller contributions to two additional published manuscripts. One was led by Dr. Cross (Ecology, 2013; Appendix I), for which I conducted a statistical power analysis justifying the strength of the manuscript’s findings. The other was led by my brother, Luke Manlove (L. Manlove et al. 2016; Appendix
J), and investigated the capacity of heterologous vaccination and the impending general immune response to clear B-Acute lymphoblastic leukemia in murine hosts. I designed and implemented a principle component analysis to characterize general relationships between immune response markers, which was used to justify subsequent funding that allowed for specific knock-out experiments, and I also provided general statistical consultation and manuscript revisions.

One additional lead-authored project quantified the capacity of public safety campaigns to alter seat belt compliance rates in the mountain West (K. Manlove et al., Accident Analysis and Prevention, 2015). This work was conducted in collaboration with the Western Transportation Institute as part of a grant received prior to initiating my PhD, and is not included here.
Chapter 2
Pathogen invasion induces a phase-transition in bighorn sheep demography

2.1 Abstract

Ecological theory suggests that pathogens are capable of regulating or limiting host population dynamics, and this relationship has been empirically established in several settings. However, although studies of childhood diseases were integral to the development of disease ecology, few studies show population limitation by a disease affecting juveniles. Here, we present empirical evidence that disease in lambs constrains population growth in bighorn sheep (Ovis canadensis) based on 45 years of population-level and 18 years of individual-level monitoring across 12 populations. While populations generally increased ($\lambda = 1.11$) prior to disease introduction, most of these same populations experienced an abrupt change in trajectory at the time of disease invasion, usually followed by stagnant-to-declining growth rates ($\lambda = 0.98$) over the next twenty years. Disease-induced juvenile mortality imposed strong constraints on population growth that were not observed prior to disease introduction, even as adult survival returned to pre-invasion levels. Simulations suggested that models including persistent disease-induced mortality in juveniles qualitatively matched observed population trajectories, whereas models

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that only incorporated all-age disease events did not. We use these results to argue that pathogen persistence may pose a lasting, but under-recognized, threat to host populations, particularly in cases where clinical disease manifests primarily in juveniles.

2.2 Introduction

Disease-induced die-offs are often viewed as random events, after which host populations quickly return to disease-free, “healthy” dynamics. This perception rests on assumptions that host population dynamics are relatively stable, and that perturbations from disease-induced die-offs do not permanently alter host vital rates. In reality, some pathogen introductions push host populations onto trajectories headed toward new equilibria. Pathogens that generate these “phase transitions” in host dynamics are capable of regulating or limiting host population growth [36]. Pathogen-induced phase transitions shape population trajectories of several well-studied host species. Macroparasite dynamics help switch host populations into and out of cyclic dynamics by altering fecundity in adult females as opposed to mortality of offspring [37,38]; and pathogens that reduce adult survival can directionally suppress host population growth, pushing these populations into periods of sustained decline (e.g., [23,39]). Yet there are few examples of juvenile diseases regulating or limiting vertebrate host populations.

To produce phase transitions, pathogens must exert lasting pressure on host vital rates. This is rarely possible for pathogens that fade out (i.e., go locally extinct) rapidly, but it can occur when pathogens persist. On-going effects of pathogen persistence are visible in systems with temporally autocorrelated disease incidence. For example, epidemics of acute, perfectly immunizing pathogens like measles and mumps in humans buffer populations against future epidemics by temporarily depleting the pool of susceptible hosts [40]. Other diseases, such as chronic wasting disease [41] or bovine tuberculosis [42], gradually accumulate in host populations so that the risk of new infections increases with time since invasion [43]. In both cases, current disease intensity shapes future disease intensity, and this autocorrelation keeps host population dynamics confined to pathogen-determined trajectories.

Host vital rates that drive long-term shifts in population trajectories may not
be the rates most altered by pathogen introduction events. Disease invasions often reduce survival for all members of a host population, regardless of age. Over time, however, morbidity and mortality from chronic diseases may become most severe in older individuals with long infection histories. By contrast, pathogens that induce lifelong immunity in their hosts gradually become sequestered in the youngest host age classes as older individuals become immune. Detecting the effects of these latter agents in nature is particularly challenging, since juvenile survival is both hard to measure and often subject to more natural variability than that of adults (e.g., [44]).

As a consequence, although phase transitions in host population dynamics due to disease-induced changes in fecundity and adult survival are well-known, the hypothesis that sustained juvenile disease could drive phase transitions in host dynamics remains untested.

Here, we ask whether pneumonia in bighorn sheep (*Ovis canadensis*) induces a phase transition in host population dynamics by placing long-term constraints on juvenile survival. This disease is caused by invasion of pathogens commonly carried by domestic sheep (*O. aries*) and goats (*Capra hircus*) into naïve wild sheep populations usually accompanied by easily recognizable all-age die-offs; indeed, managers often rely on these die-offs as a proxy for pathogen presence [45]. Although adult bighorns appear to acquire protective immunity following exposure to pneumonia-causing agents [46], disease continues to impede lamb survival in the years-to-decades following disease introduction events [1]. However, most existing models assume that the impact of disease invasion on juvenile survival is relatively short-lived (e.g., [47,48]). In this study, we evaluate the evidence supporting that assumption, along with its consequences for expected population growth.

We use timeseries data from 12 well-studied bighorn sheep populations to examine how temporal autocorrelation and demographic variation in disease intensity shape population dynamics. Our study addresses two basic questions: first, whether disease persistence causes a phase transition in bighorn sheep population dynamics; and second, whether stagnant population growth during the disease persistence phase is attributable to juvenile mortality. We employed individual-level survival and lamb rearing data to identify specific vital rates that shift when populations transition from the pre-invasion “healthy” phase to the post-invasion “disease persistence” phase. We then applied these estimates to compare the immediate costs of pathogen invasion and the long-term costs of pathogen persistence. Finally,
we simulated population trajectories under different autocorrelation and disease intensity scenarios to explore the predictive capacity of models that make varying assumptions about the disease persistence period.

2.3 Methods

2.3.1 Data collection

We analyzed data collected on radio-collared ewes and their lambs in 12 bighorn sheep populations from the Hells Canyon metapopulation in Idaho, Washington, and Oregon. Bighorn sheep populations in Hells Canyon have experienced periodic pneumonia outbreaks since the first reintroduction in 1971. In 1995-96 several northern Hells Canyon populations underwent a major pneumonia-induced die-off, and disease spread through much of the metapopulation by 2002 (Figure 2.1a). From 1997 to 2015 we collected demographic data from marked individuals and conducted annual population surveys [1]. Prior to 1997, our analyses rely solely on annual population composition counts.

Throughout the entire timeseries (1971-2015), we assigned each population to one of three disease states in each year: disease-free and healthy; disease introduction (defined as the first reported pneumonia event in a given population); and post-invasion disease persistence. Disease status for each year was based on criteria described in [1]. Healthy estimates were primarily based on data from one population with no evidence of invasion and no detection of disease through 2011, but also included data from other populations during years with no evidence of active disease.

For each year, we calculated age-specific ewe survival and productivity (measured as lamb survival to weaning). Lambs born to radio-collared ewes that survived to October 1st were classified as surviving to weaning. Recruitment was based on ground or helicopter surveys conducted in late February or March.
Figure 2.1: Empirical patterns of pneumonia in bighorn sheep. (a) Disease status of twelve focal Hells Canyon bighorn populations since 1995. Open squares are years with no documented pneumonia. Grey circles are years with documented pneumonia in adults. Black circles are years with documented or suspected pneumonia in lambs. Population-years with no data are left blank. (b) Lambda and recruitment (March lamb:ewe ratio) for every population-year in the Hells Canyon study. Colors represent different disease classes, and point size scales with population size. Dashed lines are at lambda = 1 (i.e., static population size) and recruitment = 0.2, (this equates to 20 lambs per hundred ewes, a current Western Association of Fish and Wildlife Agencies guideline for a healthy population). Boxplots show the marginal distributions of recruitment and estimated lambda for healthy years (blue) as compared to years with any documented disease (red). Boxes contain the 25th to 75th quantiles, and lines extend to 2.5th and 97.5th quantiles.
(c) Disease status classifications as a function of years since the first recorded large die-off for each Hells Canyon population. Number of populations contributing data for each year following invasion are reported above the columns. (d) Recruitment (March lamb:ewe ratio) as a function of years since disease introduction in Hells Canyon. Number of populations with recruitment for each year following invasion is reported below the columns. The blue zone is the middle 50% of recruitment values for population-years with no detected disease.

Throughout these analyses, we use confirmed presence of clinical pneumonia (i.e., from necropsies of animals with pneumonic lesions in their lungs) or low summer lamb survival paired with observed pneumonia signs (coughing, nasal discharge, lethargy; [31]) to classify population-years as “persistently diseased” or “healthy”. This definition is explicitly independent of the presence or absence any specific causal agent. Although a large body of evidence – much of it from this system [49,50] – suggests that Mycoplasma ovipneumoniae is the primary pathogen driving pneumonia dynamics in bighorn sheep, our animal handling intensity was insufficient to confirm M. ovipneumoniae status by pathogen detection for every population-year included here. Additional details on field data collection are available in Appendix C: Field methods.

2.3.2 Empirical data analysis

We applied hierarchical generalized linear models to the dataset from 1997 onwards to assess whether recruitment and health status changed in the years following disease introduction. In each case, we modelled the response variable (lambs per 100 ewes in the first case; proportion of years classified as healthy in the second) as a function of years since invasion, with a hierarchical term capturing heterogeneity across populations (Appendix C: Post-invasion empirical trends).

We then applied hierarchical change-point models [51] to the entire timeseries of population counts to determine whether trends in female population counts changed before and after the first disease detection. Change-point models identify the location of shifts in a timeseries trajectory, and describe the slope before and after the shift. We used trajectories of female counts from nine populations that were established well before the initial disease introduction event. Population-specific timeseries ranged in length from 13 to 45 years (median = 33 years).
Populations 2, 8, and 9 in Figure 2.1a were excluded due to very limited data prior to disease introduction. Trajectories were aligned so that the first year of observed disease was set to zero for all populations. The model incorporated data from all nine populations simultaneously, while accounting for variation in baseline population size, growth rate before and after the change-point, and change-point’s position in the time series through hierarchical effect terms (Appendix C: Trajectory change-point models).

We used an integrated population model (“IPM”) [52] to combine ewe annual productivity and survival with lamb counts (i.e., lamb survival to weaning) from 1997 onwards to estimate survival and population growth under healthy and persistent disease conditions. However, we could not estimate vital rates during disease introduction due to limited individual-level data collected during die-offs. The IPM simultaneously leverages individual- and population-level data to estimate age-specific survival and lamb-weaning probabilities.

The IPM incorporated data from 217 known-age ewes monitored for a total of 1555 ewe-years. We combined ewes into age classes of 1, 2-3, 4-7, 8-13, ≥14, following Festa-Bianchet et al. [53], except that unlike Festa-Bianchet, we combined two- and three-year-olds due to a sparsity of two-year-old records in healthy years. Productivity data consisted of 525 lambs born to 141 known-aged ewes (Table 2.1). We also used ewe and lamb counts from 10 Hells Canyon populations over the 18 years from 1997 to 2014 (a total of 156 population-years). Recruitment was empirically measured as the lamb:ewe ratio in March of each year. In the IPM, it was estimated by combining individual-level summer lamb survival data with population-level counts of overwinter lamb survival, since lamb status was not determined for specific ewes after October 1st. In its entirety, the IPM estimated 24 parameters, 11 each – one over-winter lamb survival term, five age-specific ewe survival terms, and five ewe-age-specific lamb survival-to-weaning terms – for healthy and diseased years, and two hierarchical year effects associated with adult survival and reproduction (Appendix C: Integrated Population Model). The IPM was fit using JAGS [54] accessed through R [55].
2.3.3 Population projections and simulations

We used age-structured Leslie matrices to project population growth and demography separately for disease-free and persistent-disease states. The matrices were constructed so that births were pulsed [56]. Since population counts occur prior to lambing in the spring, we used a female-only post-breeding model with a 1:1 sex ratio at birth (Appendix C: Population Projection Models). Leslie matrices were parameterized using draws from posterior vital rate distributions generated under the IPM. Population growth rate under each disease state was estimated as the dominant eigenvalue of the Leslie matrix parameterized from posterior estimates for that state (denoted $\lambda_{\text{Healthy}}$ and $\lambda_{\text{Persist}}$, respectively).

We then compared recovery time – years required to return to pre-die-off population size – for die-offs followed by immediate return to healthy rates to recovery time for die-offs followed by a specified period of persistent disease. A population must increase by $k\%$ of its original size to return to pre-die-off size following a die-off killing $k\%$ of the population. Under immediate recovery, this occurs when $(1 - k)\lambda_{\text{Healthy}}^t = 1$. As long as $\lambda_{\text{Healthy}} > 1$, recovery is achieved when $t = y_r$, and

$$y_r = -\frac{\log(k)}{\log(\lambda_{\text{Healthy}})}$$ (2.1)

When disease persists, populations continue declining until persistence ends (if $\lambda_{\text{Healthy}} < 1$), and begin growing again only after pathogen fade-out. In a case where pathogens persist for $p$ years, recovery is achieved when $(1 - k)\lambda_{\text{Persist}}^p\lambda_{\text{Healthy}}^t = 1$. Years to recovery ($y_r$) for a population experiencing a die-off killing $k\%$ of the population, with persistence of $p$ years, was calculated as

$$y_r = -\frac{\log(k) + p\log(\lambda_{\text{Persist}})}{\log(\lambda_{\text{Healthy}})} + p$$ (2.2)

Finally, we simulated population growth under varying periods of disease persistence. We assumed that populations experience three disease states, healthy, disease introduction, and persistent disease, and transition through disease states according to a Markov process. In this model, populations are initially healthy,
but experience disease introduction events with some probability, $\alpha$. When the population is in either an introduction or a persistent disease state, another introduction event can occur with probability $\alpha$, the disease can fade out (returning the population to a healthy state) with probability $(1 - \alpha)\gamma$, or the disease can persist and shift the population into a disease persistence state with probability $(1 - \alpha)(1 - \gamma)$. The expected number of years between disease introduction events is equal to $\frac{1}{\alpha}$; the expected number of years from disease introduction to fade out (i.e., return to healthy) is $\frac{1}{\gamma}$. Transitions among disease states are summarized in the row-stochastic Markov transition matrix in (3).

$$
C = \begin{pmatrix}
\text{Healthy} & \text{Spillover} & \text{Post - Invasion} \\
1 - \alpha & (1 - \alpha)\gamma & (1 - \alpha)\gamma \\
\alpha & \alpha & \alpha \\
0 & (1 - \alpha)(1 - \gamma) & (1 - \alpha)(1 - \gamma)
\end{pmatrix}
$$ (2.3)

We ran 100 replicate simulations for each scenario. All simulations started with a population of 500 ewes and we initiated the disease process after 30 years of healthy growth. For each year, we first updated the disease status using binomial distributions based on that simulation’s disease model (i.e., age structured vs all-age models). Then we parameterized the Leslie matrix with values drawn from the posterior distribution generated for that disease state by the IPM. Since we had limited age-specific survival data during invasion years, we assumed that disease introduction years experienced a 30% reduction in adult survival (although many published accounts exceed this value, e.g., [57], we suspect that many smaller outbreak events go undetected). We also examined simulations that did not account for age-specific disease effects (i.e., in these simulations, every “persistent disease” year was given “disease introduction” demographic rates), and simulations in which disease years occurred at random, instead of in sequence.

We do not incorporate density-dependence in these simulations, because these are small populations (median population size 75) with an estimated 6 sq km of potential habitat per sheep (Idaho Department of Fish and Game unpubl. data), and we observed no evidence of density effects on vital rates prior to pathogen introduction (Figure 2.2). Population size accounted for less than 7% of the variation in recruitment or population growth in healthy years. We recognize the
importance of density-dependent population regulation in healthy bighorn sheep populations [58] but density did not play a role in the parameter space of these data and simulations.

2.4 Results

2.4.1 Bighorn sheep populations did not fully recover following die-off events.

Disease is known to persist in bighorn populations following pathogen invasion (e.g., [1]). Here we show that disease presence is associated with both stagnant growth and low recruitment (Figure 2.1b), and that disease costs are not alleviated over time (Figure 2.1c). The probability of a year being classified as “Healthy” after pathogen invasion was 0.23 and decreased by a multiplicative factor of 0.84 with each successive year-post-invasion, although this trend was not statistically significant (95% posterior credible interval [0.54, 1.25], Figure 2.1c, Appendix C: Table B.2). Recruitment was not associated with time since disease introduction, suggesting that the magnitude of disease in lambs remained constant (lamb:ewe median = 0.32 in the year of invasion; 95% posterior credible interval on yearly change in lamb:ewe ratio = [-0.01, 0.01]; Figure 2.1d, Appendix C: Table B.1). Taken together, these results indicate that population dynamics do not return quickly to pre-die-off rates; instead, growth remains constrained as long as pathogens persist in the host populations. Full descriptions of model posteriors are included in the online supplement.

Die-offs during pathogen invasion events may eliminate weaker hosts, selecting for the most fit individuals and leading to accelerated population growth in the absence of disease transmission (as shown by [59] for tuberculosis in African buffalo). However, we saw no evidence of improved population growth after die-offs, even in healthy years (mean $\lambda$ in healthy years = 1.14 before die-offs and 1.10 after die-offs). There is no evidence in this system that hosts who survive all-age die-off events have post-die-off vital rates that facilitate rapid return to pre-die-off population dynamics; instead, growth remains constrained as long as pathogens persist in the host populations.
2.4.2 Population trajectories changed in tandem with disease invasion.

Ewe count trajectories in six of nine populations with data before and after disease introduction showed a statistically significant decline associated with the first observation of pneumonia (posterior median year of trajectory change = 2.57 years prior to reported pathogen introduction, 95% posterior credible interval from 7.31 years prior to reported introduction to 2.20 years after reported introduction; Appendix C: Table B.4).

Populations generally grew prior to pathogen introduction (posterior median increase in numbers per year = 5.54, 95% posterior credible interval for the population change per year before the change-point, \( \mu_\beta = [4.26, 7.08] \)), but stagnated or declined thereafter (posterior median change in count per year = -3.17, 95% posterior credible interval for the population change per year after the change-point = [-8.08, 1.59]). We detected no evidence of a second change-point corresponding to recovery in the aggregated post-invasion timeseries. Instead, a weakly-characterized second change-point occurred in several populations during the first 15 years following invasion (posterior median = 4.42, 95% posterior credible interval for \( \mu_u = [1.18, 15.70] \); Appendix C: Table B.5), and was associated with accelerating decreases in population growth (posterior median = 8.61, 95% posterior credible interval for the mean annual change in population size before the change-point was [2.71, 15.25], whereas after the change-point the posterior median was -3.76 with a 95% credible interval of [-11.75, 3.23]). Credible intervals for the specific change-points for three populations fell entirely outside the posterior credible interval for the mean change-point for all populations. These populations initially grew following disease introduction, but declined 6-8 years later. No populations showed long-term recovery to pre-invasion population size or growth rates (Figure 2.2).

2.4.3 Adult survival returned to pre-die-off rates following disease introduction, but juvenile survival remained low.

Adult female survival was not significantly different in disease-free and post-invasion disease persistence years (Figure 2.3a; Appendix C: Table B.6). In fact, we saw some weak suggestion that adult survival might be marginally better in diseased years
Figure 2.2: Change-points in complete ewe count trajectories. Populations are aligned so that the first year of documented pneumonia is defined to be zero such that all negative years are disease-free. The grey shaded region showed the 95% posterior credible intervals for the mean change-point calculated across all populations. Solid black sections of the individual trajectories show 95% posterior credible intervals for that particular trajectory’s change-point. (a) Trajectories for all populations whose individual change-points fell within the 95% posterior credible interval for the mean change-point. The highest peak-to-trough includes removal of 72 sheep (43% of estimated mortalities), but removing that trajectory had negligible effects on posterior estimates. (b) Three populations’ individual 95% change-point credible intervals fell entirely outside the 95% posterior credible interval for the mean change-point.

than healthy years (Figure 2.3a), though this effect was not significant. Estimated lamb weaning rates were lower in disease persistence years than in healthy years, especially for prime-aged ewes (indicated by the non-overlapping posterior credible intervals in Figure 2.3b; Appendix C: Table B.6). We did see weak evidence that disease mortality may be somewhat compensatory in lambs, since estimated overwinter lamb survival in health years approximately 12% lower than in infected years (Appendix C: Table B.6). However, this effect was insufficient to overcome the dramatic costs disease imposed on lamb survival until weaning. Consequently, population age structure was predicted to shift toward older age classes under
Figure 2.3: Posterior estimates for vital rates and $\lambda$. Posterior mean and 95% credible intervals for (a) age-specific ewe survival and (b) probability of weaning a lamb in persistent diseased (grey) and healthy (black solid) years. (c) Posterior distribution of stochastic population growth rate in healthy (black solid) and diseased (grey dashed) years.

disease persistence (Appendix C: Fig. S1B).

### 2.4.4 Disease persistence period plays a major role in determining time to population recovery.

Projections based on the Leslie matrices predicted population growth in disease-free conditions (posterior median = 1.11; 95% posterior credible interval = [1.05, 1.14]; Figure 2.3c), but gradual decline under disease persistence (posterior median = 0.98; 95% posterior credible interval = 0.95, 1.01; Figure 2.3c). As expected, the simulation studies suggested that increasing persistence sharply increased expected time to recovery. A five-year increase in disease persistence had a similar effect on time to recovery as doubling the mortality in the initial outbreak (Figure 2.4a). This is reflected in the distance on the y-axis between immediate fade-out and increasing persistence time lines in Figure 2.4a: the 25 year difference in projected recovery time between initial population declines of 10% vs 90% (reflected in the bottom curve in Figure 2.4a) was similar to, but slightly less than the difference in projected recovery time for populations with immediate disease fade-out vs. 20-year persistence, regardless of initial die-off size (Figure 2.4a). These effects remained apparent even after incorporating stochasticity (Figure 2.4b). In general, population
Figure 2.4: Simulated effects of disease persistence. (a) The number of years required for a population to recover, i.e., regain pre-die-off sizes, over varying persistence periods. (b) Population projections based on 500 simulations under the Markov transition model in equation (3). Populations were subject to continuous healthy growth rates until year 0, and experienced their first disease event between years 0 and 1. The expected invasion rates was set to one invasion per five years, and expected persistence periods were set to 1 (light grey), 5 (medium grey), or 20 (black) years. Polygons bound the middle 50% of the simulated trajectories. Additional simulation outputs are shown in Appendix C: Figs. A.2 - A.4.

growth projections were lower when age-structure was overlooked, but this effect was less pronounced when dynamics were dominated by disease introduction (right panels of Appendix C: Fig. S2 and S3) as opposed to persistence (left panels of Appendix C: Fig. S2 and S3). Temporal sequencing of disease was not crucial to population dynamics following disease introduction: random vs. ordered disease states had relatively little effect on population growth, so long as disease impacted juveniles, and not all age-classes (Appendix C: Fig. S4). This was likely because the vast majority of years following invasion were diseased.
2.5 Discussion

Pathogens constrain host vital rates, and therefore push host population trajectories toward new equilibrium dynamics including extinction [60, 61]. This may be of limited importance for transient pathogens that go locally extinct shortly after introduction, where the period of pathogen-constraint is short with healthy dynamics resuming quickly. When novel pathogens persist, however, they can induce phase transitions in host population dynamics, sometimes with serious consequences for host species conservation and management. Our analysis suggests that such a phase transition may be underway in pneumonic populations of bighorn sheep.

The majority of the bighorn sheep populations in this study did not resume pre-disease-invasion growth rates during up to twenty years following recorded disease-induced die-off events. Instead, most populations declined abruptly near the time of the first all-age disease event (Figure 2.2a), and remained stagnant-to-declining thereafter. While pathogen invasion events reduced survival regardless of age (Figure 2.2), we found that as the pathogen persisted, its effects differentiated across age classes. Adult survival returned to pre-invasion rates even as juvenile survival-to-weaning remained low. The projected cost of persistent disease on host population growth could rapidly exceed the cost of all-age die-offs (Figure 2.4a). A steady onslaught of short-lived spillover events could produce population trajectories similar to those observed in the field (middle row of Appendix C: Figure B.2). However, that pattern would require years of strong recruitment between all-age events, which is inconsistent with our field observations of sustained lamb disease ([1], Figure 2.1a). The complete suite of disease dynamics was therefore best captured by simulations that accounted for long-term juvenile disease persistence; neither a disease process consisting of only die-off events, nor a process of persistent disease in all age classes could accurately capture both observed population trajectories and vital rates (Figure 2.4b, Appendix C: Figure B.2-B.4).

Assumptions about how long populations are subject to disease-structured growth rates have important consequences on the accuracy of population models, because increasing persistence period dramatically increased the expected time to population recovery in our study (Figure 2.4). Relatively rapid short-term recovery does sometimes occur, even in the Hells Canyon populations. Three of our populations did not show immediate downward shifts in population trajectories
following die-off events (Figure 2.2b). Additionally, we regularly documented at least one year of good recruitment during the 3-5 years following disease introduction, and our study populations regularly surpassed the management agency threshold for “healthy” vital rates (greater than 0.2 lambs recruited per ewe; [62]), even in the presence of clinical disease (Figure 2.1b). Yet despite this fact, and the observation that local bighorn sheep populations remain well below historic or habitat-based carrying capacity estimates, neither recruitment nor population growth returned to pre-die-off rates in the twenty years following die-off events (Figure 2.1, Figure 2.3).

Our models illustrate that even pathogen introduction events corresponding to very small initial die-offs could have severe long-term consequences on population growth. While our results may overestimate the cost of disease persistence following very mild initial outbreaks (since mild initial outbreaks may reflect underlying factors like low-virulence pathogen strains or strong spatial structuring that might also affect persistence dynamics), this scenario remains empirically undercharacterized, and merits further consideration.

Disease-induced mortality rates in bighorn sheep vary substantially in the field, and this is reflected in both the empirical data analysis (Figure 2.2) and the simulation study (Figure 2.4). Variation could be attributable to multiple processes including contact heterogeneity and social substructuring [63], pathogen virulence, host susceptibility to pathogen establishment, and factors associated with each individual’s unique mucosal immunity and carriage status. The IPM did not parse these drivers, but they are accounted for in the breadth of the posterior estimates, and pushed forward directly into the simulations. As a consequence, the simulations capture a realistic range of disease impacts on host vital rates, insofar as those rates are empirically represented within our dataset. Partitioning the variance attributable to each source of heterogeneity is critical for management, and remains an area of active inquiry. Even in the presence of heterogeneity, the basic trend remains clear: most bighorn sheep populations experience lasting reductions in juvenile survival in the aftermath of disease-induced die-off events, and this reduction may persist until the causal agent goes locally extinct.

Evidence that pathogen persistence might induce phase transitions in host vital rates is beginning to emerge from a number of other wildlife disease systems. Acutely immunizing infections produce negatively autocorrelated prevalence oscillations, which can then drive host population cycles. This has been demonstrated for
macroparasites of red grouse [37] and reindeer [38], and is increasingly evident for morbilliviruses like canine [20,64] and phocine distemper viruses [65], hantavirus [66], and plague [67]. Oscillating population trajectories are usually centered above zero, so the major conservation concern is whether host populations can persist through epidemic peaks.

Invading chronic pathogens, however, can shift previously stable populations into monotonic declines toward zero. Small but sustained downward shifts in host viral rates “redden” the host demographic spectrum (e.g., [68]). As a consequence, persistent chronic pathogens can severely diminish the host population’s long-term growth, resilience, and viability. There are increasing indications that this may be the case for chronic wasting disease in elk at Rocky Mountain National Park [23], and simian immunodeficiency virus in chimpanzees [69], both of which appear to produce phase-transitions in adult survival. Brucellosis is projected to have a similar directional effect in African buffalo, this time through a phase-transition in fecundity [70]. Fungal pathogens of amphibians and bats also produce directional shifts in population growth rates, although the precise demography may vary between particular host species [71,72].

If invading chronic pathogens do not cause high mortality initial die-offs, as occurred in a third of the bighorn sheep populations in this study, they may go undetected, reducing opportunities for early control. Overlooking persistence and modeling only highly-visible disease introductions produces a dramatically inflated projection of future population growth for bighorn sheep, and this bias may also apply to other disease conservation systems. Wildlife and conservation biologists must be alert to invading and establishing pathogens – even those with apparently negligible costs. When left unchecked, these agents have the potential to quietly but dramatically alter long-term population trajectories.
Chapter 3  
Costs and benefits of group living for bighorns with disease

3.1 Abstract

Group living facilitates pathogen transmission among social hosts, yet temporally stable host social organizations can actually limit transmission of some pathogens. When there are few between-subpopulation contacts for the duration of a disease event, transmission becomes localized to subpopulations. The number of per-capita infectious contacts approaches the subpopulation size as pathogen infectiousness increases. Here, we illustrate that this is the case during epidemics of highly infectious pneumonia in bighorn lambs (*Ovis canadensis*). We classified individually-marked bighorn ewes into disjoint seasonal subpopulations, and decomposed the variance in lamb survival to weaning into components associated with individual ewes, subpopulations, populations, and years. During epidemics, lamb survival varied substantially more between ewe-subpopulations than across populations or years, suggesting localized pathogen transmission. This pattern of lamb survival was not observed during years when disease was absent. Additionally, group sizes in ewe-subpopulations were independent of population size, but the number of ewe-subpopulations increased with population size. Consequently, although one might reasonably assume that force of infection for this highly communicable disease scales with population size, in fact host social behaviour modulates transmission.

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such that disease is frequency-dependent within populations, and some groups remain protected during epidemic events.

### 3.2 Introduction

Host aggregation can facilitate pathogen transmission, and this is thought to be a major cost of group living [73]. The extent to which host social dynamics shape transmission depends on both the ease with which the pathogen is transmitted from host to host, and host group stability during epidemics. For easily-transmitted pathogens, like those with aerosolized transmission routes, transmission is often modelled as density-dependent: the rate at which susceptible hosts become infected is thought to increase with increasing population size, with the consequence that pathogen persistence and epidemic size are both directly related to population size [74].

By contrast, pathogens that require more intense contact for transmission, like those relying on sexual transmission routes, have forces of infection independent of population size [74]. This is because the number of disease-transmitting contacts is expected to be similar regardless of population size, a a transmission function often referred to as “frequency-dependent”. For hosts living in highly compartmentalized societies with consistent group sizes and rare between-group interactions, however, stable social bonds can modulate force of infection by making transmission of all pathogens more frequency-dependent at the population scale even though transmission rates may be higher in larger groups (density-dependent). This occurs when infectious agents are isolated to specific host groups [75], as was shown for bovine tuberculosis transmission among badgers in the United Kingdom [76], and for the single-host enzootic phase of *Yersinia pestis* outbreaks among prairie dogs [77]. Pathogen sequestration to particular social groups is predicted even for highly infectious agents that might otherwise be assumed to have density-dependent transmission. In these settings, predictions from mean-field models that describe disease at the population scale are incorrect, since the population does not accurately represent the pool of susceptible hosts [74,78–80].

Contact estimates based on population-wide mixing may be biased high during periods of group stability, with important ramifications for intervention during disease epidemics in human, domestic, and free-living animal populations. First,
since individual contacts are biased high, transmission and disease-induced mortality rates estimated at the population level will be biased low [81]. Second, choices about management actions such as culling, antibiotic treatment, or vaccine application which aim at altering the number of susceptible hosts may be misguided, since these decisions rely on theoretical threshold values which make assumptions about the relevant number of susceptible hosts [78]. Third, theory suggests that if transmission scales with population size (i.e., transmission is density-dependent), then pathogens cannot invade and persist in host populations below a threshold population size [79,82]. Thresholds, however, do not exist if disease-transmitting contact rates and population size are decoupled due to host contact structure (i.e., transmission is frequency-dependent) [83]. As a consequence, while pathogens subject to density-dependent transmission are generally predicted to fade out prior to driving their hosts extinct, frequency-dependent transmission is both theoretically [61] and empirically [84] linked to host population collapse.

Alternatively, group stability may sometimes benefit social hosts during infectious disease events. While extensive empirical work explores the role of well-connected “super-spreader” individuals in increasing epidemic size (e.g., [85]), relatively few studies investigate the inverse effect, e.g., whether temporally stable of host aggregations inhibit pathogen transmission. This is true despite the fact that many animal species live in genetically well-mixed populations which consistently break into discrete subpopulations for portions of the year. For example, the social dynamics of harem breeding species like zebras [86] and horses [87], or species like mouflon [88] and saiga antelope [89] which rear their offspring in stable nursery groups, may serve to buffer those populations against pathogen transmission during periods of increased susceptibility simply by eliminating social bridges along which pathogens could move. For these species, group stability may temporarily limit pathogen transmission by acting as a form of organizational social immunity [90].

Here, we address the question: How does spatiotemporal stability in social connections affect disease-induced mortality patterns in a social host? We then explore how the relationship between social patterns and observed mortality events relate to the choice of pathogen transmission function, and consider the implications of that choice on management of wildlife species which experience high levels of disease-induced mortality. We treat these questions in the context of a detailed study of an infectious pneumonia which regularly produces epidemics in populations...
of bighorn sheep. Existing work on this system focuses on preventing and managing all-age mortality associated with pathogen spillover from domestic sheep and goats [30,48,91]. Here we focus instead on the enzootic phase of disease characterized by recurrent annual pneumonia outbreaks among bighorn lambs [1,46]. By analysing lamb mortalities as they relate to ewe social connections, we find that disease impacts some portions of bighorn populations much more intensely than others, and explain why disease events in chronically infected populations may be unlikely to respond to population-level management strategies.

3.3 Materials and methods

3.3.1 Study system

Epidemics of contagious pneumonia challenge bighorn sheep restoration throughout the western United States [30,91]. Pneumonia epidemics in bighorn sheep are attributed to spillover of directly transmitted bacteria from domestic sheep [45,92]. Following pathogen introduction, initial disease events affect all age classes with ewe mortality rates ranging from 30% to 60% [1]. Subsequently, some populations continue to experience periodic outbreaks of pneumonia in lambs prior to weaning for a decade or more [1].

Bighorn sheep live in sexually segregated societies for much of the year [93]. During summer, ewes rear their lambs in nursery groups. Pneumonia outbreaks likely start with transmission from one or more chronically infected ewes to their lambs [1,48,94], with gregarious juvenile behaviour providing a plausible mechanism for subsequent lamb-to-lamb transmission. This study focused on animals living in the Hells Canyon area of Idaho, Washington, and Oregon (Figure 3.1a). The preponderance of diagnosed lamb mortality in Hells Canyon during the study period was attributable to infectious pneumonia (true for 92 of 104 necropsied lambs for whom a cause of death could be confirmed) [1]. In light of this observation, we assumed that lamb mortality was a reasonable proxy for lamb infection throughout this analysis. Lamb mortality rates varied dramatically from year to year, regardless of whether disease was present (Figure 3.1b).

We investigated the capacity of four factors to account for variability in lamb mortality: population, year, ewe-subpopulation, and individual ewe. We used these
factors to specify a hierarchy of four possible transmission scales during enzootic lamb pneumonia events. Follow-up analyses examined the relationship between ewe-subpopulation size and population size. If most lamb mortalities result from direct ewe-to-lamb transmission, then we would expect that some (presumably chronically infected) ewes would consistently lose their lambs while others would consistently rear theirs successfully, producing heterogeneity and high variation in individual ewe outcomes. If disease is localized within ewe-subpopulations, then we would expect some subpopulations to experience very poor lamb survival, whereas other subpopulations in that same year might have high lamb survival. This would produce heterogeneity and high variation at the subpopulation level. If transmission is localized to populations, we would expect some populations to consistently have high summer lamb survival, whereas other populations would consistently perform poorly, producing high variation at the population level. Follow-up analyses examined the relationship between ewe-subpopulation size and population size.

### 3.3.2 Data collection and preparation

We followed lambs born to marked ewes from 1997 through 2010 in four intensively monitored bighorn sheep populations in Hells Canyon (Figure 3.1a). Location data from 162 radio-collared ewes over a combined 664 ewe-summers in the Asotin, WA; Black Butte, WA; Immaha, OR; Redbird, ID; and Wenaha, OR and WA populations were used to build summer contact networks for 66 lamb cohorts born in the years following an all-age pneumonia outbreak in 1995 - 1996 [1,31]. We defined populations as geographically distinct groups, with no exchange of females and low levels of mixing by males. A lamb cohort was defined as a year-class of lambs within a population. As in previous analyses [1,46] each lamb cohort was considered an independent observation from its population, since there are no clear trends in disease severity or population trajectories once pathogens were present in the study populations (Appendix B: Fig. S1). The resulting dataset includes between 10 and 13 cohorts from five populations (Appendix B: Table C.1). The median proportion of ewes radiocollared per population was 0.25 (Appendix B: Table C.1). Ewes almost always gave birth to a single lamb in May (85%) and weaned the lamb at approximately four months of age [95]. We excluded seven cohorts where the
median interval between successive locations of a given ewe was greater than ten days. Lambs were dependent on, and tightly paired with, their dams throughout the summer (May 01 to September 30). A detailed description of summer ewe social network construction, including details on the data used in construction, are included in the online supplementary information. We used ewe social networks as a basis for identifying temporally stable ewe-subpopulations, which we defined as clusters of marked ewes observed associating directly or indirectly (i.e., were not observed directly associating, but were both observed associating with a third marked individual at different points in time; Figure 3.1c) over the course of the summer. All ewe-subpopulations were entirely nested within populations in this study.

Daily group size and composition were recorded at each observation; in this analysis, we use “group size” to refer to the number of sheep seen together in these “daily groups”. Ewes were classified as having a lamb when they were observed nursing, bedded with, or in body contact with, a lamb. The survival of lambs born to marked ewes was calculated through September 30th (age approx. 18 - 20 weeks). Diseased and healthy cohorts were classified on the basis of the presence or absence of recorded or suspected lamb pneumonia [1,31], and were analysed separately. Appendix B: Table C.1 contains information on each cohort, including the number of ewes and lambs followed, population health status, number of relocation events, and number and size of all ewe-subpopulations.

3.4 Analysis

Bootstrap-based methods were used to check for relationships between number of detected ewe-subpopulations and number of radiocollars, and for differences in numbers of groups between diseased and healthy years (see online supplementary material). Network-based autocorrelation approaches [96] were used to evaluate the stability of ewe-subpopulation membership from year to year, and are included in the online supplement.

To determine the spatial and temporal scales of pneumonia transmission, we decomposed variance in summer lamb survival using a hierarchical logistic regression model. We estimated variance attributable to: populations, years within populations (“years”), ewe-subpopulations, and ewes. Because daily group membership was
not stable over the timescale of epidemic events, we omitted daily groups from the variance decomposition in favour of the coarser but more realistic ewe-subpopulation membership. We took years to be nested in populations because disease severity was not temporally synchronized across all Hells Canyon populations (Appendix B: Fig. S1). The nested structure allowed each population to experience a unique disease status in the same year. Ewe effects were estimated across all years during which a ewe reproduced, regardless of that year’s disease status. Since ewe effects are estimated across all years that she was observed with a lamb, they are partially crossed with all other terms in the model. Comparisons were based on a variance decomposition performed using a hierarchical logistic regression model with random effects for ewes, ewe-subpopulations, years, and populations. For the $i^{th}$ lamb born to the $j^{th}$ ewe in the $k^{th}$ ewe-subpopulation during the $l^{th}$ year in the $m^{th}$ population, this corresponded to the following hierarchical linear model:

$$\log \frac{p_i}{1 - p_i} = subpop_k[i] + ewe_j[i]$$ \hspace{1cm} (3.1)

$$subpop_k \sim N(year_{l[k]},$$
\[ \tau_{\text{subpop,PN}} \times I(\text{PNStatus})_k + \tau_{\text{subpop,healthy}} \times (1 - I(\text{PNStatus})_k) \] (3.2)

\[ \text{year}_l \sim N(\text{pop}_m[l]) + \delta I(\text{PNStatus})_l, \]
\[ \tau_{\text{year,PN}} \times I(\text{PNStatus})_l + \tau_{\text{year,healthy}} \times (1 - I(\text{PNStatus})_l) \] (3.3)

\[ \text{pop}_m[l] \sim N(0, \tau_{\text{pop}}) \] (3.4)

\[ \text{ewe}_j \sim N(0, \tau_{\text{ewe}}) \] (3.5)

We use the subscript notation \( z_{q[r]} \) to denote which of the \( q \) levels of factor \( z \) was experienced by individual \( r \). For examples, \( \text{ewe}_j[i] \) indicates which of the \( j \) ewes produced lambs \( i \). \( I(\text{PNStatus}) \) are indicator terms that take on the value 0 for years classified as healthy, and 1 otherwise. These terms control the variance estimate to which each observation contributes. Indicator terms were generated separately for each level of the model, but always retained the same meaning: any ewe-subpopulation present in a year classified as having pneumonia is assigned an \( I(\text{PNStatus}) \) term of 1, as is any year classified as having pneumonia. A schematic of model hierarchy is shown in Figure 3.2.

For our purposes, the critical attributes of this model were the precision parameters, \( \tau_{\text{subpop,PN}} \), \( \tau_{\text{subpop,healthy}} \), \( \tau_{\text{year,PN}} \), \( \tau_{\text{year,healthy}} \), \( \tau_{\text{pop}} \), and \( \tau_{\text{ewe}} \), which we inverted to variances following model fitting. We were particularly interested in the relative size of each variance component. High variance at a particular level indicated that lamb survival differed between units at this level, whereas low variance meant that lamb survival did not differ. Since we were specifically interested in whether models that incorporated ewe-subpopulations outperformed mean-field models which treated all ewes in a population as well-mixed, we used DIC to compare models with and without a ewe-subpopulation level in their hierarchical structures.

We then tested whether the number of ewe-subpopulations during the \( l^{th} \) study year in the \( m^{th} \) population (\( \lambda_l \)) depended on population size. Population size was estimated from the total number of ewes counted in annual aerial surveys. We used a hierarchical Poisson model containing a fixed effect for total ewes counted ("TotEwes"), a random intercept for each population ("pop"), and an overdispersion term, \( \psi \), to evaluate this relationship [97]. Formally, this model was
\[
\log(\lambda_l) = \beta_0 + \beta_1 TotEwes_{m[l]} + pop_{m[l]} + \psi_l
\]  
\[
pop_m \sim N(\mu_{pop}, \tau_{pop})
\]

In this model, our inferential focus was on the posterior density associated with the \( \beta_1 \) parameter, which links the observed number of ewe-subpopulations to the total number of ewes in the population. We used a similar structure to model median daily group size as a function of total number of ewes, however in that case we used an identity link function, and treated the residuals as normally distributed. See Appendix B for detailed descriptions of statistical methods and model fits.

We fitted a piecewise linear regression model [51] to describe how the number of ewe-subpopulations related to median daily group size. Piecewise regression allows a process to abruptly change forms at some (model-estimated) point along a covariate axis. Median daily group sizes were modelled as a function of the number of ewe-subpopulations present in each population, in a framework that allowed for a possible change in the relationship between daily group size and number of subpopulations. In this model, \( \lambda_k \) represents the median daily group size observed for members of the \( k^{th} \) subpopulation in the \( j^{th} \) year. Let \( \beta_1 \) be the linear relationship between number of subpopulations (“NumSubpops”) and median daily group size prior to the changepoint; let \( U \) be the changepoint, and let \( \gamma \) be the adjustment to the relationship between number of subpopulations and daily group size for numbers of subpopulations exceeding the changepoint. Then the changepoint model is

\[
\log (\lambda_k) = \beta_0 + \beta_1 NumSubpops_{j[k]} + \gamma(NumSumpops_{j[k]} - U) \times step(NumSubpops_{j[k]} - U)
\]  

Our inferential focus was on whether the relationship between daily group size and number of subpopulations was constant over the range of number of subpopulations observed. We were also interested in the specific shape of that
relationship. Thus, inference was based on estimates of $\beta_1$, $\gamma$, and $U$. The hierarchical linear models were fitted using the \texttt{lme4} package [98], and the piecewise regression model was fitted using the \texttt{SiZer} package [99] in R [?]. The variance decomposition was fitted using MCMC samplers implemented in JAGS [54] and accessed through R. Five chains of length 100,000 were run for each model, with the first 50,000 steps omitted for burn-in. Gelman-Rubin statistics [100] were used to evaluate chain mixing and model convergence. Credible intervals were extracted from the joint posterior distributions produced by the MCMC algorithm following convergence. We used noninformative priors wherever possible. Specific parametrizations, convergence diagnostics, and posterior estimates for all emergent quantities are included in Appendix B.

3.5 Results

The number of ewe-subpopulations that contributed to a lamb cohort ranged from one to nine, with a median of 3 (Appendix B: Fig. S2). Twenty-eight of the 53 cohorts with multiple ewe-subpopulations were classified as healthy, and 25 as diseased. To test for ewe-subpopulation-specific effects on lamb survival in diseased years, we analysed survival of the 274 lambs born in cohorts with more than one ewe-subpopulation present. In years with lamb disease events, 50.8% of the observed variation in lamb mortalities was attributed to ewe-subpopulations (95% posterior credible interval: 28.6%, 77.0%), whereas in healthy years, subpopulations accounted for only 28.9% of observed variation (95% posterior credible interval from 2.2% to 64.6%). The subpopulation a lamb was reared in explained more variation than year or ewe during years when disease was detected, whereas in healthy years, group explained no more variation than individual or year (Figure 3.2b). Furthermore, a model that did not incorporate ewe-subpopulation was a significantly poorer fit than a model that included ewe-subpopulations (DIC for the full model = 580.5; DIC for the reduced model = 600.8). The variance in lamb survival among subpopulation was significantly higher in years with disease. Lambs in a subpopulation with survival one standard deviation below average have greater than three times the odds of mortality [$\exp(\sqrt{1.46}) = 3.35$] when epidemics were detected. By contrast, in healthy years the odds of mortality for lambs born in a subpopulation with survival one standard deviation below average was less than
Figure 3.2: Variance decomposition. (a) Schematic representing hierarchical structure of variance decomposition. Y = “year”, SP = “subpopulation”, E = “ewe”. (b) Variance attributed to each random effect (ewe-subpopulations, years, specific ewes). Grey lines indicate 95% posterior credible intervals associated with each variance component. Black is used for variance decomposition when disease is present; grey is used years when no disease was detected. Variance decompositions are based only on years with multiple components. Ewe effects were estimated across all years (healthy and diseased), and are thus shown as a single distribution applied across all disease classes. Variance in the hierarchical (ewe, subpopulation, year, population) logistic regression model in diseased cohorts was estimated to be 1.41 at the subpopulation level (95% credible interval from 0.77 to 2.21), and 0.58 at the year level (95% credible interval from 0.03 to 1.45). In healthy years, variance based on 167 lambs was estimated as 0.43 at the subpopulation level (95% credible interval from 0.02 to 1.32), and 0.279 (95% credible interval from 0.01 to 0.96) at the year level. Variance attributable to ewe effects across all years was estimated at 0.406 (95% credible interval from 0.02 to 0.95); variance attributable to population was estimated at 0.280 (95% credible interval from 0.01 to 1.46).

twice \[\exp(\sqrt{0.42}) = 1.91\] those of lambs born in an average subpopulation. In an analysis which excluded ewe-subpopulation, ewe-subpopulation level effects in diseased years were re-attributed to years, whereas healthy year effects remained relatively unchanged (Appendix B: Fig. S4).

Ewe-subpopulations were defined such they that were never observed to mix with one another over the course of the summer. While some unobserved mixing between subpopulations almost certainly occurred, our sampling intensity limits the likelihood that strong connections between marked animals (e.g., “bridge vertices”) escaped detection in this dataset. However, subpopulations did undergo internal fission-fusion dynamics, with some subpopulation members forming ephemeral groups that lasted one or several days before splitting. Median total daily group size was 9 (approximately 6 ewes with a mixture of three juveniles and (usually
young) rams). The number of ewe-subpopulations did not differ between healthy and diseased years (p = 0.289 on 10000 bootstrapped replicates; online supplementary material). The number of ewe-subpopulations increased when the population size increased (= 0.013; 95% confidence interval = (0.0024, 0.0245); Figure 3.3a). The relationship between number of ewe-subpopulations and ewe-subpopulation size was characterized by an abrupt change once multiple ewe-subpopulations were present. Median daily group size declined dramatically when the number of ewe-subpopulations increased from one to two, but remained relatively constant for two or more subpopulations (95% confidence interval for slope with less than three groups = (-14.176, -0.228); 95% confidence interval for slope with two or more ewe-subpopulations = (-0.725, 3.215); Figure 3.3B). The number of ewe-subpopulations did not differ by health classification (healthy and diseased) (p = 0.289 on 10000 bootstrapped replicates). We found no compelling evidence of a relationship between total number of ewes observed and daily group size (95% confidence interval for = (-0.064, 0.042); Figure 3.3c); daily group size remained relatively constant across a range of observed population sizes. We observed similar patterns regardless of whether we used total daily group size or number of ewes in a group in these models.

3.6 Discussion

We found that lamb mortalities were affected by female social connections during years when disease was present, but not in the absence of disease. Ewe-subpopulations accounted for most of the variation in lamb mortality, while individual ewe, year, and population-level variation were relatively unimportant. Mortalities were structured at the subpopulation scale and therefore the assumption that all ewes in a population mix homogeneously during lamb disease epidemics is not supported.

For the five months following parturition, ewe group sizes were independent of population size (Figure 3.3B; Figure 3.3c). Group size during lamb-rearing may represent an ecologically optimal aggregation for foraging efficiency or protection from predators [101], but the size and stability of these aggregations also have consequences for disease transmission and management. Specifically, our data suggest that lamb mortality, and by proxy pathogen transmission, is largely localized
within ewe-subpopulations. These results have several important ramifications. First, disease-induced mortality rates calculated at the population level likely underestimate true impacts on exposed lambs. Rather than successfully controlling their own infections, surviving lambs may simply be protected by social barriers that reduce or eliminate their risk of exposure. In this situation, ewe social connections provides a form of organisational social immunity [90] in which some individuals remain protected simply because existing contact processes do not provide a direct chain of transmission linking them to infected individuals. In this way, stable female-offspring groups may provide population-level resilience in the face of infectious disease despite high juvenile susceptibility. This resilience comes at a cost, however: since ewes in this system appear to consistently form groups of similar sizes, regardless of population size, then disease die-offs are likely to precipitate social reorganization patterns that may foster continued pathogen
transmission even as populations decrease in size. In short, the advantage of a critical population size below which pathogens fade out may be lost.

Choice of transmission function often shifts from density-dependent to frequency-dependent when systems are viewed from increasingly coarse scales. While phocine distemper in harbour seals generally follows density-dependent transmission processes within cohorts, between-cohorts transmission is frequency-dependent [19]. The relative role of different transmission modes shifts dramatically between spillover (between-species) and enzootic (within-species) persistence in prairie dog populations infected with *Yersinia pestis* [77, 102]. Seasonally variable host aggregation and disease incidence patterns are consistent with a shift between frequency- and density-dependent transmission of chronic wasting disease incidence in white tail deer [103]. However, despite the growing empirical support for scale-dependent approaches to transmission function, transmission in a system is still usually assumed to follow the same process: it is either frequency- or density-dependent, regardless of the scale of observation (but see [104]). This assumption may hinder model development and disease management, especially for systems transitioning from spillover to endemic, or with long-lived infectious stages [105].

Research on the relationship between group stability and disease often focuses on systems in which transmission is enhanced during periods of increased group mixing or group size. This is the case, for example, in measles among school children [14], and brucellosis in elk [106]. In this study, we explore the other end of that spectrum. Disease events in our system occur when populations are compartmentalized into subpopulations, and we argue that this compartmentalization constrains epidemic size. This finding has important implications about the evolutionary pressures that pathogens place on host societies.

As is inevitable with analysis of field data, ours has a number of assumptions and limitations. First, since not all ewes were marked, we were unable to build saturated contact networks for each cohort. This may have resulted in an underestimate of the amount of mixing among subpopulations and introduced some upward bias on the number of subpopulations detected. The relationship between number of collared ewes and ewe-subpopulations was not significant after accounting for population size, however (Appendix B). Frequent relocations and the general fidelity of collared ewes to their resident groups (Appendix B: Fig S5) suggest that this weakness does not jeopardize our overall findings. Second, we could not directly measure ewe-
subpopulation size. Although subpopulations were defined based on associations of marked individuals, they also consisted of an unknown number of unmarked sheep that were not individually identifiable. Since the number of subpopulations increased with population size but daily group size did not (Figure 3.3) we assumed that the number, but not the sizes of both subpopulations and groups were linked with population size. Third, this analysis focuses entirely on enzootic disease events in ewes and lambs; the potential roles of adult rams and domestic sheep during lamb pneumonia events are not considered here. Disease in lambs is unlikely to come from rams since, consistent with previous work [93], we rarely observed adult rams with ewes and lambs during summer. Apparent population closure paired with high ewe survival during lamb epidemics is consistent with the hypothesis that infection in lambs is not due to spillover, but to chronically infected ewes.

Finally, we attribute the observed variation in lamb mortality between subpopulations to disease transmission, but subpopulations may be subject to varying risk from other sources, particularly genetic susceptibility and habitat. We feel confident attributing risk to contact as opposed to genetic factors because ewes are not completely nested in subpopulations. Instead, some reshuffling in subpopulation membership occurs between years (Appendix B: Fig S5). If genetics drive susceptibility in this system, then a strong ewe effect should have emerged in the variance decomposition, but no such effect was observed. From a habitat perspective, while there could be some microsite differences between populations, all populations studied here are at low density, and are unlikely to be nutritionally limited and forage quality and availability are unlikely to be vary substantially between ewe-subpopulations.

Our results reflect a growing resource allocation dilemma for bighorn sheep management: research and management efforts have focused on all-age die-offs that occur after pneumonia is first introduced to a naïve population; however, the long-term enzootic persistence of pneumonia may be the greater threat to species recovery. Management aimed at reducing disease transmission through limiting or reducing population size would likely result in fewer ewe subpopulations of the same size and might have no effect on the probability of within-subpopulation disease transmission during lamb rearing. A reduction in population size without a corresponding change in ewe group size is unlikely to alter contact rates within ewe-subpopulations, which appear to be most critical for disease transmission to
juveniles. An alternative strategy is to target removal efforts at animals that are likely to be chronically infected, a strategy we are currently exploring in the Hells Canyon system. Although disease transmission within subpopulations may proceed via density-dependent transmission, transmission is frequency-dependent when viewed from the population scale [9]. As a consequence, the critical community size required for pathogen persistence may be much lower than previously expected, and this will hamper efforts to manage enzootic disease by limiting population size.

Limited knowledge of the mechanisms underlying pathogen persistence inhibits our ability to make management decisions that facilitate population recovery after disease invasion. Reducing spillover risk is unquestionably critical for bighorn sheep restoration, but following spillover, risk mitigation is not necessarily sufficient to spur pathogen fade-out and population recovery. We showed that when groups are stable, social structure can be directly linked to mortality patterns, and that this relationship may be discernible from commonly-available data like the relocation dataset used here. Such a relationship can serve as a basis for drawing inference about the extent of disease transmission, with direct implications on disease management. Incorporating social networks into survival analyses is a powerful yet underutilized approach for investigating and managing disease in wildlife.
Chapter 4  
Contact and contagion: Bighorn sheep demographic groups vary in probability of transmission given contact

4.1 Abstract

Both contact and probability of transmission given contact shape epidemic progress, and understanding both is key to managing wildlife diseases. However, wildlife disease research tends to focus on contact heterogeneity, since probability of transmission given contact is notoriously difficult to measure. Here we present a first step toward empirically investigating probability of transmission given contact in free-ranging systems. We used measured contact networks to test whether bighorn sheep demographic groups vary systematically in infectiousness or susceptibility to Mycoplasma ovipneumoniae, an agent responsible for bighorn sheep pneumonia. We built covariates using network metrics, demographic information, and infection status, and used logistic regression to relate those covariates to lamb survival. The covariate set contained degree, a classic network metric describing node centrality, but we also built covariates that broke the network metrics into particular categories to differentiate between probability of transmission given contact with yearlings,

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1This chapter is under review at *Journal of Animal Ecology*, as Manlove, K.R., E.F. Cassirer, P.C. Cross, R.K. Plowright, and P.J. Hudson. Contact and contagion: Bighorn sheep demographic groups vary in probability of transmission given contact.
ewes with lambs, and ewes without lambs. Yearlings, ewes with lambs, and ewes without lambs showed similar group membership patterns, but direct interactions involving touch occurred at a rate two orders of magnitude higher between lambs and reproductive ewes than between any classes of adults or yearlings, and one order of magnitude higher than direct interactions between lambs. Although yearlings and dry ewes regularly carried Mycoplasma ovipneumoniae, our models suggest that the probability of transmission given contact was approximately five times higher for reproductive ewes than for non-reproductive animals. Consequently, management actions targeting infected animals might lead to unnecessary removal of young animals who carry pathogens but rarely transmit.

4.2 Introduction

Understanding factors that drive variation in host transmission rates is critical for predicting and managing infectious disease events [107]. For directly-transmitted pathogens, transmission can be distilled into two stages. First, a susceptible and an infected host must contact one another; and second, the pathogen must take advantage of the contact and move between hosts and establish in the new host. In the absence of either host contact or pathogen movement and establishment, transmission does not occur. This compartmentalization is evident in a classic reconstruction of the transmission term in Susceptible - Infectious - Recovered (“SIR”) models, which expresses transmission rate, $\beta$, and $-\kappa \log(1 - c)$. Here, $\kappa$ reflects the contact process and $c$ is the probability of transmission and establishment given contact (e.g., [108]). Partitioning observed heterogeneity into separate stages attributable to contact, and pathogen movement and establishment is the first step towards understanding and managing pathogen transmission. Theoretical and empirical studies describe how contact heterogeneity shapes transmission [109–111], but a key question is whether measured networks can also inform inference about pathogen transmission and establishment probabilities.

To date, network-based studies of disease transmission fall into two general groups. The first group simulates epidemics forward on empirical contact networks to assess the relative importance of well-connected individuals or groups for pathogen transmission (e.g., [109,111–114]). Inferences usually focus on population-level epidemic outcomes, such as epidemic size (e.g., [115]), duration (e.g., [116]), or
serological signal (e.g., [117]). The second group of studies directly measures transmission using physically or genetically marked pathogens (e.g., [118–121]), and empirically evaluates the relationships between host behavior and transmission. The majority of both sets of studies tacitly attribute all transmission heterogeneity to the contact process, so that epidemic features are treated as a function of contact, with probability of transmission given contact assumed constant.

However, contacts alone do not explain all the variation in observed transmission. Transmission sometimes depends more on the timing and load of pathogen shedding, or the dose received and susceptibility of potential recipient hosts than on variation in host behavior (e.g., [122]). Since load, shedding rate, and susceptibility are difficult to measure in free-ranging hosts, a key next step in epidemiological network modeling is to constrain contacts with empirical observations, and shift the inferential focus to probability of transmission given contact. Whereas the probability of transmission given contact is assumed constant in models attributing heterogeneity to contact, models could condition on empirically measured contacts, allowing direct inquiry about probability of transmission and establishment given contact.

A few studies have conditioned on contact without investigating the probability of transmission given contact per se. Grear et al. [123] used a model conditioned on contacts to find optimal temporal lags for capturing transmission dynamics in two classes of macroparasite, and Godfrey et al. [124] conditioned on contact to examine host sex differences in transmission of three tuatara parasites. Nevertheless, studies that condition on measured contacts to better estimate other aspects of transmission remain rare.

Probability of transmission given contact depends on both the infectiousness of the infected host and the susceptibility of the recipient host. Contacts involving infected hosts who shed low pathogen doses, or susceptible hosts who require high doses for pathogen establishment are less-likely to result in transmission than identical contacts involving infected hosts who shed high doses, or susceptible hosts with lower pathogen establishment thresholds. Sometimes these factors vary systematically with infectious phase. In HIV, for example, the probability of transmission during a single sexual contact is much higher if the infected individual is within the first 15 months of infection than if the same contact occurred during the subsequent period of viral latency (e.g., [18]). Pregnant women infected with Plasmodium falciparum experience higher Plasmodium loads in their blood [17, 32]
and higher vector bite rates [33] than other human host groups, and may therefore contribute disproportionately to the force of infection. Individuals shed *Salmonella typhi* and *Escherichia coli* at differing levels, and thus impose variable force on their environments [125, 126].

In each of these examples, variation in host infectiousness or susceptibility was established by directly measuring pathogen load or immune dynamics within the host longitudinally through time. Acquiring those direct measurements requires extensive animal sampling, which is often not possible in free-ranging animal systems. Here, we offer an alternative approach that tests postulated differences in transmission risk simply by using social contact information and disease outcomes.

### 4.2.1 General approach

Individual-level network metrics are not independent of one another, in that one animal’s connections are not independent of its neighbors’ connections [127]. Although dependencies are usually viewed as inferentially problematic, here we take advantage of those inherent relationships to evaluate how well, shared contacts predict the covariance in epidemic outcomes between a set of hosts.

Our approach is inspired by adaptive evolutionary models that infer selective pressures over different regions of phylogenetic trees across evolutionary time [128]. These models treat the networks – in their case, phylogenetic trees; here, social contact networks – as known. Node-specific outcomes – in their case, observed phenotypes; here, individual disease outcomes – are assumed to covary in proportion to shared edge attributes. If outcomes for nodes with many edges in common do not covary strongly, that suggests their shared edges contribute only weakly to observed outcome. Hypotheses about how edge-specific effects vary can be recast as alternative weightings of the network edges, and tested using standard statistical analyses that compare how well alternative groupings or weightings of edges predict observed outcomes (Figure 4.1).

Consider an epidemic progressing along a weighted network whose nodes represent individuals and edges represent contact intensities. The epidemic produces disease occurrences along the network, with individual outcomes depending on both contact structure (edges and edge-weights) and probability of transmission given contact. If probability of transmission given contact does not vary, the
individual outcomes should covary in proportion to their shared edges (or, contacts). Alternatively, if some host groups have systematically higher probabilities of transmission given contact, then a more detailed model allowing different probabilities for different host groups will better describe the epidemic.

A purely behavioral model might treat an individual’s risk as a function of its centrality. For individual whose contacts are listed in the set $I = \{1, 2, \ldots, j\}$, the model postulates that risk of infection is a function of the sum of i’s edgeweights, $\sum_{j \in I} e_{ij}$. In this case, all edges of a given weight impose the same transmission risk, regardless of the particular animals involved. However, consider the common alternative hypothesis that probability of transmission given contact also varies with infectious dose, and its imperfect proxy, individual load. If load data are available, then the contacts can be reweighted, so that edgeweights are rescaled to also account for neighbor j’s current pathogen load, $l_j$. Under the load-weighted risk model, i’s risk becomes $\sum_{j \in I} e_{ij}l_j$. When outcomes and pathogen statuses are known, these two hypotheses can be compared with standard logistic regression models (Figure 4.1).

4.2.2 Application: Inferring transmission risk in bighorn sheep

Bighorn sheep in the United States experience recurrent spillover and prolonged persistence of pathogens that cause population-limiting pneumonia [1,129]. Bighorn sheep pneumonia is characterized by an initial all-age die-off event that typically kills between 30% and 90% of affected herds. Following all-age die-offs, some adults continue to chronically carry *Mycoplasma ovipneumoniae* (“M.ovi”), the primary causal agent underlying disease [45,49]. Chronically infected adults apparently act as reservoirs for transmission to naïve lambs, producing lamb disease events that severely reduce lamb survival to weaning in the years-to-decades following die-offs [1]. Currently, it is unclear whether all chronically infected animals impose equivalent transmission risks on lambs, and whether all lambs are equally vulnerable to death from pneumonia.

We apply our general approach to understand M.ovi transmission in bighorn sheep during summer lamb pneumonia outbreaks. Our analysis hinges on the assumption that host groups with low probability of transmission given contact will have little effect on disease dynamics, even if their contact rates are high. By
Figure 4.1: Conceptual underpinnings. Consider a focal individual, and its set of social contacts (a). For a system in which all animals impose the same infection force on their neighbors, the probability a focal animal gets infected is simply a function of the sum of its edgeweights (b). However, when different groups impose different probabilities of transmission given contact, the focal individual’s probability of infection can be compartmentalized into a set of forces coming from separate groups (c). When individual outcomes and contacts are known, individual outcomes can be regarded as binary response variables, \( Y \), and summed edge weights can be treated as covariates, \( X \). Group-specific probabilities of transmission given contact can then be estimated as regression \( Y \) and \( X \), shown in (d) and (e).

In contrast, groups with highly infectious members may influence disease dynamics even if their contact rates are low. We arrange the investigation around three research questions. First, are association and interaction patterns consistent across demographic groups? Second, does the probability of transmission given contact differ between demographic groups? Finally, how does infection status relate to transmission risk in the bighorn pneumonia system?
4.3 Materials and Methods

4.3.1 Study area and field data collection

We collected health and behavioral data on three bighorn sheep populations in southeastern Washington and northeastern Oregon during the summers of 2013-2015 over a total of six population-years (Table 4.1). The Asotin Creek, Black Butte, and Mountain View populations were reintroduced through translocations conducted between 1977 and 1997 (WDFW, ODFW). These populations experienced all-age pneumonia epidemics between 1988 and 2012, followed by pneumonia outbreaks of varying severity in lambs. Each population consisted of 42 to 65 animals. This study relies specifically on observational field data from 88 marked ewes and yearlings, monitored for 129 animal-years. We collected nasal swabs from most (82 of 129, accounting for 74% of all relocations) of the instrumented animals in the winter preceding summer observations. Samples were analyzed at the Washington Disease Diagnostic Laboratory, which derived *M.ovi* titers from serum, and active current infections with an *M.ovi*-specific PCR (e.g., Raina Plowright unpubl. data, E. Frances Cassirer unpubl. data). Animals greater than one year of age were fitted with color-coded radio-collars, and individually marked with numbered and colored ear tags. Animals under one year received only ear tags. Capture and handling were conducted according to protocols approved by the Oregon and Washington Departments of Fish and Wildlife.

Crews located radio-collared individuals approximately every three days from May 1st to July 15th. During observations, crews recorded group location, number of lambs present, and health status of all members of the group. Number of relocations and sampling intensities for each site are reported in Table 4.1. Data collection was conducted under protocols approved by Penn State (IACUC #40292) and Montana State University (IACUC #2014-59).

Direct contact, or “interaction” data came from 3234 ten-minute group follows, in which we timed, and classified all touching events between individuals. We attributed contacts to specific individuals whenever individual identities were known. Unmarked individuals involved in contacts were recorded by their demographic group (ewes with lambs, dry ewes who were never observed with lambs or lost their lambs prior to June 1st, yearlings, and lambs). We excluded data on adult rams.
Although single rams did occasionally appear in ewe groups, this occurred in less than 10 of the 1131 groups included in this analysis. Interactions were classified as body contacts (any contact except nursing or facial contacts), facial contacts, nursing, and bedded contacts (in which animals were bedded touching one another). Lambs could not be uniquely identified. We assumed that nursing and bedded contacts occurred between dam-lamb pairs unless otherwise noted by the observer; other interactions involving lambs were attributed to “unknown” lambs.

### 4.3.2 Network construction

We constructed two kinds of networks: an association network whose edges linked pairs of animals that occurred in the same group, and an interaction network whose edges linked pairs of animals that directly contacted one another. We weighted edges in the association network according to each pair’s social affinity index, $S_{i,j}$ [127]. The social affinity index is an association measure that adjusts for uneven ability to record animal identification (in our case, due to a subset of animals that were only marked with ear tags). When $m_{i,j}$ is the number of sampling units in which individuals $i$ and $j$ were associating, $n_{i,j}$ is the number of sampling periods in which both $i$ and $j$ were identified, $n_i$ is number of sampling periods with just $i$ identified, and $n_j$ is number of sampling periods with just $j$ identified, then the social affinity index for individuals $i$ and $j$ is

$$S_{i,j} = \frac{m_{i,j}}{\min ((m_{i,j} + n_{i,j} + n_i) (m_{i,j} + n_{i,j} + n_j))}$$

(4.1)

Lambs were not uniquely identifiable, but since lambs associate with their dams continuously until weaning in the fall [95], we assumed lambs were always present in the same group, and therefore had the same social affinity indices, as their dams.
Table 4.1: Study population sample sizes. In “Disease status”, “I” indicates whether a disease event occurred in lambs; “P” indicates live pathogens were detected in some hosts in the population during that population-year; “S” indicates some adult animals actively displayed symptoms in that population-year; “E” indicates some collected lamb carcasses showed evidence of exposure to pathogens in that population-year, “He” indicates no signs of clinical disease were observed in lambs. *Population estimates are based on counts at the end of the preceding biological year.

<table>
<thead>
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<th>Year</th>
<th>Population</th>
<th>Population Size*</th>
<th># ID’d Adults</th>
<th># Relocations</th>
<th># Lambs</th>
<th>Disease status</th>
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<td>554</td>
<td>11</td>
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<td>1136</td>
<td>15</td>
<td>He, P, S</td>
</tr>
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<td>13</td>
<td>718</td>
<td>11</td>
<td>I, P, S, E</td>
</tr>
<tr>
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<td>Asotin</td>
<td>60</td>
<td>33</td>
<td>1442</td>
<td>23</td>
<td>He</td>
</tr>
<tr>
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<td>45</td>
<td>16</td>
<td>574</td>
<td>11</td>
<td>He, P</td>
</tr>
</tbody>
</table>
In the interaction networks, nodes again represented individuals, and edges represented direct contacts between individuals, as recorded through focal follows. In this case, we collapsed the interaction network down so that nodes reflected demographic groups instead of individuals. We denote estimated interaction rates between individuals of two demographic groups, Grp1 and Grp2, as \( \hat{\gamma}_{Grp1-Grp2} \). Rates were estimated as simply the total minutes of observed direct contacts between individuals of Grp 1 and Grp 2, divided by the total duration of pair-minutes in which animals of those classes were observed. We built 95% bootstrap confidence intervals by resampling focal follow events, calculating contact rates for each bootstrapped replicate, and extracting the 2.5th and 97.5th quantiles of the bootstrapped distribution of contact rates.

### 4.3.3 Network analysis

Classic measures of centrality for temporally static networks were calculated, as were several temporally dynamic network features. Static networks were used to estimate two centrality metrics: degree (the total number of other animals with which a given animal associates), and eigenvector centrality (connectedness of a given animal that up-weights connections to well-connected neighbors, and down-weights connections to poorly-connected neighbors). Dynamic networks were used to estimate the rate of individual degree accumulation, as well as temporal variation in each individual’s short-term degree. In all cases, metrics were compared between three demographic classes: reproductive ewes, dry ewes, and yearlings.

A series of networks, one for each population-year during each day of the field season, were built to model degree accumulation. Each day’s network used all data acquired through that day. Degree was calculated (the number of identifiable animals that each marked animal co-occurred with) for each individual using each day’s network, creating a time-series of individual degrees. Each individual’s degree on each day was divided by the total number of marked animals in that population, so that degree values were bounded above by one. Since degree captured any contact with a particular animal (e.g., did not account for association strength), degree was constrained to increase monotonically through time, from a minimum of associations with 0% of the marked animals in a given population-year up to a maximum of associations with 100% of the marked animals in that population-year.
This constraint allowed use of a logistic function with a common upper bound across all population-years, even though the number of marked animals varied from population to population. Logistic functions were fit using the `nls` function in R's stats package (55).

A second series of networks, each extending over sequential seven-day intervals (e.g., the first network included data from days 1-7, the second network included data from days 2-8, etc.) was built to model temporal changes in number of associates. For each network, each individual’s degree was calculated, creating a time-series of degrees for each animal. Each individual’s average degrees were arranged into a $1 \times (\text{Timesteps} - 6)$ vector ($Y_i$), which could decline through time during periods when animals were isolated or in smaller-than-average groups. We then fit generalized additive models (GAMs) to the time-series of node strengths and allowed the smoothing functions ($q_i$) to vary across reproductive groups. We included a fixed intercept adjustment ($\alpha_j$) for each of the $j$ population-years. Formally, these models can be written

$$Y_i = \alpha_{j[i]} + q_i (\text{ReproductiveGroup}_i) + \epsilon_i$$

where $\epsilon_i \sim N(0, \sigma^2)$. GAMs were fit using R’s mgcv package [130].

We evaluated whether association rate and interaction rate were reasonable proxies for one another by modeling centrality within the association network (measured here with eigenvector centrality; [131]) as a function of interaction rate ($\text{inter}_i$), after adjusting for population, year, and demographic group. We used interaction rate as our measure of interaction centrality, since that allowed us to normalize over unbalanced numbers of focal follows including each animal. Formally, the eigencentrality for the $i^{th}$ animal, $e_i$, was modeled as

$$e_i \sim N (\beta_0 + \beta_1 \text{inter}_i + a_{\text{Pop-Year}}, \sigma_r)$$

where $a_{\text{Pop-Year}} \sim N (0, \sigma_{\text{Pop-Year}})$. 55
Table 4.2: Hypotheses, covariate definitions, and expected relationships. $I_X$ denotes all of lamb $i$’s edges connected to individuals of group $X$. $S_{i,j}$ is the social affinity index for individuals $i$ and $j$; $\gamma_{Grp1-Grp2}$ is the estimated interaction rate between individuals of demographic group 1 and demographic group 2.

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>Covariate</th>
<th>Calculation</th>
<th>Expectation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Association centrality predicts transmission</td>
<td>$C_{Dam}$</td>
<td>$\sum_{j \in I_{Dam}} S_{i,j}$</td>
<td>Lambs with many dam associations have high $P(Death)$</td>
</tr>
<tr>
<td></td>
<td>$C_{Dry-Yrl}$</td>
<td>$\sum_{j \in I_{Dry-Yrl}} S_{i,j}$</td>
<td>Lambs with many dry ewe or yearling associates have high $P(death)$</td>
</tr>
<tr>
<td>Transmission requires direct contact</td>
<td>$D_{Dam}$</td>
<td>$\sum_{j \in I_{Dam}} S_{ij} \times \hat{\gamma}_{Dam-Lamb}$</td>
<td>Lambs with high interaction rates have high $P(death)$</td>
</tr>
<tr>
<td></td>
<td>$D_{Dry-Yrl}$</td>
<td>$\sum_{j \in I_{Dry-Yrl}} S_{ij} \times \hat{\gamma}_{Dry-Yrl-Lamb}$</td>
<td>Lambs with high interaction rates have high $P(death)$</td>
</tr>
<tr>
<td>Inference is strengthened by looking only at associations with infected animals</td>
<td>$PA_{Dam}$</td>
<td>$\sum_{j \in I_{Infected \ dams}} S_{ij}$</td>
<td>Lambs with high association rates with infected dams have high $P(death)$</td>
</tr>
<tr>
<td></td>
<td>$PA_{Dry-Yrl}$</td>
<td>$\sum_{j \in I_{Infected \ dry-yrl}} S_{ij}$</td>
<td>Lambs with high association rates with infected dry ewes and yearlings have high $P(death)$</td>
</tr>
<tr>
<td>Inference is strengthened by looking only at interactions with infected animals</td>
<td>$PI_{Dam}$</td>
<td>$\sum_{j \in I_{Infected \ dams}} S_{ij} \times \hat{\gamma}_{Dam-Lamb}$</td>
<td>Lambs with high interaction rates with infected dams have high $P(death)$</td>
</tr>
<tr>
<td></td>
<td>$PI_{Dry-Yrl}$</td>
<td>$\sum_{j \in I_{Infected \ dry-yrl}} S_{ij} \times \hat{\gamma}_{Dry-Yrl-Lamb}$</td>
<td>Lambs with high interaction rates with infected dry ewes and yearlings have high $P(death)$</td>
</tr>
<tr>
<td>Dam’s antibody titer</td>
<td>$M$</td>
<td>Dam’s antibody titer (measured in preceding winter)</td>
<td>Lambs born to seropositive ewes have lower $P(death)$</td>
</tr>
</tbody>
</table>
4.3.4 Linking network measures to lamb survival

Lamb survival to October 1st was the response variable in all transmission models. Although some lambs died of factors other than disease, most summer lamb mortality is attributable to pneumonia [1]. Our dataset consisted of lamb survival and covariate measures for 56 lambs born to 43 separate ewes over five population-years. We eliminated lambs born to Black Butte ewes in 2013, when fewer than 50% of the ewes were marked, and none had been tested for *M.ovi*.

Static association and interaction network metrics provided a suite of covariates associated with transmission risk. Covariates were calculated for each lamb based on the set of individuals observed interacting or associating with that lamb (or that lamb’s dam). From the outset we wanted to compare transmission risk posed by dry ewes and yearlings with transmission risk posed by reproductive ewes. To that end, some of our models allow separate risks incorporated through separate covariates for contacts with reproductive ewes, versus contacts with dry ewes or yearlings. Covariates, along with details of their construction, are outlined in Table 4.2. To increase power, and because of their relatively similar contact rates (Figure 4.2a), we combined data from yearlings and dry ewes in all transmission analyses. Bivariate plots of all covariates are shown in Appendix D Figure D.1.

We fit a suite of logistic regression models that captured all combinations of the hypotheses in Table 4.2. The association centrality, interaction intensity, and infection-weighted interaction hypotheses were all tested using variants on the same theme: that the risk posed on a given individual by a certain peer group can be measured by summing over total contacts or interactions with members of that peer group (Table 4.2). We also considered models with more conventional approaches to infection risk, for example, a model that treated an individual’s risk as a function of the sum of that individual’s edge-weights in the interaction or association network.

Lambs born to each marked ewe (*j* ∈ 1 : 56) were assigned binary responses equal to 1 when the lamb survived to weaning, and 0 otherwise. Marked animals never observed with lambs did not contribute response values to the analysis, but did contribute to the covariate sets. All models included a random effect for population-year. We compared models conditioned on contacts to models that overlooked contact in order to determine whether conditioning on contact allowed for new insights. Models without contact used the number of reproductive
ewes, dry ewes, and yearlings in each population as covariates (in some models, we limited these counts to just numbers of infected animals in each group). We included maternal antibodies in these models so they were directly comparable to contact-conditioned models.

Results from the transmission models led us to conduct two follow-up analyses. The first was a Fisher’s exact test comparing prevalence (as determined by *M.ovi* PCR status in the preceding winter) in yearlings (n = 22) to prevalence in adults (n = 60). The second follow-up analysis was a logistic regression of lamb survival status (0 or 1 for each lamb) as a function of two indicator variables: one indicating whether a lamb had any associations with infected reproductive ewes, and one indicating whether a lamb had any associations with infected yearlings or dry ewes.

### 4.4 Results

#### 4.4.1 Are association and interaction patterns consistent across demographic groups?

Association networks that focused on animals being present in the same group were generally well-connected, with all animals exhibiting similar connection numbers and strengths (Figure 4.4ab; Figure D.2-D.3). All animals accumulated associates at a relatively constant rate, although yearlings were somewhat slower than reproductive and dry ewes (Figure 4.2a; Table D.1; demographic groups showed statistically significant differences in patterns, but qualitatively similar behaviors). While groups were large and stable prior to the birth pulse, all demographic groups showed low association strength during the birth-pulse month. Association strengths increased again about a week after the peak of the birth pulse, and remained high throughout the summer (Figure 4.2b; Table D.2; patterns are statistically significantly different, but qualitatively consistent across groups), consistent with formation of nursery groups. A few yearlings were lost to follow-up midway through the field season (Figure 4.2b), possibly as a result of yearling rams migrating to bachelor groups [132].

We recorded data on 1131 groups, and observed an average of 27.4 identifiable animals and 6.6 groups during each 48-hour sampling interval. Interaction patterns varied dramatically between demographic groups, with all the most common forms of interactions involving lambs (Figure 4.3; Table D.3). The most intense interactions
Figure 4.2: Temporal association dynamics. (a) Cumulative node degree through time for all animals in our study (grey lines), and fits from logistic functions derived from dams, dry ewes, and yearlings. (b) Node strength through time in the study. Node strength can decrease when animals are consistently observed separately.

were between lambs and their dams, and typically consisted of nursing or bedding together. Yearlings and adults of all reproductive states almost never directly interacted with other animals. Interactions and associations were not significantly related to one another at the individual level ($\beta = 0.004$; standard error = 0.002; conditional $R^2$ from the regression of total interactions on association eigencentrality = 22.6%).

4.4.2 Does the probability of transmission given contact differ between demographic groups?

The strongest model (AIC weight = 0.59) included interaction-weighted, demographic-class-specific contacts with infected animals, as well as dam’s antibody titer (Figure 4.4c, Table 4.3). A second model that included group-specific associations with infected animals and dam’s antibody titer was competitive with the top model (AIC weight = 0.39; Table 4.3). Both high-performing models supported separate probabilities of transmission given contact coming from reproductive vs. non-reproductive animals, suggesting probability of transmission given contact varied
with reproductive status. Models that overlooked neighbor infection status, or accounted for connectedness (either through associations or interactions) without adjusting for differences between demographic groups, and models that did not include dam’s antibody titers received little-to-no support (Table 4.3). There were not significant differences in antibody titers among adult animals across all population-years included in this analysis ($F = 2.0708, p = 0.101$).

In the top model, more interaction time with yearlings and dry ewes was associated with a significant increase in lamb survival ($\beta = 0.940; \text{SE}(\beta) = 0.352; p = 0.001$; Table 4.4), whereas interactions with dams was associated with decreased lamb survival ($\beta = -0.712; \text{SE}(\beta) = 0.222; p = 0.008$; Table 4.4). Higher antibody titers in a lamb’s dam was associated with a small but significant increase in the likelihood the lamb survived ($\beta = 0.069; \text{SE}(\beta) = 0.024; p = 0.003$; Table 4.4).
Figure 4.4: Inferred transmission risk. (a) Social contact network from the Asotin population in 2014. (b) The same network, with edges separated into groups reflecting the hypothesis that infected animals in different demographic groups exert differing forces on their neighbors. (c) Covariate effects from the strong lamb survival model.

Figure 4.5: Lamb survival by association with infected animals of differing demographic groups. (a) Infection status in winters preceding our field sampling for adults and yearlings (e.g., rising lambs). (b) Proportion of lambs that died by association status with infected dams (left). Proportion of lambs that died by association status with infected yearlings or dry ewes (right). Bars represent 95% binomial confidence intervals in all panels.
Table 4.3: Hypotheses, models, and AIC diagnostics.

<table>
<thead>
<tr>
<th>Lamb survival predictors</th>
<th>Model</th>
<th>AIC</th>
<th>Delta-AIC</th>
<th>AIC Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Interactions with specific groups of infected animals</td>
<td>$\beta_0 + \beta_1 PI_{Dams} + \beta_2 PI_{Dry-yrl} + \beta_3 M$</td>
<td>48.41</td>
<td>0</td>
<td>0.59</td>
</tr>
<tr>
<td>2. Dam’s antibody titers</td>
<td>$\beta_0 + \beta_1 PA_{Dams} + \beta_2 PA_{Dry-yrl} + \beta_3 M$</td>
<td>49.25</td>
<td>0.84</td>
<td>0.39</td>
</tr>
<tr>
<td>1. Associations with specific groups of infected animals</td>
<td>$\beta_0 + \beta_1 D_{Dams} + \beta_2 D_{Dry-yrl} + \beta_3 M$</td>
<td>55.46</td>
<td>7.06</td>
<td>0.02</td>
</tr>
<tr>
<td>2. Total population size</td>
<td>$\beta_0 + \beta_1 PopEst$</td>
<td>57.13</td>
<td>8.72</td>
<td>0.01</td>
</tr>
<tr>
<td>1. Interactions with specific groups of animals, regardless of infection status</td>
<td>$\beta_0 + \beta_1 PA_{Dams} + \beta_2 PA_{Dry-yrl}$</td>
<td>60.37</td>
<td>11.96</td>
<td>No support</td>
</tr>
<tr>
<td>2. Dam’s antibody titers</td>
<td>$\beta_0 + \beta_1 C_{Dams} + \beta_2 C_{Dry-yrl} + \beta_3 M$</td>
<td>63.51</td>
<td>15.1</td>
<td>No support</td>
</tr>
<tr>
<td>1. Associations with specific groups of animals, regardless of infection status</td>
<td>$\beta_0 + \beta_1 D_{Dams} + \beta_2 D_{Dry-yrl}$</td>
<td>64.47</td>
<td>16.06</td>
<td>No support</td>
</tr>
<tr>
<td>2. Dam’s antibody titers</td>
<td>$\beta_0 + \beta_1 M$</td>
<td>67.24</td>
<td>18.83</td>
<td>No support</td>
</tr>
<tr>
<td>1. Constant risk</td>
<td>$\beta_0$</td>
<td>70.75</td>
<td>22.34</td>
<td>No support</td>
</tr>
<tr>
<td>1. Total associations with infected animals, regardless of demographic group</td>
<td>$\beta_0 + \beta_1 (PA_{Dams} + PA_{Dry-yrl})$</td>
<td>72.25</td>
<td>23.84</td>
<td>No support</td>
</tr>
<tr>
<td>1. Associations with specific groups of animals, regardless of infection status</td>
<td>$\beta_0 + \beta_1 C_{Dams} + \beta_2 C_{Dry-yrl}$</td>
<td>71.81</td>
<td>23.40</td>
<td>No support</td>
</tr>
<tr>
<td>1. Total associations with all animals, regardless of infection status or demographic group</td>
<td>$\beta_0 + \beta_1 (C_{Dams} + C_{Dry-yrl})$</td>
<td>77.11</td>
<td>28.69</td>
<td>No support</td>
</tr>
<tr>
<td>1. Total interactions with all animals, regardless of infection status or demographic group</td>
<td>$\beta_0 + \beta_1 (D_{Dams} + D_{Dry-yrl})$</td>
<td>77.91</td>
<td>29.49</td>
<td>No support</td>
</tr>
</tbody>
</table>
Table 4.4: Coefficient estimates from the top model.

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Coefficient estimate</th>
<th>Standard error</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>-1.55</td>
<td>0.98</td>
<td>0.11</td>
</tr>
<tr>
<td>Interactions with infected dams</td>
<td>-0.71</td>
<td>0.22</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Interactions with infected dry ewes and yearlings</td>
<td>0.94</td>
<td>0.35</td>
<td>0.01</td>
</tr>
<tr>
<td>Dam’s antibody titers</td>
<td>0.07</td>
<td>0.02</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

Ewes who lost lambs had lower antibody titers on average than ewes whose lambs survived ($F = 6.099$, $p = 0.0167$), though this effect is likely confounded with ewe age, as younger ewes were born after the major disease transmission event at Asotin Creek in 2011, and also had a higher propensity for losing their lambs than older, more experienced ewes. Since lambs born to two-year-old ewes generally had low survival, we refit the entire model suite using a dataset that omitted the six lambs born to two-year-olds. Model rankings were nearly identical (Table D.4), as were coefficient estimates for the best-performing model.

4.4.3 How does infection status relate to transmission risk in the bighorn pneumonia system?

Yearlings had marginally higher M.ovi prevalence than adults (Fisher’s exact test $p = 0.054$; Figure 4.5a). However, lambs that never associated with infected reproductive ewes had significantly higher survival rates than lambs that associated with at least one infected reproductive ewe ($\beta = 1.552$; SE($\beta$) = 0.690; $p = 0.024$; Figure 4.5b). By contrast, lambs that never associated with infected dry-ewes or yearlings had significantly worse survival than lambs that associated with at least one infected dry-ewes or yearlings ($\beta = -2.994$; SE($\beta$) = 1.131; $p = 0.008$; Figure 4.5b).

4.5 Discussion

We proposed a simple logistic regression approach that incorporated contact structure into model covariates and used disease outcome as a response variable to test between a series of hypotheses about probability of transmission given contact.
When we applied the approach to *Mycoplasma ovipneumoniae* transmission dynamics in free-ranging bighorn sheep, we found that association (presence in the same group) and interaction (direct contact) patterns were not predictive of one another, and networks incorporating both interactions and associations, along with dam antibody titer, provided the best explanation for observed epidemic patterns. Incorporating individual-specific infection status improved model fits even further.

Our approach extends existing epidemiological network methods, which traditionally focused on network topology as the primary determinant of disease transmission patterns (reviewed e.g., in [80,133]). Contact heterogeneity certainly produces some heterogeneity in transmission, but probability of infection given contact can also drive heterogeneity, especially when some host groups exhibit categorically higher probabilities of transmission given contact than others. While many wildlife disease transmission studies focus on contacts, which are relatively straight-forward to measure, detailed studies in humans show that probability of transmission given contact varies dramatically and predictably for many pathogens, including HIV and malaria. Our approach allowed us to infer differential risks using measured networks and known disease outcomes, without measuring probability of transmission given contact directly. Although some authors have used similar approaches to address other questions (for example, Perkins et al. 2009 [134] and Grear et al. 2013 [123] both compared the ability of various network construction methods to predict pathogen transmission; Godfrey et al. 2010 [124] modeled differential tick transmission risk between sexes of tuatara), we are unaware of other studies using networks to draw inferences about probability of transmission given contact per se. This approach therefore provides wildlife disease researchers some access to a previously unavailable area of ecological inquiry.

In the bighorn system, degree accumulation and association strengths were similar for reproductive ewes, dry ewes, and yearlings (Figure 4.2), but direct contact rates varied dramatically between these groups (Figure 4.3). While reproductive ewes, dry ewes, and yearlings all followed similar movement patterns, they did not follow common interaction patterns with lambs. Qualitatively, we observed that animals without lambs tended to be spatially peripheral to nursery groups, and rarely interacted directly with other group members. That discrepancy in direct interaction frequency appears to have a bearing on disease transmission. While a simple evaluation of prevalence by reproductive status suggested yearlings might
act as an important local pathogen reservoir (Figure 4.4a), our analyses suggested that yearlings and dry ewes rarely transmit pathogens to lambs, and probability of transmission given contact was approximately five times higher for reproductive ewes than for non-reproductive animals.

Association strengths declined during the birth pulse, and then rapidly increased (Figure 4.2b), corresponding to “creching”, the formation of ewe-lamb nursery groups. From a disease transmission standpoint, creching elevates an animal’s number of potential infectious contacts. However, we did not see a clear ceiling in node strength, suggesting that animals spend increasing time with their associates (through either increased group size or increased fission-fusion rates) throughout much of the summer. Degree, however, accumulated rapidly during June, with most animals encountering all other population members by early-to-mid July. Whether degree or association strength is the best measure for rate of acquiring new potentially infectious contacts likely depends on the intensity of contact required for transmission, as well as the interaction rates, pathogen loads, and symptoms of the particular animals involved. While each of these mechanisms clearly contributes to disease dynamics, our existing data are insufficient to separately quantify their effects here.

On a similar note, ewe antibody titers were associated with small but significant increases in lamb survival, but we hesitate to overemphasize this relationship for several reasons. First, models that did not incorporate interaction rates or association patterns into the covariates could not identify an antibody titer effect (Table 4.4). Second, titer data were collected 2-6 months prior to lamb birth, and are therefore likely subject to both process error from antibody waning in the dam, and measurement error associated with the sampling protocol and subsequent ELISAs. Even if the detected effect reflects a meaningful biological signal, it is unclear what a relationship between dam antibody titers and lamb survival actually means in terms of biological mechanisms. We saw similar antibody titer levels for older dams, regardless of their current infection status (Figure 4.6a). This suggests that magnitude of the immune response is not strongly associated with pathogen clearance (in which case we would expect to see higher titers among uninfected animals). Younger dams, who typically had lower titers, never had active infections in this study (Figure 4.6a). While there was no clear relationship between dam antibody titers and lamb survival status in older dams (Figure 4.6b), in younger
Figure 4.6: Dam’s infection status and lamb outcomes by antibody titer for older and younger ewes. Sample sizes in each group are shown in parentheses above each box. (a) Antibody (Ab) titers by dam’s infection status for ewes aged 4+ (dark grey) and 2-3 (light grey). The younger dams studied here had low antibody titers, and none had active infections. (b) Antibody (Ab) titers by lamb survival status and ewe age. Antibody titers were high for ewes aged 4+, regardless of their current infection status. Antibody titers had no relationship with lamb survival for lambs born to older dams (Wilcoxon rank-sum p-value = 0.383). There may be a relationship between antibody status and lamb survival for lambs born to 2-3 year-old dams (Wilcoxon rank-sum p-value = 0.114), but our current dataset lacks the statistical power to reliably characterize that relationship.

In older dams there was some evidence that such a relationship might exist, though our current dataset is insufficient to explicitly quantify that relationship. Supposing this apparent relationship is attributable to biological process and not just random chance, we still cannot determine whether the antibodies themselves are protective, or whether antibody titers are correlated with some other trait actually driving the lamb survival discrepancy. Despite these lingering questions, we include the antibody result in hopes of motivating additional investigation in the future.

Our analysis relies on a few additional assumptions that could shape the underlying results. First, we used lamb survival as a proxy for transmission and infection, and assumed that all infected lambs died, and all uninfected lambs survived. In reality, we know that some infected lambs do survive, and that some lambs die of other causes. However, our previous work on this system showed that the vast majority of local summer lamb mortality is attributable to disease, and that summer lamb survival in the absence of disease is in excess of 80% [1].
Therefore, although misclassification error adds noise to this analysis, we see no reason to suspect that this noise introduced systematic biases in our findings (but see [135]).

Second we assumed that direct contact patterns during focal follows reflected direct contact patterns at other times of day. Importantly, we have no observations during night. We assume that animals are less active at night, and spend most of their time bedded in groups. Since lambs probably bed preferentially with their dams, the data presented here likely underestimate direct dam-lamb contact rates. If this bias exists, it might explain the much-higher force of infection from infected reproductive ewes than from infected yearlings and non-reproductive ewes. However, we would expect that force to apply disproportionately to lambs born to infected ewes (as opposed to lambs who associated with infected ewes, but born to uninfected ewes), and that effect did not emerge in our analyses.

Finally, we assumed that contact rates were homogeneous within demographic groups. This assumption was made partially out of necessity: we could not uniquely identify lambs unless they were interacting with their own dams via nursing or bedding together. For the demographic groups in which animals were uniquely identifiable, rarity of contacts paired with unbalanced observation times complicated formal assessments of within-group heterogeneities in interaction patterns. Therefore, while within-group heterogeneities in interaction rates likely exist, we overlook them in the analyses presented here.

Despite these caveats, our results strongly suggest that pathogen transmission risk is not constant across all infected hosts, even after accounting for differences in association patterns and interaction rates. We saw major differences in transmission probabilities between demographic groups after accounting for differences in contact intensity. Specifically, although yearlings were infected at high rates (Figure 4.5a), they nevertheless imposed a lower-than-expected force of infection on susceptible lambs (Figure 4.3c). This discrepancy may reflect a number of different underlying processes, including changes in infection intensity for infected ewes in the period between health sampling and lamb birth, or demographic-group-specific differences in contact quality (for example, whether contacts were nose-to-nose vs. body-to-body). Our results nevertheless reinforce one important point for bighorn management: if yearlings pose limited transmission risk to lambs, then perhaps test-removal strategies should primarily target infected ewes.
This analysis is an early effort to leverage empirically measured contacts in order to test hypotheses about probability of transmission given contact. We anticipate that this line of inquiry will increase in importance as the set of measured animal contact networks continues to grow, and research efforts shift to the remaining variation in transmission not attributable to contacts alone. For wildlife systems, inferring probability of transmission given contact using social network analyses may be more plausible than conducting the analogous infection and transmission trials in captivity.
Chapter 5  
Formalizing the common ground among spatial, social, and group size representations of animal contact

5.1 Abstract

Ecological network analyses describe and predict contact patterns that drive processes like gene flow and disease transmission. These analyses are constrained by implicit assumptions made during network construction, yet to date few studies have asked the questions, “How do animal behavior processes relate to social and spatial network metrics?” and “How do spatial and social network metrics relate to one another, to group size, and to space use?” We outline a bipartite approach to animal networks that directly relates different construction methods under a single overarching structure. We use simulation to examine how three different animal movement drivers, home-range affinity, social affinity, and habitat heterogeneity, influence spatial and social network topology. We investigate when spatial and social network metrics are correlated, and when these metrics can be reliably predicted using data on space use and group size.
5.2 Introduction: Why a unified approach to animal social networks is necessary

Network analysis organizes and characterizes relationships and identifies central players in complex systems [136]. Ecologists frequently use networks to study contact processes underlying disease transmission and gene flow, but the particular network structure employed varies from study to study. At this time, there is no clear method relating animal behavioral patterns like space use and aggregation preferences to spatial and social network topology. Even within taxonomic orders, species differ dramatically in their grouping affinities and space use. For example, both defensibility – the ratio of day-range to home-range size – and group-size vary over several orders of magnitude (e.g., [137]).

The theoretical gap separating animal behavior and network topologies is problematic, since correlations between animal behavior patterns and network topologies could provide a theoretical framework for inferring network structure in emergency settings when network data are unavailable. Land-use and aggregation patterns can be inferred from studying a relatively small number of individuals, and these data are available for a wide range of taxa, but inferring social contact patterns requires instrumenting a large proportion of the focal population. Behavioral ecologists, disease ecologists, and landscape geneticists would benefit from clear guidance about when social contact patterns can be inferred from spatial network metrics and other readily measurable features like space use, group size, and animal competition behaviors.

Animal space use and aggregation patterns may hold inferential power for social contact patterns because space use, aggregation patterns, and contact networks are all emergent properties of animal movements, which are in turn jointly governed by habitat quality, predation, territoriality, sociality, and competition. When resources are distributed heterogeneously over a landscape, many animals may spend a disproportionate amount of their time in resource-rich areas, with the consequence that resource distribution structures dispersion of the animal’s utilization distribution (the extent to which an animal concentrates its time in a few patches and only rarely visits the majority of its home range) and animal group size (the extent to which animals occur in large groups, as opposed to small).
Habitat resources are important drivers of animal space use/aggregation patterns for animals participating in scramble competition at waterholes and mineral licks in desert and mountain environments (e.g., [138]), and at clear patches or feeding areas during severe winters (e.g., [139]). By contrast, territorial species that partition space through contest competition must regularly visit most of their home ranges, potentially leading to low spatial dispersion and small group sizes, as in lion prides [111], and wolf packs [20]. Yet formal relationships between space use, aggregation patterns, and network metrics have not been systematically explored.

Social networks, spatial networks, and aggregation patterns are all used to characterize intraspecific animal interactions. Social networks consider animals themselves, connecting specific individuals on their frequency of co-occurrence in space and time. Social network analyses identify well-connected individuals (e.g., [111]), and reveal the social structure of groups (e.g., [135,140,141]), but provide no specific insights into spatiotemporal patterns of behavior. Spatial networks connect specific points in space either through co-occurrence of the same animal (as in circuit-based approaches, e.g., [142,143]), or by treating space on a continuum, with edges depending on distance between locations (e.g., [144,145]). Spatial networks identify highly used locations and spatial structure, but provide no specific insights about the relative importance of different individuals in forming connections.

A few authors have linked social, spatial, and aggregation patterns in the wild or simulation. Onnela et al. [146] saw that geography was correlated with fine-scale modularity in human cell phone networks, but this effect eroded with group size. Madden et al. [147] found that large group sizes were associated with shorter path lengths in social networks of wild meerkats, though this result rested on a temporally aggregated definition of group size. In some preliminary work structured around a primate system, [148] found no strong relationship between group size and network modularity [148]. Best et al. [149] explored group size and individual sociality (in their case, shyness) in grey kangaroos, and found shy animals were more likely to live in groups. [150] linked social and spatial contacts in a study of bat roosting trees, and that approach was emphasized in a recent review [136].

Despite these early explorations, there is limited guidance for researchers trying to design efficient animal movement studies. Whether inferences from spatial and social networks and group size distributions diverge, and due to what factors, is
not well-characterized. Researchers are left making ad hoc decisions driven by general impressions of the system and its important features as opposed to a clear theoretical framework.

Here, we begin addressing the questions, “How do animal behavior processes relate to social and spatial network metrics?”, and “How do spatial and social network metrics relate to one another, to group size, and to space use?” We first introduce an organizing structure that unifies social and spatial network construction methods, group size patterns, and animal space use by expressing each as a particular projection of intersecting spatiotemporal movement paths. We then empirically assess how animal space use and aggregation patterns relate to network construction decisions in the published literature. We use simulation to quantify relationships between factors driving animal space use and emergent properties like network topologies and group size. Finally, we examine correlations between spatial and social network metrics. We identify regions of the behavioral space in which network topologies are not well matched, and regions where spatial networks, group size, and defensibility provide clear predictions for social network topology.

5.3 Methods

5.3.1 A probabilistic framework for animal movements

Network structures, group sizes, and occupancy patterns are all emergent properties driven by a common set of underlying animal movements. Consider a particular individual, \( i \in \{ I : 1, ..., N \} \) in a population of size \( N \), moving within some spatial domain, \( D_S \subset \mathbb{R}^d \), continuously over time, \( D_T \subset \mathbb{R} \). The individual’s location at any point in space and time can be written as the ordered triad \((i, s, t)\), which is a draw from the joint probability density function \((I, S, T)\). This joint density function describes occurrence probabilities for an infinite set of triads, in which \( I \) is a categorical variable indicating identity, \( S \) denotes a position in space, and \( T \) denotes position in time. Network structures, space use patterns, and group size distributions can be probabilistically related because they all emerge from \((I, S, T)\).

Relationships between \( I \), \( S \), and \( T \) vary with animal behavior and spatial preferences. In species that migrate as groups, blocks of individuals are continuously near one another in \( S \) and \( T \), as a cohesive group moves together on the landscape.
Space and time are strongly correlated for migratory herds, so that space can be accurately predicted if time is known. No such correlation exists for societies undergoing rapid fission-fusion dynamics in the absence of migration. Group size distributions in fission-fusion societies may remain consistently relatively low, however, unless the habitat is highly heterogeneous. The highest-quality patches may generate very large aggregations that would have low probabilities of occurrence in the absence of heterogeneity in patch quality.

Social behaviors also structure the joint probability density function of \((I, S, T)\). For territorial species like wolves, lions, or kudu, individuals partition space so that even if probability of use by some animal is relatively constant across a landscape, probability of use by a particular individual changes abruptly depending on whether the site is within that individual’s territory. As a result, we expect \(I\) to becomes increasingly correlated with \(S\) in the joint distribution of \((I, S, T)\) for increasingly territorial species. For matriarchal species like elephants and bison which move as nomadic, yet relatively stable groups over broad spatial domains, members of the same maternal group co-occur disproportionately in space and time, even as members of different groups rarely co-occur. In matriarchal societies, then, two individuals should either consistently co-occur in \(S\) and \(T\) (if they are in the same group), or almost never co-occur (if they are members of different groups).

### 5.3.2 Mapping animal movements to network constructions

Network constructions vary in how they define “connection” and marginalize \((I, S, T)\). Most empirical animal networks use edges that can be described as particular intersections in \((I, S, T)\), and the levels of the variable between which intersections are evaluated form the network’s nodes. For example, two individuals, \(I_1\) and \(I_2\), might have movement paths denoted \(X_1 = \{I = I_1, S, T\}\) and \(X_2 = \{I = I_2, S, T\}\). The intersection of those paths, \(X_1 \cap X_2\), contains all points in \((S, T)\) where \(I_1\) and \(I_2\) co-occur – in other words, their complete set of contacts. Edges in static social networks are defined by the frequency of observed spatiotemporal intersections in all animal pairs’ movement paths, scaled by the frequency with which each animal’s movement path was observed. Since social networks use intersections between individuals (levels of \(I\)), individuals form the network’s nodes. If intersections were measured between different locations in space – levels of – then
Table 5.1: Common ecological analyses, and how they relate to \((I,S,T)\).

<table>
<thead>
<tr>
<th>Analysis type</th>
<th>Variable marginalized out</th>
<th>Density function employed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Home-range analyses</td>
<td>(T)</td>
<td>(\int_{t \in T} f(I,S,T)dt)</td>
</tr>
<tr>
<td>Spatiotemporal population-level</td>
<td>(I)</td>
<td>(\int_{s \in S} f(I,S,T)ds)</td>
</tr>
<tr>
<td>analyses</td>
<td></td>
<td>(f_s = \sum_{i \in I} \int_{t \in T} f(I,s,T)dt)</td>
</tr>
<tr>
<td>Individual survival analyses</td>
<td>(S)</td>
<td></td>
</tr>
<tr>
<td>Occupancy analyses</td>
<td>(I) and (T)</td>
<td></td>
</tr>
</tbody>
</table>

the network’s nodes would be locations in space.

Although connections underlying all networks stem from realizations of the complete joint density \((I,S,T)\), most network construction methods do not operate directly on that complete joint density. Instead, each construction method relies on a particular marginal distribution of the joint density. Marginal distributions are formed by summing or integrating the joint distribution over one (or several) variable(s). This process eliminates all information unique to the variable integrated out, and projects the joint density function down to a lower-dimensional space. Marginals of \((I,S,T)\) form the basis for an array of ecological analyses (summarized in Table 5.1).

Ecological home-range analyses typically retain all information on individuals and space \((I,S)\) but aggregate data over time \((T)\), and home-range overlap networks rely on this same projection. Nodes are individuals, and connections are based on how extensively individuals overlap in space (intersections between individuals in \((I,S)\)), regardless of whether overlapping space use also overlapped in time. Circuit-like networks also aggregate information over \(T\) to use an \((I,S)\) marginalization of \((I,S,T)\), but unlike home-range overlap networks, they define connections based on multiple sites sharing the same individual (intersections between sites in \((I,S)\)) so that nodes in circuit-like networks are points in space.

Purely spatial networks define nodes in space, but use Euclidean distance between locations to define connections. These networks therefore reproject \((I,S,T)\) by marginalizing out individuals (and sometimes also time), so that observations are reduced to \(s\), and edges are defined as distances between spatial points (that is, distance functions relating \(s_1\) and \(s_2\), sometimes scaled by the density of observations at each point). This configuration is commonly used for classic metapopulation
networks.

Dynamic social networks retain all information on both individual identities, \( I \), and intersection timing, \( T \) in their construction. Although they usually do not retain the spatial information in \( s \) per se, \( s \) is used to determine whether a given dyad gets an edge at a given value of \( T \). Dynamic social contact networks do not marginalize, and are thus robust to changes in social contact pattern through time, at the cost of high sampling variability in all three raw variables, \( I, S, \) and \( T \). The cost of that complete information is inferential power: at a fine-enough time scale, all edges become binary, and no smoothing is available. This limits the network’s inferential power.

### 5.3.3 Correlations between \( I, S, \) and \( T \) determine consistency across network constructions

Marginalizing over a variable removes all information unique to that variable from \((I, S, T)\), so the information costs of a particular marginalization depend on the degree of independence between \( I, S, \) and \( T \).
5.3.4 Empirical data analysis

We used data from ungulates, a taxonomic group with highly varied ecologies, to characterize general patterns in animal behavior. For each species, we used existing datasets and primary literature to determine day-range distance (i.e., the typical distance an animal travels in a given day, [151]), home-range size, and group size. Group sizes were estimated from published distributions wherever possible. In the absence of published data, we used mean group sizes reported in PanTHERIA [152], or by [151]. We followed [137] and calculated a defensibility score for each species, as the ratio of day-range (in km) to home-range size (in km$^2$). Defensibility values are high when species traverse their home ranges completely (or multiple times) in a single day, and they are low when species occupy only a small part of their home-range in a given day. We then compared relationships between defensibility and group size for 28 ungulate species, along with a set of well-studied animal systems that have been characterized via social networks.

5.3.5 Simulations

We used simulation to explore dependence between $I$, $S$, and $T$ as a function of three animal behaviors: social affinity ($\rho$), spatial fidelity ($\gamma$), and habitat heterogeneity ($\delta$). All animals were subject to a constant spatial diffusion coefficient, $\alpha$, which was fixed to 0.01 for all simulations. Relatively high values of $\rho$ produced movements that were predominantly governed by the position of the focal individual’s group leader. In nature, $\rho$ values are approximately equivalent to half-weight indices (“HWI”, e.g., [127]), or group fission rates. HWI ranges in value from an average of $\sim 0.1 – 0.2$ in onagers, and the probability that two individuals in a group on one day are together the next ranges from $\sim 0.37$ in roe deer to $\sim 0.80$ in African buffalo. Relatively high values of $\gamma$ produced movements that were mostly localized within territories. $\gamma$ is comparable to an animal’s preference for small regions within its home-range. Relatively high values of $\delta$ led to greater habitat heterogeneity, in which animals moved freely to resource-rich patches spread over large home ranges. $\delta$ approximates the extent to which animal home-ranges overlap in space, ranging from very low home-range overlap for species like kudu, wolf packs, and lion prides, to complete overlap in species that form massive aggregations like elk, wildebeest, and some species of migratory waterfowl. Movement from by an individual from
site $s_i$ to site $s_j$ in a single timestep was specifically defined as

\[
\alpha \times \frac{1}{d(s_i, s_j)} + \gamma \times I(s_j \in \text{individual's neighborhood})
+ \rho \times I(\text{individual's group leader is at } s_j) + \delta \times X_{s_j} \tag{5.1}
\]

where $X_{s_j}$ is a randomly chosen desirability of site $s_j$. For each simulation, we recorded each animal's position at each timestep. We simulated over different values of $\rho$, $\gamma$, and $\delta$.

In the animal social network and disease literature, two metrics, degree ([109,110]) and modularity [153], see particularly frequent use, and we focus on those metrics here. A node’s degree is the number of other nodes to which it is connected. In a social network, this is the number of other animals a given animal associates with. Quantifying degree in spatial networks varies. Some authors take all spatial nodes to have identical degrees, with movements only between neighboring patches (e.g., [21]), while other approaches use measured animal movements between patches to characterize degree (e.g, [154]). We use the latter approach here, connecting spatial locations that are visited by the same animal, with edgeweights reflecting the frequency with which animals use both sites.

Modularity ($Q$) measures the extent to which the network breaks down into local processes or units. Societies with low social modularity do not exhibit consistent group membership, whereas societies with high social modularity have consistent groups. Societies with low spatial modularity do not partition space, but all move throughout the entire available spatial domain (as is the case for wildebeest, elk). Societies with high spatial modularity partition space, with animals being localized into particular regions of the complete domain (as is the case for territorial species like lions, kudu, etc.).

We also measured network “small-world-ness” [155], describes how quickly information, pathogens, or genes can traverse a system. Small-world-ness is quantified as the ratio of global transitivity on an observed graph ($C_{obs}$) to global transitivity on a random graph ($C_{rand}$) with the same number of nodes and edges, divided by average path length on the observed graph ($L_{obs}$) over average path length on the analogous random graph ($L_{random}$). Formally,
\[ S^\Delta = \frac{C_{obs}}{C_{random}} \frac{L_{obs}}{L_{random}} \] (5.2)

The observed graph is increasingly small-world as \( S^\Delta \) values increase away from one.

Each simulation occurred on a 10 \times 10 gridded torus, and consisted of 50 animals moving over 100 timesteps. Animals were randomly distributed in groups of 5 across the grid at the simulation outset. Each animal was assigned a preferred area and a preferred group at the start of the simulation (and began simulations in their preferred group and area), and movements followed (2). The simulated movement paths were used to build social and spatial networks, group size distributions, and utilization distributions. We calculated a maximum likelihood estimate for a Poisson distribution fit to spatial and social degrees aggregated over all simulated timesteps, and calculated modularity and network small-world-ness for both the spatial and social networks following the last simulated timestep. We recorded the lambda estimate of a Poisson fit to the distribution to group sizes over the entire simulation, and calculated average area of 95\% kernel and 50\% kernel utilization distributions for each animal over the entire simulation. Example simulation output for various points in the movement parameter space are shown in Figure 5.2.

We used simple correlation coefficients to describe the relationship between each movement driver (home-range affinity, social affinity, and habitat heterogeneity), each alternative metric (group size, core area:home range, 50\% kernel area) and spatial and social network metrics.

### 5.4 Results

#### 5.4.1 Animal societies show a relationship between group size and habitat defensibility

Ungulate and other mammalian social systems showed a clear negative correlation between defensibility, the ratio of day-range to home-range size, and average group size. Animals with lower defensibility indices generally resided in larger groups, although these groups varied in stability (Figure 5.1). Network models of these
Figure 5.1: Group size and space defensibility (e.g., day-range divided by home-range size) for a selection of ungulates. Note the negative correlation between group size and defensibility: species that can cover most of their home-ranges within a particular day tend to live in smaller groups.

systems did not show consistent patterns of network construction. Most of the studies we investigated used individual-level social contact networks, although in a few cases, focal animals were used as indicators for groups. A few studies used home-range overlap networks, and a few (usually colony-forming rodent species) used purely spatial networks. The inconsistency in construction approaches suggests that researchers may often make ad hoc decisions about how they build their networks.

5.4.2 How do animal behavior processes relate to social and spatial network metrics?

When habitat quality was heterogeneous, heterogeneity in both spatial and social degree was lost as all animals came together around resources. This effect was strengthened when spatial and social affinities were low (top row of Figure 5.2). Higher spatial affinity led to lower spatial and social network degrees (Figure 5.2 rows 2 and 4), though this effect was stronger in spatial networks than social networks (Figure 5.3). Social degree was nearly always lower, and showed lower variation, than spatial degree (Figure 5.4), and that likely reflects biological reality in many cases.

Heterogeneous habitat quality also reduced spatial and social modularities. Modularity was almost always low, and only rose under conditions of low habitat
heterogeneity and high spatial affinities. Both spatial and social modularity scaled more strongly with spatial affinity than social affinity, but social affinity becomes important for spatial modularity when habitat heterogeneity was low. When habitat heterogeneity was high, all animals used the entire space. Kernel size was limited by spatial, but not social, fidelity. The ratio of core area:home range size did not change systematically with spatial or social affinity. In contrast to spatial and social degrees and modularities, group sizes were predominantly driven by social affinity, and was surprisingly robust to changes in habitat heterogeneity.

Spatial degree nearly always overestimated social degree, except when spatial affinity was very high, and habitat heterogeneity was relatively low (shown in the dominant left-to-right changes within and between panels in the top row of Figure 5.3). When habitat heterogeneity was low, the capacity of spatial degree to predict social degree depended strongly on the animal’s home-range affinity. The relationship between spatial and social degree also depended on social affinity, but this effect was weaker, and limited to only the highest social affinities under conditions of low habitat heterogeneity (Figure 5.3).

Spatial and social modularities generally agreed closely (bottom row of Figure 5.3), except when home-range affinities were very high, in which case social modularity exceeded spatial modularity (regardless of social affinity). When social affinity was high, social modularity sometimes exceeded spatial modularity, but this effect was weak, and required social affinities in excess of 0.8 (Figure 5.3 bottom row).

5.4.3 How do animal behavioral processes relate to group size and defensibility?

Group sizes generally declined with increasing spatial affinity, and increased with increasing social affinity, and were always largest when habitat heterogeneity was highest (Figure 5.4 top row). Defensibility declined with increasing spatial affinity, up until a ceiling that was likely contingent upon the spatial extent of the simulator (bottom left panel fo Figure 5.4). There was no clear relationship between social affinity and defensibility (bottom right panel of Figure 5.4).
Figure 5.2: Simulation output. Simulation output under varying parameter conditions. The first column shows movement paths for 6 animals from each simulation (full simulations consist of 50 animals). The second column shows a static association network based on each simulation. The third column shows a circuit-like spatial network under the same simulation. The fourth column shows group sizes under each simulation. The fifth column shows the distribution of 50% kernel home range areas for all animals in the simulation (scaled as a percentage of the total simulation area). When parameter values are unspecified in the panel row labels, they are low. “Universally low” parameter values map to simulations in the low left-hand corner of each panel in Figures 5.3 and 5.6. “High social and spatial affinity” parameter values map to the upper right-hand corner of each panel in Figures 5.3 and 5.6. “High habitat heterogeneity” simulations are the left-most panels of Figures 5.3 and 5.6.
5.4.4 How do spatial and social network metrics relate to one another?

Spatial degree consistently underestimated social degree when group sizes were relatively small, although in occasional unusual cases, social degree was much higher than spatial degree. In the upper end of the parameter space, spatial degree greatly exceeded social degree, though this may have been due to the structure of the simulator (left-most panel of Figure 5.5). Spatial modularity consistently underestimated social modularity, and in this case, unusual cases showed substantial underestimation of social modularity by spatial modularity (center-left panel of Figure 5.5). Average spatial distance was essentially independent of social distance, up until a threshold spatial distance. Social distances were typically low regardless of spatial distance, until spatial distance crossed a threshold and social
distances rose rapidly (center-left panel of Figure 5.5). Spatial transitivity bore a non-linear relationship to social transitivity, likely due to the particular spatial constraints of the simulator. In general, scenarios in which animals had very low defensibility indices (that is, when animals lived over broad home ranges, most of which were rarely visited, as is true of fission-fusion and migratory societies) produced networks with high spatial transitivity, but low social transitivity. When defensibility was moderate, networks generally had moderate social transitivity, but spatial transitivity was polarized as either very high or very low. When defensibility was high (that is, when animals spent the vast majority of their lives in small, intensively patrolled home ranges), social and spatial transivities were high as well.

5.4.5 How do animal movement parameters relate to network small-world-ness?

Social networks were much more variable in small-world-ness than spatial networks (Figure 5.6). Spatial networks showed an abrupt drop in small-world-ness commensurate with increasing home range fidelity. Social networks showed high small-world-ness when home range affinity was low and social affinity was high. This pattern persisted until the point when habitat heterogeneity became so high that all animals mixed completely, and small-world-ness became undefined (right-most panel of Figure 5.6).

5.5 Discussion

Spatial and social degree measure fundamentally different aspects of animal behavior, but are nonetheless correlated in nature. Our results suggest that while this correlation is relatively strong, spatial degree scales much more dramatically over varying levels of spatial affinity than does social degree. This means that spatial networks may overestimate the intensity of connections, and actual connectivity on the landscape.

Modularity strength (either social or spatial) depended strongly on animal responsiveness to heterogeneity in habitat quality. When animals responded strongly to good patches on the landscape, spatial and social modularity were both essential
Figure 5.4: Relationships between movement parameters, group size, and defensibility. A. Group size generally declines with increasing spatial affinity. High habitat heterogeneity (purples and blues) keeps group sizes higher by pulling animals to habitat hot-spots where they interact. B. Increasing social affinity is categorically associated with increasing group size. The largest groups occur in scenarios with high habitat heterogeneity. C. Defensibility increases with increasing spatial affinity when habitat heterogeneity is low. The apparent downswings in defensibility with increasing spatial affinity is attributable to complete mixing through the spatial domain in the low-affinity simulations gradually giving way to incomplete mixing. D. Social affinity and defensibility are independent under the assumptions used in this simulator.
Figure 5.5: Correlations between spatial and social network features, colored by defensibility. Relationships from simulations in which hosts had very low defensibilities, for example, migratory societies, or rapid and wide-ranging fission-fusion societies, are shown in red. Hosts with very high defensibilities (for example, highly territorial hosts who cover much of their home-ranges multiple times per day) are shown in purple and blue. Hosts with moderate levels of defensibility are shown in green throughout.

Figure 5.6: Network “small-world-ness” by movement parameters. Social small-world-ness declines with increasing home-range affinity, and is highest when social affinities are high (top left corner of top row). Spatial small-world-ness declines abruptly at a critical level of small-world-ness, beyond which animals localize entirely to their own home ranges.
absent. However, when sociality or spatial affinities trumped responsiveness to resources, modularity patterns emerged. Both social (e.g., consistent, structured groups) and spatial (e.g., partitioned space use) were strongest when spatial affinities were very strong, and spatial modularity was further strengthened by strong social modularity.
<table>
<thead>
<tr>
<th>Parametric description</th>
<th>Biological description</th>
<th>Social network metrics captured by spatial networks, group sizes, or defensibility</th>
<th>Implications for contact studies</th>
<th>Expected relative transmission rate (low to high)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High spatial affinity, low habitat heterogeneity</td>
<td>Territorial host with well-defined, well-defended home ranges</td>
<td>Yes</td>
<td>S and I highly correlated; can use S as a proxy for I. Group sizes should be relatively constant and small.</td>
<td>1</td>
</tr>
<tr>
<td>Low spatial affinity, high habitat heterogeneity</td>
<td>Non-territorial, gregarious host that migrates or aggregates at resource host-spots</td>
<td>No</td>
<td>Som animals will have very high centrality, and these animals will be hard to identify without marking a high proportion of the population</td>
<td>3</td>
</tr>
<tr>
<td>High social affinity, low spatial affinity, variable habitat heterogeneity</td>
<td>Matriarchical or harem societies without well-defined territories, like Elephants, bison, female kudu</td>
<td>No, but few marks required (1 per group).</td>
<td>Can mark indicator animals in each group. Expect relatively constant group size. Space use should be well-mixed</td>
<td>2*</td>
</tr>
<tr>
<td>Low social affinity, low spatial affinity, low habitat heterogeneity</td>
<td>Solitary species without well-defined territories, like moose</td>
<td>No</td>
<td>May need many marks, but expect small group sizes and relatively low contact rates</td>
<td>2*</td>
</tr>
</tbody>
</table>
In some (perhaps many) cases, group size may be an inherent preference like sociality or spatial affinity, as opposed to an emergent property like degree or modularity (e.g., [156–158]). We chose to treat it as an emergent property here to constrain our parameter space to three dimensions, and because at least for some societies (for example, fission-fusion societies), it likely is an emergent feature.

As with any social or spatial analysis, our results are contingent upon the spatial and temporal scales at which the system is observed. Our simulations do not capture demography (all animals survive the entire simulation), nor do our network analyses explore the role of variation in sampling scale on network metrics. We checked to see whether network metrics saturated over the temporal course of the simulations, and saw that degree and modularity both saturated in most simulations over time.

Our goal was to describe broad patterns of animal behavior and network features, but our methods likely overlook some important drivers of network connections. In particular, our comparisons of group size and degree rest on Poisson fits, which may not capture important attributes of distribution tails (in particular, the heavy-tailed structure common to power-law networks). We compared likelihoods under Poisson and power-law fits to degree, and saw a generally superior performance of Poisson fits, which we use to justify our decision to use those fits here. In reality, however, tail dynamics likely fall somewhere between those two models. Further work should flesh out relationships between animal space use and structure of the degree distribution’s tail.
Chapter 6  
“One Health” or Three? Publication silos among the One Health disciplines\(^1\)

6.1 Abstract

The One Health initiative is a global effort fostering interdisciplinary collaborations to address challenges in human, animal, and environmental health. While One Health has received considerable press, its benefits remain unclear because its effects have not been quantitatively described. We systematically surveyed the published literature and used social network analysis to measure interdisciplinarity in One Health studies constructing dynamic pathogen transmission models. The number of publications fulfilling our search criteria increased by 14.6 percent per year, which is faster than growth rates for life sciences as a whole, and for most biology subdisciplines. Surveyed publications clustered into three communities: one used by ecologists, one used by veterinarians, and a third diverse-authorship community used by population biologists, mathematicians, epidemiologists, and experts in human health. Overlap between these communities increased through time in terms of author number, diversity of co-author affiliations, and diversity of citations. However, communities continue to differ in the systems studied, questions

asked, and methods employed. While the infectious disease research community has made significant progress toward integrating its participating disciplines, some segregation – especially along the veterinary/ecological research interface – remains.

6.2 Introduction

Understanding and containing emerging infectious diseases (EIDs), especially those crossing the wildlife, human, and domestic animal interfaces, is a global health imperative. Novel diseases have major implications on geopolitics [159–161], animal and human well-being, and wildlife conservation [162–164], and effective management hinges on understanding disease emergence and persistence processes. Disentangling the mechanisms underlying these processes is a fundamentally trans-disciplinary endeavor, and disease research efforts must blend expertise on the behavior, health, and immune dynamics of a variety of host-pathogen pairs [7,165].

Efficiently disseminating knowledge and methodologies across disciplinary boundaries is essential for a cohesive reaction to emerging threats. However, researchers tend to organize themselves into discipline-specific “silos” that contain robust internal research communities, but only rarely interact with one another. This is particularly true of the disciplines studying infectious disease: workplaces range from hospitals, to microbiological laboratories, to ecological field sites, to mathematical computing facilities, and communicating across these physical and cultural boundaries is difficult [166]. Modern ecological, epidemiological, medical and veterinary programs advocate “One Health” approaches to facilitate cross-disciplinary communication and research [167]. One Health targets three basic objectives: 1) achieving human health, 2) achieving animal health, and 3) developing resilient, sustainable ecosystems [168]. Although research disciplines vary in the relative importance they ascribe to each objective, all objectives should retain value within each discipline.

Despite efforts to integrate, we continue to observe limited participation by all relevant viewpoints in our collective work on diseases breaching the domestic livestock-wildlife interface, leading us to question One Health’s efficacy. While recent papers discuss One Health applications and perspectives [?,165], we are not aware of empirical analyses measuring temporal collaboration trends within the infectious disease research community. Here, we fill that gap with a quantitative
study of cross-disciplinary research patterns surrounding one important aspect of zoonotic disease, between-host pathogen transmission.

In order to control our database’s size we confined our study to dynamic disease transmission models. Dynamic transmission models are integral to understanding and managing zoonotic disease [107, 169]. While dynamic modelling is not the sole objective of One Health, model development requires integrating empirical and theoretical knowledge about pathogen virulence, ecological context (population densities, interaction patterns, and so on), environmental setting, and animal immunology and physiology. Experts at each level of the system contribute meaningful information that is distinct from knowledge at other levels. Model building requires sustained communication and cooperation across disciplinary boundaries, so we assumed that collaborative patterns surrounding dynamic models form a good proxy for collaborative patterns across the broader One Health spectrum.

We hypothesized that growth of the One Health initiative was associated with an erosion of traditional academic disciplinary “silos”. In particular, we expected the number of papers fulfilling our search to grow at a rate faster than overall growth rates in the biological and medical sciences. We suspected citations in recently published papers would reference a wider spectrum of disciplines than similar work published earlier. We also anticipated that journals today publish papers led by authors from more academic domains, and that individual authors now publish in more domains than in the past. Finally, we posited that approaches, methodologies, and research questions are increasingly shared across all contributing silos, indicating improved communication across historic disciplinary boundaries.

6.3 Methods

We followed standard structured review protocols [170] to conduct a literature search of papers containing dynamic models [107] that captured nonlinear aspects of the disease transmission process (search terms and modifications are listed in Appendix E: Table F.1-F.3). We constructed three networks to examine connectivity between papers in the paper bank. The first was a citation network connecting papers (nodes) along edges that represented citations between papers. A walk-trap algorithm [171] with four steps was used to characterize/identify structure in each network and to identify the number and composition of node communities. The community
structure of the journal network formed the basis for our follow-up comparative analyses. Each paper’s lead author was assigned to a particular discipline depending on their stated affiliation.

Paper inclusion by era and discipline are shown in Appendix E: Table F.4. We compared the proportion of within-community citations in each community for publications in the years prior to 2010, to publications from 2010 to 2014 using simple binomial tests with Bonferroni adjustments accounting for the three communities. We used a pair of generalized additive models (GAMs) to describe author diversity and within- to between-community citation ratios through time. Both forms of diversity were then modeled as a function of year and journal community. Descriptions of the GAMs are included in the supplementary information. We identified factors leading to high citation rates by modeling the total number of times each paper was cited as a function of that paper’s within-paper authorship diversity, citation diversity, and publication year.

We read a stratified sample of 236 papers from the paper bank to compare modeling objectives, approaches, and data incorporation across journal communities over time, and by paper impact. Strata were defined by journal community, annual paper citation rate, and year of publication. In each sampled paper, the study system was classified as human, domestic animal, wildlife, plant, or hypothetical. Papers were identified as being predictive, descriptive, or both. They were further classified as primarily seeking insights about basic science, applied science, or management. All recorded fields are listed and defined in Table F.10. We identified highly cross-disciplinary journals in dynamic modeling of infectious disease by comparing the composition of lead author affiliations across all papers in our paper bank to the composition of lead author affiliations within paper bank papers from each particular journal.

A complete description of all aspects of the literature search, network construction, stratified sampling, and analysis are included in the online supplement.

We followed standard structured review protocols [170] to conduct a literature search for papers containing dynamic models [107] with nonlinear aspects of disease transmission (search terms and modifications are listed in Appendix E: Table F.1-F.3). We constructed three networks – one of papers, one of authors, and one of journals – to examine relationships in our paper bank. A walk-trap algorithm [171] with four steps was used to characterize structure in each network and to identify
the number and composition of node communities. Community structure identified for the journal network formed the basis for our follow-up analyses. We classified text strings in author institute affiliations (for example, “vet”, “ecol”, “math”) to determine a discipline for each paper’s lead author. Paper inclusion by era and discipline are shown in Appendix E: Table F.4.

We estimated the paper bank’s growth rate using a linear model. Let $P_t$ be the number of papers returned by our search in year $t$. Then we assumed a Gaussian error structure and fit the model $\log(P_t + 1) = \beta_0 + \beta_1 t + \epsilon$. In this model, percent growth is estimated with the $\beta_1$ term; approximate time to doubling is calculated as $\frac{\log(2)}{\log(1+\beta_1)}$. Due to the high number of zeros in the dataset prior to 1990, we fit this model on data from 1990 to present.

We read a stratified sample of 236 papers from the paper bank to compare modelling objectives, approaches, and data incorporation across journal communities over time, and by paper impact. Strata were defined by journal community, year of publication, and annual paper citation rate (total number of citations per year, divided by years since publication). In each sampled paper, the study system was classified as human, domestic animal, wildlife, plant, or hypothetical. Papers were identified as being predictive (using models to project future scenarios under varying conditions) or descriptive (using models to describe and gain insights from existing datasets), and were classified as primarily seeking insights about basic science, applied science (basic science questions addressed in a system of management or conservation importance), or management. Recorded fields are listed and defined in Appendix E: Table F.11. We identified highly cross-disciplinary journals by comparing the composition of lead author affiliations across all papers in our paper bank to the composition of lead author affiliations within paper bank papers from each particular journal.

We used generalized additive models (GAMs) to describe two measures of author diversity, and community-to-community citation rates through time. We used Shannon’s diversity index, $H'$, a metric that accounts for both number and relatively frequency of different unit types, to quantify author affiliation diversity. Under Shannon’s diversity, a community with $I$ distinct categories, each represented at proportion $p_i$, is assigned the value $H = \sum_{i=1}^{I} p_i \log(p_i)$. We measured author diversity within papers ($H'_C$) and lead author diversity within communities ($H'_L$), and modelled each as a function of journal community and year (Appendix E:
Saturated models included a community-specific intercept $\alpha_j$, a spline function describing changes over years ($s_1$), and a spline term capturing interaction between year and community ($s_2$), where community is denoted by indicator variables $Comm_1$, $Comm_2$, and $Comm_3$. For the $i^{th}$ observation ($H'_{Li}$ and $H'_{Ci}$) in journal community $j$, this model can be written as

$$H'_i = \alpha_{j[i]} + s_\alpha(Year_i) \times Comm_{j[i]} + \epsilon_i$$  \hspace{1cm} (6.1)$$

where $\epsilon_i \sim N(0, \sigma^2)$. Community-to-community citation rates were modelled using the count of papers published in community $k$ that were cited by papers published in community $l$. Counts were treated as Poisson, and model covariate structure was identical to the author diversity models described previously. Saturated models were reduced in accordance with AIC. We identified factors leading to high citation rates by modelling how many times each paper was cited (denoted $Y_i$, $Y_i \sim Poisson(\lambda_i)$) as a function of that paper’s within-paper authorship diversity ($H'_{C_i}$), citation diversity ($R$, the ratio of between-community citations to total citations), and publication year ($Year$). The model allowed for community-specific intercepts ($\alpha_{j[i]}$), slopes associated with author diversity ($\delta_{j[i]}$), and slopes associated with citation diversity ($\phi_{j[i]}$). The model had a log-link and an offset term for years since publication, and can be written as

$$\lambda_i = \exp[\alpha_{j[i]} + \beta Year_i + \delta_{j[i]} H'_{C_i} + \phi_{j[i]} R_i + \epsilon_j]$$  \hspace{1cm} (6.2)$$

A complete description of the literature search, network construction, stratified sampling, and analysis is included in Appendix E.

### 6.4 Results

#### 6.4.1 Journals divided into two traditional disciplines and a diverse third group

A literature search using Web of Science returned 2,258 papers containing dynamic disease transmission models; examination of titles and abstracts reduced this
number to 1,628 suitable papers published in 108 journals, with 4,219 unique authors. We described citation patterns between journals using a network with nodes representing journals and directed, weighted edges representing citation rates between journals. Descriptive metrics associated with the journal network, as well as metrics from networks describing relationships between authors and between publications, are shown in Appendix E: Table F.5. We used a walk-trap algorithm [171], which accounted for both undirected and weighted edges, to identify communities in the journal network. We compared clustering under the walk-trap to clustering under a variety of other algorithms for undirected networks (fast-greedy, eigen, Louvain detection methods, and so forth).

The walk-trap algorithm suggested that journals segregated into three communities (Appendix E: Figure F.1, Figure F.2), two of which corresponded to easily identifiable subject areas: ecology and veterinary medicine. Participants in a third community, which contained the preponderance of our papers, represented a wide range of disciplines (Figure 6.1a). Since this group defied a clear disciplinary label, we refer to it as “Group 3” throughout. Most algorithms that relied on undirected edges (which is to say, a reference from journal A to journal B was equivalent to a reference from journal B to journal A), partitioned Group 3 into separate epidemiological and mathematical biology communities. However, the walk-trap algorithm is a better approach for our purposes, since we are interested in identifying journal groups with reciprocal, rather than directional, citations. A full list of journals in the three largest communities is included in Appendix E.

The three largest communities encompassed 79 journals capturing 95.3% (1551 of 1628) of the papers returned by our search. Veterinarians tended to lead papers in the veterinary journal community; ecologists and evolutionary biologists tended to lead papers in the ecology community; and mathematicians, statisticians, or health-informatics experts disproportionately led papers in Group 3 journals (Figure 6.1a). In the veterinary community and Group 3, the majority of citations were directed towards papers within the same community, with 30.2% and 15.5%, respectively, of citations pointing outside the community. In the ecology community, the division between within-community and outside-community citations was close to equal, with 51.7% of citations pointing outside the community (Appendix E: Table F.6). A list of the ten most-cited papers in each community is provided in Appendix E.
Figure 6.1: Participant diversity and publication growth. (a) Frequency of lead author affiliation disciplines across all 1551 papers published in journals in the three major journal communities. “Math” here encompasses “math” and “stat” affiliations; “ecol” encompasses “eco”, “evo”, and “biol” affiliations; “vet” captures “vet”, “animal health”, and “animal science”; “Med” captures “med” and pharmacy affiliations. (b) Number of papers captured by our search through time. Blue = veterinary community; gold = ecology community; red = group 3. Numbers are the annual percent growth rate within each community.

6.4.2 Number of publications and author diversity both increased with time

The number of papers fulfilling our search increased at a rate of 14.6% per year (95% CI[13.0%, 16.2%], Appendix E: Figure F.5, Table F.7) and doubled approximately every 5.06 years (95% CI [4.62, 5.66]). This puts publication growth rates for our search well above publication growth rates for most biological sub-disciplines (e.g., a 10.4% growth rate for ecology and evolution [172]), and for medicine and health sciences (growth rate estimated around 8-9% [173]). Lead author diversity â€” low when all lead authors in a community have the same disciplinary affiliation; high when lead authors come from a wide variety of disciplines – increased with time in all three communities (Figure 6.1b), but this was strongly correlated with...
Figure 6.2: Venn diagrams of cross-community authorship through time. Each year’s Venn diagram is scaled to reflect the number of authors with two or more papers in our paper bank over the preceding five years. Number of authors with two papers in the same journal community are represented by disjoint regions of the circles, and number of authors with papers in two different communities are represented by the area of the intersections. Each circle is scaled to reflect the total number of authors with papers in that community during the five years prior to the label year. Areas are on a log-scale, and total number of authors with multiple papers each year is reported below each Venn diagram.

The number of authors contributing papers to two or more communities also increased with time (Figure 6.2). The proportion of authors with two or more papers who contributed papers to multiple communities also increased up until 2012, when it was likely halted by the rapid growth of Group 3.

the number of papers (Appendix E: Figure F.6). The best description of temporal dynamics in lead author diversity was a generalized additive model (GAM) that let each community follow its own trajectory (AIC for the saturated GLM = 48.33; AIC for GAM with common trajectory structure = 71.9; AIC for the GAM with varying trajectory structure = 21.5). That model explained 92.7% of observed deviation (coefficient estimates in Table F.9). Author diversity within papers also increased through time (Appendix E: Figure F.7a-b, Table F.8), and was not strongly correlated with the number of authors on the paper (Appendix E: Figure S6a). However, both lead author diversity and author diversity within a paper were lower for the two traditional disciplines than for Group 3.
6.4.3 Journal communities varied in study system, question, and analytical approach

We read a random stratified sample of 236 papers designed to capture research trends in each journal community through time. Fifty-three (22.5%) of those papers did not include dynamic models of disease transmission and were therefore removed, leaving 183 papers: 50 from the ecology journal community, 64 from the veterinary journal community, and 69 from Group 3.

Perhaps the most profound content difference among the three journal communities was the study system. Fifty-nine of the 64 manuscripts (92.2%) from the veterinary journal community focused on domestic animal systems; the ecological journals were dominated by studies of plant and wildlife systems (35 of 50 papers; 70.0%); and human systems were primarily studied in Group 3 (28 of 69 papers, 40.6% human) (Figure 6.2a). The communities also differed fundamentally in their approaches, objectives, and methodologies. Veterinary studies were more likely to be predictive and management-focused than studies in ecology or human-focused epidemiology (Figure 6.3b, Figure 6.3c). Analyses aimed at gaining basic science insights were common in the ecological community and Group 3, but rare in the veterinary community (Figure 6.3b).

The veterinary community often included data in their analyses (Appendix E: Figure F.3a-b), while this was not the case in the ecology community or Group 3; however none of the communities regularly reported model performance of fit (Appendix E: Figure F.3c). Simulation – both stochastic and deterministic – was widely used in all communities (Appendix E: Figure F.4a-b), with a heavy emphasis on model sensitivity in the veterinary community (Appendix E: Figure F.4c), and deterministic predictions based on mathematical analysis in the ecology community (Appendix E: Figure F.4a, Figure F.4d).

6.4.4 Publications increasingly cited members of their own community and Group 3

All three communities cited other papers from their own community at increasing rates through time (Figure 6.4, Appendix E: Table F.10). Internal citation rates were highest for Group 3, due in part to its dramatically increasing size. Over
that same period, the ecology and veterinary communities also increased the rate at which they cited Group 3, and Group 3 increased the rate at which it cited ecology parallel to ecology’s increase in internal citations. However, the ecology and veterinary communities did not increase citation rates toward one another, suggesting strong segregation in their respective bodies of literature. The apparent decline in recent citations (Figure 6.4) is consistent with other bibliometric analyses showing that citation distributions from papers published in a given year peak three years prior [174].

The effect of a focal paper’s author diversity on its citation rates – calculated as the number of papers within our search that cited the focal paper per year – varied between communities (Figure 6.5, Appendix E: Table F.10). In ecology, increased author diversity was associated with a substantial increase in a paper’s expected citation rate, but this effect was significantly lower for papers published in the veterinary community or Group 3.
Figure 6.4: Cross-disciplinary citations through time. (a) Citations from papers in ecology journals to papers in each journal community. (b) Citations from papers in veterinary journals to papers in each journal community. (c) Citations from papers in Group 3 journals to papers in each journal community. Shaded regions are 95% confidence intervals from a Poisson generalized additive model fit to each journal community’s time series.

Figure 6.5: Citation benefits of author diversity. Associations between author diversity and citation rate for papers in each journal community. Model estimates are derived from a Poisson mixed effects model with an offset term for years since publication, and coefficient estimates are reported in Appendix E: Table F.8. Predictions are calculated for papers published in 2010, with 25% of citations to other journal communities, and 75% of citations to the paper’s own community.
6.5 Discussion

We found three pieces of evidence suggesting the dynamic disease modelling community grew and diversified in tandem with increasing calls for One Health. First, the number of publications presenting dynamic infectious disease models grew rapidly relative to publication growth rates in the natural and health sciences as a whole (Figure 6.1b), indicating an intensifying research interest in this aspect of One Health. Second the number of authors who contributed papers to journals in two or more journal communities also increased with time (Figure 6.2). Third, the fastest-growing community (“Group 3”) contained a broad and balanced range of contributing disciplines (Figure 6.1a), and captured the majority of papers fulfilling our search criteria (Figure 6.1b, Appendix E: Table F.6); we tentatively suggest this journal community could actually be labelled “One Health”.

Not all of our findings are so positive, however. Two traditional publication silos, ecology and veterinary medicine, remained segregated from one another. While system may be the nominal factor separating these disciplines (Figure 6.3a), their publications also partitioned along methodological and theoretical axes. All three communities asked unique questions (Figure 6.3b), and address those questions with unique methods (Appendix E: Figure F.3, Figure F.4); we saw limited evidence of information movement between silos. Therefore, although our analysis uncovered an active interdisciplinary disease modelling publication forum, some fundamental challenges remain. These challenges are especially important for emerging infectious diseases with both domestic and wildlife hosts, since those systems are the purview of the segregated veterinary and ecological communities.

One additional observation merits mentioning: only three of the 1628 papers included in this analysis derived from high-profile medical journals. Of the twenty-five highest ranked medical journals in 2014, only New England Journal of Medicine and PLoS Medicine contained any papers fulfilling our search. While epidemiology journals were well-represented in our survey, only 83 of the 6450 ranked medical journals fall into that group. Limited medical representation in disease transmission studies (Figure 6.1a) poses an obstacle for strengthening the interface between human medicine and other facets of One Health.

Some continued disciplinary isolation is neither surprising nor problematic. Expertise on within-host processes, between-host processes, and mathematical
modelling are the realms of distinct academic domains, and each domain requires intensive study on the way to expertise. Yet research teams that bridge traditional research silos are essential for efficient science, and building those teams is difficult. For example, a survey of researchers working on coupled human-natural systems (CNS) documented tension between collaborating departments and institutions as a common hindrance to interdisciplinary work [175], and the majority of respondents in that study also reported difficulties in finding publication venues for interdisciplinary projects. Funding was cited as the primary obstacle to forming interdisciplinary collaborations, and researchers across participating disciplines reported limited credit for interdisciplinary work as a major barrier to interdisciplinarity [175]. In a different study of interdisciplinary geography faculty [176], participants expressed general scepticism toward researchers whose expertise differed from their own. If these sentiments also apply to researchers in One Health, they may form a barrier to future collaboration.

One Health training programs must provide students with sufficient depth in their own domain, while also providing them with sufficient cross-disciplinary perspective to participate in multidisciplinary work. In traditional academic settings, graduate students are subject to expectations from their home departments, which may not coincide with an interdisciplinary team’s goals. Explicitly interdisciplinary graduate programs come with their own challenges. One study of 45 interdisciplinary neuroscience doctoral students [177] found that both students and faculty were dissatisfied with time constraints and imperative trade-offs of depth for breadth required by their interdisciplinary curriculum. Parallel sentiments arose among geography research faculty members [176], and graduate students from three different IGERT programs [178,179]. Interdisciplinary collegiate and graduate programs continue to grow in favour and number [180], however, so designing programs in the quantitative, ecological, veterinary, and medical sciences that balance discipline-specific and cross-disciplinary perspectives is a critical next-step for One Health.

Researchers incur a cost – in visibility, productivity, and income – for failing to specialize [181]. One study found a significant positive relationship between specialization under a variety of metrics and productivity, and between productivity and income; researchers need strong incentives to overcome that cost and engage in cross-disciplinary collaborations. Horizontal funding structures (e.g.,
training centers (e.g., the National Institute for Mathematical and Biological Synthesis [NIMBioS], Mathematical Biosciences Institute, Los Alamos National Laboratory’s Center for Nonlinear Studies), and academic consortia combat cost disincentives, and form an active presence in today’s One Health community. Similar collaborations in the physical and medical sciences served as a basis for building interdisciplinary research teams [182] and increasing participant productivity [183]. Cross-disciplinary participant collaboration increased after attending workshops at NIMBioS [184], and similar, albeit undocumented, patterns probably surround many One Health gatherings.

In order to move forward, we must determine which projects intended to enhance One Health succeeded, and identify factors that continue to impede interdisciplinarity. More information on the costs and benefits of different kinds of interactions (for example, participating in a common workshop, occupying a common institutional building, attending common seminars, and so on) would help clarify the most efficient means for building One Health teams.

One Health collaborations will likely continue increasing in frequency. Emerging quantitative methods like Bayesian hierarchical modelling, approximate Bayesian computation methods [185], and partially observed Markov models [186] offer new platforms for integrating data from veterinary studies, theoretical concepts from ecology, and detailed mechanistic models from mathematical epidemiology. Knowledge from captive or domestic animal systems may help researchers constrain some parameters in dynamic models of wild systems, and knowledge from disease transmission studies in wildlife may help refine captive study design. However, these benefits will not accumulate overnight.

Ideally, each One Health researcher should appreciate all aspects of field, modelling, and captive study, and interact with several of these components directly. Short of that goal, One Health requires at a minimum open-mindedness to varying approaches among individuals, training cultures, and publication venues. Funding agencies incentivized One Health Collaborations by restructuring grants to favor interdisciplinary teams, and research institutions could do the same by restructuring tenure expectations to account for interdisciplinary effort (for example, by incorporating performance measures that account for cross-disciplinarity [187]). Editors in all contributing disciplines – but especially ecology and veterinary medicine – could help by accepting for review more disease papers that were historically perceived
as too far afield. We see clear evidence of an emerging One Health community; however, without sustained efforts to integrate ecology, veterinary, and human medicine, disease modelling efforts will remain in some sense more “Three Healths” than “One”.
Chapter 7  
Conclusions

7.1 What we learned

The bighorn sheep chapters of this dissertation were designed to add teeth to common perceptions about disease in bighorn sheep, in the form of clearly illustrated empirical arguments. While our major empirical findings – that local pathogen persistence limits population growth for decades after disease introduction, that population substructuring during summer pushes lamb disease events toward frequency-dependent transmission processes, and that infected dry ewes and yearlings pose lower transmission risks than infected dams – are not particularly surprising, prior to this work these ideas were largely unsubstantiated. As an empiricist, my task was to map those ideas onto an actual dataset in an effort to quantify the weight of evidence supporting them. While this is somewhat less intellectually exciting than generating new theory, quantifying evidential burdens is imperative for systems regularly caught up in legal conflict (as is the case for bighorn pneumonia).

If nothing else, I hope that this dissertation unequivocally illustrates the importance of disease persistence in driving bighorn population dynamics. Chapter 2, which delineated the long-term effects of disease invasion and persistence, was not among my originally planned chapters; it was added following several conferences and discussions that underscored the lack of consensus about whether disease-induced lamb fatalities were actually relevant to bighorn population dynamics. While an earlier study from our research team [1] clearly documented severely reduced summer lamb survival and recruitment in the presence of pneumonia,
that project did not emphasize temporal patterns of disease, it did not explore whether mortality rates were changing with time since invasion, nor did it project demographic disease costs forward. All of those topics were taken up explicitly in Chapter 2.

The population substructuring patterns documented in Chapter 3 provide some important insights into management options for bighorn sheep. At the moment, many agencies take a density-based approach to management, in which populations are reduced upon reaching a given (albeit somewhat ad hoc) size, in an effort to mitigate disease risk. Although we did not address the relationship between density and spillover risk, our work showed that density reductions likely have very limited implications on pneumonia persistence. Consequently, current management aimed to limit pneumonia risk through density reduction requires substantial financial investments, along with human and animal health risks, with very little hope of actually solving the problem. Emily Almberg (Montana Fish Wildlife and Parks) and I are currently exploring whether density reductions and various other management actions might mitigate long-term disease persistence in a follow-up SIR-like model.

Preliminary analyses suggest that infection risk in this system varies with age (e.g., R.K. Plowright and E.F. Cassirer, unpublished data), with young and old animals exhibiting higher prevalences than prime-aged animals. For managers interested in halting pathogen transmission, removal of young animals is often controversial, especially for a long-lived host of conservation importance like bighorn sheep. As such, understanding the transmission potential for young vs. old infected animals was important: if young are simply infected without posing transmission risk, their removal may not be relevant for spurting population recovery. While a number of authors have reported demographic group structures of bighorn sheep that suggest juveniles of both sexes mix with ewe groups during summer [132], prior to this study, no clear data existed documenting direct contact patterns between juveniles and susceptible lambs. Our data suggest that these contacts are rare, and that infected juveniles rarely transmit directly to lambs, providing some evidence that removal of young infected animals may not effect local transmission.

The Hells Canyon bighorn populations do not subscribe to consistent space use and social interaction patterns. While many ungulate hosts can be readily classified as living in territories (e.g., for male eland, [188]), living in matrilineal groups [140,189],
or adopting fission-fusion dynamics [112], no such blanket statement applies to the Hells Canyon bighorn herds. Some populations abruptly partition space while others exhibit fission-fusion dynamics; some reside in groups of highly consistent membership whereas others are subject to many switches in associates. This heterogeneity begs the question, “Can we quantify the extent to which spatial networks approximate social networks for each of these herds?”, which served as the jump-off point for Chapter 5. We were surprised by the extent to which space use dominated spatial and social network topology, and suggest that disease and behavioral ecology might benefit from more studies exploring the interplay between space use and social contact.

Bighorn sheep disease ecology rests on processes that reside in fundamentally different academic domains, each with their own social cultures, methods, and traditional questions. Unfortunately, these academic “silos” remain highly segregated. Bridging interdisciplinary gaps is crucial in advancing our knowledge of this system and others. Only through stronger interdisciplinary collaboration can we efficiently leverage information aggregated on different aspects of the system.

### 7.2 Other sides of the bighorn story

While the work presented here focuses mainly on disease dynamics at the between-host scale, within-host dynamics – and especially the processes that generate and propagate chronic infections – are major driving factors in the bighorn system. Pursuant of understanding within-host dynamics and cross-scale relationships, our team has analyzed existing data and longitudinally sampled animals in captivity and field settings in tandem with our behavioral field work. From the existing data, we detected some signal that adults had increased resistance to disease in the aftermath of an epidemic event, but that that resistance was not passed on to lambs [46]. In the follow-up longitudinal samples, we saw evidence that infection status partitioned into clear classes, with some animals consistently resisting pathogen invasion, others occasionally acquiring intermittent infections, and a few animals testing consistently positive (examination of serum antibodies suggested that all animals experienced consistent exposure)(R. Plowright and E. F. Cassirer, unpubl. data). This observation piqued our interest in the role that chronic pathogen carriers play in perpetuating disease persistence in bighorn herds, and information from the
longitudinal sampling informed the dataset used in Chapter 4.

In the midst of the longitudinal sampling trials, and commensurate with one of our field seasons, we opportunistically observed a novel Mycoplasma ovipneumoniae strain introduction event. Since we were already intensively monitoring that herd as part of the behavioral and longitudinal sampling studies, we were able to capture detailed data on symptom emergence in all resident animals. An all-age die-off event occurred, despite the fact that a different Mycoplasma ovipneumoniae strain had previously circulated in the local group. By the termination of the all-age die-off (at which point all surviving animals were moved into captivity), the initial strain had been completely replaced by the novel strain. This unplanned natural experiment demonstrated that bighorns lack protective cross-stain immunity. Since that observation has important implications from a management standpoint – namely, that even infected herds need to be protected from novel pathogen introduction events – we wrote this “natural experiment” up in a forthcoming article at the *Journal of Wildlife Management* [190].

### 7.3 What next for bighorn sheep?

Pneumonia remains an outstanding threat to bighorn population recovery. While our work has begun to delineate some of the underlying processes both within- and between-hosts, we have yet to uncover clear management strategies that lead to pathogen fade-out and recovery. Two major open questions shape our planned work on this system.

1. What are the processes that allow for chronic *Mycoplasma* colonization in adult bighorn sheep?

2. What management actions might spur population recovery?

Understanding the mechanisms that allow some individuals to carry *Mycoplasma ovipneumoniae* asymptptomatically, even as others clear infections, and others still succumb to disease, is crucial for surgical population management to facilitate pathogen clearance. Our current modeling project suggests that only very extensive culling operations could result in pathogen clearance, unless culls are targeted toward infected animals. Some animals (especially younger animals) are potentially
capable of eventually clearing a long-term infection; oldest animals appear to be at disproportionate risk for chronic infections. Yet the mechanisms that allow for chronic infections in some animals are not well-characterized. A major challenge in exploring chronic infections in the bighorn host is the extreme difficulty in handling and maintaining bighorns in captivity. Unless animals are hand-reared from birth, achieving a sampling resolution at a finer temporal resolution than approximately once per month has proven impossible.

Nevertheless, there are a number of lines of evidence that could inform the first question. At the population level, some insights might be gained by mapping population-level carriage prevalences to ecological drivers like habitat quality, age structure, or genetic diversity. At the within-host level, generating a set of a priori hypotheses, and evaluating these hypotheses in a mathematical context might provide key insights about crucial knowledge gaps (since at this time, our hypothesized list of within-host drivers ranges from IgA efficacy to complement capacity to fine-scale heterogeneities in Mycoplasma’s capacity to secrete hydrogen peroxide to host-specific factors related to cilia binding affinities and resistance to damage in the presence of hydrogen peroxide). One factor that is simultaneously a challenge and an opportunity is that bighorn and domestic sheep show very different responses to Mycoplasma colonization. This means that contrasting responses between host species could provide insights into the processes leading to pathogenicity in bighorn sheep. At the same time, infection progressions within domestic sheep – which are far more amenable to high-resolution longitudinal sampling and captive study than bighorns – cannot serve as a fully accurate proxy for infection in bighorns. Prioritizing questions to address using captive bighorns, and implementing those captive studies under appropriate control conditions, remain outstanding challenges in this system.

On the management side, four different management actions are currently debated in the bighorn community. These are

1. A “do nothing” approach, in which populations are allowed to clear pathogens (or go extinct) in their own time;

2. Density reductions that agnostically remove a subset of animals for infected herds;

3. A targeted reduction that removes *M.ovi*-positive animals;
4. Depopulation and reintroduction, in which infected herds are completely extirpated and then reseeded from other stock.

A fifth option, treatment or vaccination, remains elusive despite over twenty years of intensive research. Of the above actions, our research group strongly favors the targeted cull, which we are currently field-testing at our Hells Canyon sites. A west-wide adaptive management program aims to experimentally compare the different options in the enumerated list on bighorn herds throughout the western United States, however field efforts for that project are still in their infancy.

7.4 Toward stronger disease inferences in data-weak systems

Dynamic models of infectious disease are notoriously hard to fit, even for very data-rich systems like measles in humans. Trying to gain insight from dynamic frameworks in wildlife systems, which are subject to lower-quality data with higher stochasticity due to small sample sizes (often arising from truly small populations), is even more difficult. Over the course of my research, I took a “kitchen-sink” approach, trying a wide variety of statistical methods to glean any available insights. En route, a few overarching themes emerged.

First, wildlife biologists would likely benefit in general from simultaneously leveraging multiple datasets to estimate key parameters. Integrated population models, like the one we used in Chapter 2, provide a new framework for doing just that. By utilizing the statistical architecture of Bayesian hierarchical models, integrated population models provide a framework for incorporating all kinds of ecological data, and leveraging all those data toward a common framework. While our approach focused exclusively on host vital rates and population dynamics, other authors have begun to explore IPM applications to disease dynamics directed (e.g., [191]). Future exploration in that direction might provide a powerful tool for disease ecologists to harness otherwise disparate wildlife field data toward addressing questions of disease transmission.

Second, disease ecologists would be much better able to understand transmission dynamics in emerging infectious disease settings if a general framework for assessing animal contact existed. Accurately constructing social networks requires very
information-rich data, and a near-census of host populations, neither of which are available for most wildlife taxa. On the other hand, more readily available datasets, like habitat use data and group size are available. Clear frameworks that link these readily available datastreams to social network dynamics would be extremely valuable in rapid modeling settings that rely exclusively on data in-hand. Additionally, better general frameworks for within-host processes, including sampling protocols aimed at characterizing likely regions of within-host carriage (e.g., explicit sampling of immune organs and other immune-priviledged sites in hosts dying of disease) would dramatically improve our ability to forecast the consequences of pathogen establishment in novel wildlife hosts.
<table>
<thead>
<tr>
<th>Data form</th>
<th>Aspect of disease</th>
<th>Availability</th>
<th>Disease parameter(s)</th>
<th>Management utility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host spatial data</td>
<td>Introduction risk, contact structure</td>
<td>Widespread (pop level), Moderate (individual level)</td>
<td>Contact rate, $c$</td>
<td>Mitigate spillover risk, identify well-connected groups for targeted management, determine whether density reduction might reduce transmission</td>
</tr>
<tr>
<td>Wild host incidence / survival data (unmarked animals)</td>
<td>Pathology, causative agent, disease interactions with other biotic factors</td>
<td>Moderate</td>
<td>(Qualitative)</td>
<td>Identify causal agent, and potential treatments</td>
</tr>
<tr>
<td>Wild host incidence / survival data (marked animals)</td>
<td>Disease incidence, mortality by cause, seroprevalence</td>
<td>Moderate</td>
<td>Disease-induced mortality rate, $\alpha$</td>
<td>Quantify demographic costs of disease</td>
</tr>
<tr>
<td>Cross-sectional serosurvey</td>
<td>Past pathogen prevalence</td>
<td>Moderate</td>
<td>Force of infection, $\lambda$ (if animals are aged)</td>
<td>Understanding extent of transmission</td>
</tr>
<tr>
<td>Longitudinal serosurveys</td>
<td>Antibody waning</td>
<td>Very limited</td>
<td>Reversion from R to S, $\omega$</td>
<td>Forecast whether pathogen is likely to fade-out or persist locally</td>
</tr>
<tr>
<td>Cross-sectional PCR data</td>
<td>Prevalence (by strain), strain competition dynamics</td>
<td>Limited</td>
<td></td>
<td>Identify cull targets, estimate disease-induced mortality rate</td>
</tr>
<tr>
<td>Longitudinal PCR data</td>
<td>Infectious period, strain heterogeneity</td>
<td>Very limited</td>
<td>Recovery rate, $\gamma$</td>
<td>Predict effect of removing infecteds</td>
</tr>
<tr>
<td>Lab challenges</td>
<td>Dose-response relationships, relationship between dose, symptoms, and time since infection</td>
<td>Very limited</td>
<td>$P(\text{infection given contact}), \kappa$</td>
<td>Make decisions about importance of separation between host species</td>
</tr>
</tbody>
</table>
7.5 Caveats and disclaimers

7.5.1 Scope limitations due to working in a single system

Our findings here, and all findings from Hells Canyon suffer a common limitation: without parallel data from other metapopulations, we cannot say with certainty what part of the patterns we describe are general phenomena, and what part are unique to our system. This caveat is lessened by extensive population-level replication within Hells Canyon (which has 16 weakly-connected herd units), and dramatic differences in herd habitats (ranging from the high-elevation Lostine herd to nearly desert-like conditions approximately 8,000 feet lower in the Black Butte and Redbird herds along the Snake River). We also “benefit” from the presence of multiple *M.ovi* strains, which apparently sometimes occur singly within particular population-years. We have begun investigating the interplay between those strains (e.g., [190]), and will continue to do so in future projects. Nonetheless, predation, management, and anthropogenic pressures are relatively consistent throughout the system, and may drive some of the signals reported here.

7.5.2 Consistency of animal samples and test results

In Chapter 4, we classify animals as infected or not based on animal handling events that precede our study by up to 8 months, forcing us to assume that infection status is relatively constant, at least within adult animals. Our longitudinal sampling project suggests that this assumption is merited in many (but not all) cases. Ideally, we would use a real-time test that could be applied during the last trimester of pregnancy, but at this time no such test exists.

7.5.3 Animal movements between VHF relocation events

The data used to build networks in Chapter 3 were temporally coarse: locations occurred on a biweekly basis, giving us only a limited perspective on the complete suite of animal movement patterns. The trade-offs between VHF and GPS collars is a regular discussion point in this system. In Hells Canyon, we have strongly favored the long-lasting VHF collars (our collars regularly last five to seven years, and some continue transmitting for up to a decade following deployment). A great strength
of our dataset is that our locations are accompanied by visual observations, including group size and composition data, and lamb status information. Since visual observations are fundamental to our sampling protocols, the shorter life-time, higher failure rate, and higher costs of GPS units do not outweigh the benefits of higher temporal resolution in data.

That said, we recognize a real need for a more detailed analysis of bighorn spatial ecology (for both sexes) in order to understand how much sampling is needed prior to active management. To that end, we may begin deploying more GPS collars in the future.

7.6 Departing thoughts

I was incredibly lucky to stumble upon this system and its remarkable accompanying dataset for my dissertation work. Over a quarter of my PhD time was spent in the field following animals. That experience essentially altered my thinking about empirical disease ecology and data analysis. I am extremely grateful to my mentors for pushing me to spend time in the field, and in particular to Frances Cassirer, who shared her data – truly a lifetime’s worth of work – and experience studying bighorns, and modeled for me what an agency biologist can do.

My research program kept me constantly on the move, spending falls in State College, winters in Bozeman, and summers in the field. Although that schedule occasionally grated, the trifecta of different experiences – emersion in infectious disease and evolution at Penn State, interfacing with a very wildlife-management-oriented Ecology program and working with wildlife disease ecologists in Bozeman, and working on the ground with state biologists and locals in Hells Canyon – fundamentally shape my thinking as a disease ecologist.

Although on paper my degree might appear rapid, my pace was only possible due to four years of “warm-up” graduate work in statistics, which equipped me to move quickly on some of the analyses in this dissertation. The time invested in developing some statistical and computational literacy prior to beginning a PhD in biology was well worth my while.

Finally, the bighorn system was amazing and rewarding to work on. Sportsmen, conservationists, and locals all seem to love bighorns in a way that does not apply to wolves, elk, or other “charismatic” North American megafauna. While this work
by no means solves the bighorn pneumonia problem, I hope it at least clarifies the scope of the challenge, and illuminates some important paths forward.
Appendix A

A primer in bighorn biology

A.1 Evolutionary history

North American sheep of the *Pachyceros* subgenus (bighorn sheep *Ovis canadensis*, Dall sheep *Ovis dalli*, and *Ovis nivicola*) diverged from domestic sheep approximately 5.3 million years ago [192]. Hybrids between bighorn and domestic sheep are fertile, and the preponderance of species differentiation is due to non-overlapping habitats and behavioral ecology (as has been documented using evolutionary markers for thinhorn sheep, [193]). Sequestration to two glacial refugia (one in the Mojave Desert, and another near Natural Trap Cave in Wyoming) 300,000 years ago during the Illinoian glaciation and 100,000 years ago during the Sangamon interglacial period led to two distinct subspecies of bighorn sheep. Recent analyses suggest the Mojave refugia gave rise to all extant subspecies of *Ovis canadensis*, with the Desert subspecies being most diverse.

There are three distinct subspecies of bighorn sheep: Rocky Mountain (*O. c. canadensis*), the endangered Sierra Nevada (*O. c. sierrae*) and California (*O. c. californiana*) subspecies, and Desert bighorns, which further substructure into three distinct polyphyletic clades, Nelson bighorns of the Mojave, Great Basin, and Colorado Plateau (*O. c. nelsoni*), the endangered Peninsular sheep of the Peninsular Ranges *O. c. cremnobates* and the Mexican sheep *O. c. mexicana* of the Chihuahuan and Sonoran Deserts [194]. All these subspecies are geographically distinct, and exhibit admixture at the edges of their ranges [194]. The Desert clades are apparently the source of all other subspecies, with the large Rocky Mountain clade diverging from Desert lines approximately 680 plus or minus 130 thousand
years ago [194].

Despite evolutionary and spatial divergence, pneumonia has been documented as problematic in all extant subspecies of *Ovis canadensis*.

### A.2 Life history

Bighorn sheep are relatively long-lived, with females typically living 8-15 years ([195] reported that 42% of ewes alive at age 3 survived to age 10; [196] reported a maximum longevity of 19 years), and rams living to a maximum age of 14 years [197]. Density operates primarily by increasing age at primiparity [198]. Bighorns are highly sexually dimorphic, with adult rams weighing approximately $1.4 \times$ the weight of adult females [197]. Mating is sequentially polygynous and promiscuous, with rams competing for access to individual estrus ewes ([199]. The preponderance of matings going to older, heavier males with larger horns [200], and in several studies, approximately 35% of lambs are sired by the single best performer [200, 201].

Gestation is approximately 174 days [201], and births are pulsed in concentrations that vary according to habitat and latitude, with more southerly desert sheep exhibiting a more dispersed birth pulse. Most ewes give birth to a single lamb, and maintain close contact with that lamb until weaning, which occurs at approximately 4-5 months of age. Bighorns are “capital” breeders insofar as they rely on existing and previously accumulated body reserves to meet energetic costs of reproduction [202]. Therefore, under healthy conditions, ewes trade off their own fitness against investment in offspring survival.

### A.3 Behavioral and spatial ecology

Bighorns reside in sexually segregated groups [93] of typically 5 to 25 animals throughout most of the year [203], with one- and two-year-old rams sometimes splitting time between ewe-lamb groups and bachelor groups [132]. Most mixing occurs during fall rut [1], which peaks between October and December. Bighorn home ranges are typically tens of square miles, and some populations undertake regular seasonal migrations, typically of distances less than about 20 miles. Both males and females occasionally go on forays [204, 205] of varying distance, and
these also vary in destination from consistent trips to optimal sites like mineral licks, and more walk-about-style trips beyond the periphery of the animals’ home-ranges.

Bighorn spatial structure during all-age and endemic lamb disease events are fundamentally different. All-age events often cluster during the fall rut, when populations are relatively panmictic. Lamb mortalities, however, have a very clear summer timing signature.
Appendix B
Supplementary Information for Chapter 3: Demographic phase transition

B.1 Field Methods

Prior to 1996, data consist of annual population surveys conducted in the late winter or early spring. From 1997-2014, survey data are accompanied by information on survival and reproduction of radio-collared animals (described in Cassirer et al. 2013). Ewes were monitored at least monthly year-round, weekly during lambing, and weekly to monthly over the summer during lamb-rearing. Lamb disease events in Hells Canyon were associated with a median of 20% summer lamb survival (95% of observed disease years in lambs had summer lamb survival between 0% and 67%). 54 of 106 population-years studied by Cassirer et al. [1] were classified as having summer lamb pneumonia. While die-offs years would be classified as lamb disease events, we have little individual-level data during die-offs, so for our purposes, lamb disease events are generally restricted to post-die-off lamb disease events. Extensive lamb carcass collection and necropsy revealed that 88% of mortalities for 129 necropsied lambs, including 9 of 11 euthanized lambs collected in the Hells Canyon bighorn sheep system of ID, WA, and OR, were attributable to pneumonia [1], even though disease-induced mortality in adults during the same period was only about 30% (54 of 179 animals necropsied; [1]). We use confirmed presence of clinical pneumonia (i.e., from necropsies of animals with pneumonic
lesions in their lungs) or lamb survival to weaning of less than 50% (36 of 190 population-years) paired with observed pneumonia signs (nasal discharge, coughing, lethargy) to classify population-years as “persistently diseased” or “healthy” [1,31]. Ewes were considered to have a lamb if they nursed, were alone with a lamb, or had close body contact with a lamb.

**B.1.1 Determination of lamb status**

Ewes were considered to have a lamb if they nursed, were alone with a lamb, or had close body contact with a lamb.

**B.1.2 Determination of ewe age**

217 known-age ewes were monitored from 0-17 years each (median of four years), and were collectively monitored for 1555 ewe-years that could be assigned a population-level health status. We assigned age based on tooth eruption for animals that entered the study at less than four years of age (N = 94 ewes), and on post-mortem cementum analysis combined with length of time monitored (N = 123 ewes) for ewes with full dentition at initial capture. We assumed that individuals’ true ages were the midpoint of their estimated age-range when cementum analysis did not establish a specific age.

**B.1.3 Cause-specific mortalities**

Radiocollars were equipped with activity sensors, and a site investigation was conducted following detection of an inactive signal. Crews searched extensively for lambs when they went missing, which resulted in recovery of 104 lamb carcasses fresh-enough to identify cause of death. Of the known-age ewes, 107 died of causes other than pneumonia, and 28 died from pneumonia during the study period. 82 remained alive or were censored due to translocation or dead radiocollars. Where possible animals were collected whole or tissues were collected during field necropsy and submitted to the Washington Animal Disease and Diagnostic Laboratory at Washington State University. Population disease status was assigned on the basis of observed pneumonia, or lamb survival to weaning (Cassirer et al. 2013).
B.2 Post-invasion empirical trends

B.2.1 Hierarchical linear models

Recruitment as a function of Years since invasion

We modeled recruitment (the number of lambs counted in a given year per 100 ewes) as a function of years since pathogen invasion for the intensively studied populations. Although recruitment is effectively bounded above by 100 (since twinning in Hells Canyon is extremely rare), we treated it as continuous in this model. We included a hierarchical effect for each population, denoted $b_j$. Recruitment in the $i^{th}$ year and the $j^{th}$ population was denoted $R_{ij}$. Let $X_i$ denote the year post-invasion in which recruitment $R_{ij}$ was observed. Then our model was

$$R_{ij} \sim N(\mu_{ij}, \sigma_R) \quad (B.1)$$

$$\mu_{ij} = \beta_0 + \beta_1 X_i + b_j \quad (B.2)$$

We placed diffuse normal priors ($N(0, 10,000)$) on $\beta_0$ and $\beta_1$. $b_j$ values were treated as Gaussian, with standard deviation $\sigma_{\text{PopYear}}$, and we placed uniform(0, 10) priors on both $\sigma_R$ and $\sigma_{\text{PopYear}}$. The posterior was based on three Markov chains, each run for 5000 steps, with a 2500-step burn-in. The multivariate prsf was 1.03 (values less than 1.10 suggest model convergence). The model converged very rapidly (multivariate prsf = 1.02 after 5000 steps). Posterior medians and credible intervals are tabled below.
Table B.1: Caption: Posterior parameter estimates from Recruitment ∼ Years since invasion. Estimates are reported under the indentity link.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Posterior median</th>
<th>95% Posterior credible interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recruitment on the year of invasion</td>
<td>$\beta_0$</td>
<td>0.318</td>
<td>(0.268, 0.369)</td>
</tr>
<tr>
<td>Change in recruitment with each additional year since invasion</td>
<td>$\beta_1$</td>
<td>0.001</td>
<td>(-0.006, 0.007)</td>
</tr>
<tr>
<td>Standard deviation between pop-years</td>
<td>$\sigma_{PopYear}$</td>
<td>0.032</td>
<td>(0.001, 0.0.093)</td>
</tr>
<tr>
<td>Residual standard deviation</td>
<td>$\sigma_R$</td>
<td>0.189</td>
<td>(0.166, 0.214)</td>
</tr>
</tbody>
</table>

Table B.2: Posterior parameter estimates for Health status ∼ Years since invasion.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Posterior median</th>
<th>95% Posterior credible interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline probability that the year following a disease introduction is classified as healthy</td>
<td>$\beta_0$</td>
<td>0.225</td>
<td>(0.102, 0.391)</td>
</tr>
<tr>
<td>Multiplicative change in odds of being Healthy with each increasing year since invasion</td>
<td>$exp(\beta_1)$</td>
<td>0.843</td>
<td>(0.540, 1.246)</td>
</tr>
<tr>
<td>Standard deviation of pop-year effects</td>
<td>$\sigma_{PopYear}$</td>
<td>0.602</td>
<td>(0.505, 0.802)</td>
</tr>
</tbody>
</table>

Table B.3: Posterior estimates for Population growth rate ($\lambda$) ∼ Recruitment. Estimates are reported on the identity scale.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Posterior median</th>
<th>95% Posterior credible interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda$ when recruitment = 0</td>
<td>$\beta_0$</td>
<td>-0.133</td>
<td>(-0.204, -0.073)</td>
</tr>
<tr>
<td>Change in $\lambda$ with one-unit increase in recruitment</td>
<td>$\beta_1$</td>
<td>0.420</td>
<td>(0.243, 0.613)</td>
</tr>
<tr>
<td>Standard deviation of pop-year effects</td>
<td>$\sigma_{PopYear}$</td>
<td>0.025</td>
<td>(0.002, 0.080)</td>
</tr>
<tr>
<td>Residual standard deviation</td>
<td>$\sigma_R$</td>
<td>0.196</td>
<td>(0.176, 0.224)</td>
</tr>
</tbody>
</table>
Health status as a function of Years since invasion

We evaluated whether the proportion of disease-free years changed as a function of time since invasion by first classifying each population-year and healthy or not (e.g., any disease was observed, or demographic rates were consistent with a disease event). We treated population-year healthy status, $H_{ij}$ as Bernoulli, and modeled it in a hierarchical logistic regression model as a function of years since invasion. We included a hierarchical logistic effect on population. In full, the model was

$$H_{ij} \sim \text{Binomial}(1, \pi_{ij}) \quad (B.3)$$

$$\text{logit}(\pi_{ij}) = \beta_0 + \beta_1 X_i + b_j \quad (B.4)$$

The model converged very rapidly (Multivariate psrf = 1.03 after 5000 steps). Posteriors are based on three chains of 5000 steps each, with a 2500-step burn-in. Posterior medians and credible intervals are tabled below.

Population growth rate ($\lambda$) as a function of Recruitment

We modeled population growth rate, $\lambda$ (calculated as $\frac{N_t}{N_{t-1}}$, after adjusting for animals added and removed) as a function of recruitment (treated as continuous, and bounded to fall between zero and one in this case). We included a hierarchical effect for each population, denoted $b_j$. Estimated $\lambda$ in the $i^{th}$ year and the $j^{th}$ population was denoted $\lambda_{ij}$. Let $X_i$ denote the recruitment corresponding to each observed $\lambda_{ij}$. Then our model was

$$\lambda_{ij} \sim N(\mu_{ij}, \sigma_R) \quad (B.5)$$

$$\mu_{ij} = \beta_0 + \beta_1 X_i + b_j \quad (B.6)$$

We placed diffuse normal priors ($N(0,10,000)$) on $\beta_0$ and $\beta_1$. $b_j$ values were treated as Gaussian, with variance $\sigma_{PopYear}$, and we placed uniform(0, 10) priors on both $\sigma_R$ and $\sigma_{PopYear}$. The posterior was based on three Markov chains, each run for 5000 steps, with a 2500-step burn-in. The multivariate prsf was 1.01 (values
less than 1.10 suggest model convergence). Posteriors are based on three chains of 5000 steps each, with a 2500-step burn-in. Posterior medians and credible intervals are tabled below.

B.2.2 Trajectory change-point model

We used a Bayesian manifestation of a hierarchical change-point model to determine whether ewe count trends changed in the temporal vicinity of first recorded pneumonia event. Our objective was to determine whether ewe count trajectories altered behavior in proximity of the first disease detection. We also checked for the presence of a second behavioral shift, corresponding to population recovery, in the post-invasion time-series. Change-point models identify the location of a change-point in a trajectory, and describe the trajectory’s slope before and after the shift. We aligned the trajectories so that the first year of observed disease was set to zero for all populations. This configuration allowed us to model trajectories of all populations simultaneously, while accounting for variation in baseline population size ($\mu_0$), growth rate before ($\mu_1$) and after ($\mu_2$) the change-point, and change-point position ($\alpha_i = \mu_u + \delta_i$, where $\mu_u$ = grand mean change-point, and $\delta_i$ is a position adjustment to the change-point for the $i^{th}$ population) through hierarchical effect terms. In the first model, we used complete timeseries from nine Hells Canyon populations which were established well before their first recorded disease event (populations 1, 3, 4, 5, 6, 7, 10, 11, and 12). We then fit the model over shortened timeseries running from each population’s first documented disease event through the most recent year of sampling to test for a change-point associated with population recovery. In full, we modeled the ewe count ($Y_{ij}$ for the $i^{th}$ population in the $j^{th}$ year) as a function of years since disease introduction, $X_j$:

$$
Y_{ij} = \begin{cases} 
\beta_{i0} + \beta_{i1}X_j + \epsilon_{ij} & X_{ij} \leq \alpha_i \\
\beta_{i0} + \beta_{i1}X_j + \beta_{i2}(X_j - (\mu_u + \delta_i)) + \epsilon_{ij} & X_{ij} > \alpha_i 
\end{cases}
$$

(B.7)

where $\beta_{i0} \sim N(\mu_{\beta_0}, \sigma_{\beta_0})$; $\beta_{i1} \sim N(\mu_{\beta_1}, \sigma_{\beta_1})$; $\beta_{i2} \sim N(\mu_{\beta_2}, \sigma_{\beta_2})$; $\delta_i \sim Unif(s_i - \mu_u, e_i - \mu_u)$; $\epsilon_{ij} \sim N(\mu_e, \sigma_{epsilon})$. All $\sigma$ terms had $Unif(0, 10)$ priors, and $\mu_\beta$ terms had $N(0, \tau)$ priors, where $\tau = exp(log(\tau))$, and $log(\tau) \sim Unif(-100, 100)$. $\mu_u$ came from a $Unif(-22, 27)$ distribution, since our timeseries ran from 22 years before
to 27 years after die-off events. \( S_i \) and \( e_i \) are the minimum year and maximum year of observation for population \( i \), and the prior on \( \delta_i \) constrains each population’s change-point, \( \mu_u + \delta_i \), to fall within the observed range of values for that population. We placed Unif(-100, 100) priors on \( \mu_0 \) and \( \mu_1 \), and these parameters along with \( \mu_u \) and \( \mu_u + \delta_i \) were our inferential foci. Models were fit using JAGS and rjags (Plummer 2015), accessed through R [55]. Counts were high enough that their discrete nature had little bearing on the overall model, thus we treated counts as normally distributed in this setting. Posteriors were generated using JAGS, accessed through R. We ran 3 chains of 100,000 steps, and eliminated the first 50,000 steps as burn-in. Gelman-Rubin diagnostics suggested that this was sufficient time for chains converged on the posterior (multivariate prsf = 1.01).

We then tested for a second change-point by fitting exactly the same model to truncated timeseries that started with the year of pathogen introduction. In that case, a well-defined change-point might correspond to population recovery. Model posteriors are shown in Table B.4 (for the full timeseries) and S5 (for the post-invasion timeseries) below.
Table B.4: Posterior estimates for top-level change-point model parameters, for a model fit on the full timeseries. Estimates are reported on the identity scale.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Posterior median</th>
<th>95% Posterior credible interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-invasion population growth</td>
<td>$\mu_{\beta_1}$</td>
<td>5.54</td>
<td>4.26, 7.08</td>
</tr>
<tr>
<td>Post-invasion population growth</td>
<td>$\mu_{\beta_1} + \mu_{\beta_2}$</td>
<td>-3.17</td>
<td>-8.08, 1.59</td>
</tr>
<tr>
<td>change-point position</td>
<td>$\mu_u$</td>
<td>-2.57</td>
<td>-7.31, 2.20</td>
</tr>
</tbody>
</table>

Table B.5: Posterior estimates for top-level change-point model parameters, for a model fit only on the post-invasion timeseries. Estimates are reported on the identity scale.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Posterior median</th>
<th>95% Posterior credible interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-invasion population growth</td>
<td>$\mu_{\beta_1}$</td>
<td>8.61</td>
<td>2.71, 15.25</td>
</tr>
<tr>
<td>Post-invasion population growth</td>
<td>$\mu_{\beta_1} + \mu_{\beta_2}$</td>
<td>-3.76</td>
<td>-11.75, 3.23</td>
</tr>
<tr>
<td>change-point position</td>
<td>$\mu_u$</td>
<td>4.42</td>
<td>1.18, 15.70</td>
</tr>
</tbody>
</table>
B.3 Integrated Population Model

B.3.1 Demographic rate estimation in each environmental state

Data underlying the integrated population model and disease state definitions are shown in Table 2.1 and Figure 2.1a in the main text. As noted in the Methods section of the main text, an IPM estimates a single set of high-level parameters that govern multiple observation processes. Here, the high-level parameters are age-specific survival (denoted $\sigma_a$), lamb-weaning probabilities (denoted $\omega_a$), and probability of overwinter lamb survival, given that the lamb was weaned ($\eta$). These parameters govern both population-level ewe and lamb counts in the spring ($f_1(\sigma_a, \omega_a)$), and individual-level survival ($f_2(\sigma_a)$) and lamb weaning ($f_2(\omega_a)$) events. Information from both the timeseries of counts and the radiocollared survival and weaning statuses are used to estimate the high-level parameters through a Bayesian hierarchical model.

$\eta$, the probability a lamb survives winter given it was weaned, is estimated slightly differently than $\sigma$ and $\omega$. First, it is not specific to different ewe age classes. $\eta$ is estimated by comparing number of lambs surviving using observations from two separate datasets (summer lamb survival from the radiocollared ewe dataset, and recruitment from the spring population counts). The starting lamb survival is based on summer lamb survival estimated using survival of lambs born to radiocollared ewes, whereas the number of lambs the following spring is based on a population-level survey. We cannot reliably match lambs to dams after rut, and one advantage of the IPM is that it allows us to estimate this quantity, which would be latent and inestimable if either dataset were used on its own.

Population-level counts

We modeled counts under a state-space framework with a Poisson or Log-Normal distribution to estimate the true population counts of ewes and lambs on the basis of observed ewe and lamb counts. Ewe and lamb counts collected for each population ($j$ in $1, \ldots, J$) and year ($t$ in $1, \ldots, T$) are denoted $O_{Ewe,j,t}$ and $O_{Lamb,j,t}$, respectively. We used a state-space framework to account for observation error, such that

\[ O_{Ewe,j,t} \sim \text{Poisson} \left( N_{Ewe,j,t} \right) \]  

(B.8)
where \( N_{\text{Ewe},j,t} \) and \( N_{\text{Lamb},j,t} \) are the true but unknown numbers of ewes and lambs present in population-year \( j,t \), respectively. We constructed a disease-status matrix describing disease presence during each population-year, \( d : J \times T \). The \( [j,t]^{th} \) element of matrix \( d \) was set to 1 if the \( j^{th} \) population experienced disease conditions in the \( t^{th} \) year, and 2 otherwise. \( N_{\text{Ewe},j,t} \) is the sum of ewes of all ages greater than 1 year present in population-year \( [j,t] \), \( N_{\text{Ewe},j,t} = \sum_{a=2}^{A} N_{a,j,t} \), where \( N_{a,j,t} \) is the (unobserved) number of ewes in age-class \( a \) during population-year \( j,t \). We assumed a binomial ewe survival distribution in each age-class, such that

\[
N_{a,j,t} \sim \text{Binomial} \left( \frac{\exp[\sigma_{a-1,j,t-1} + r_t]}{1 + \exp[a\sigma_{a-1,j,t-1} + r_t]} \right) \quad (B.10)
\]

where \( \sigma_{a-1,j,t-1} \) is the appropriate element from the 5 x 2 matrix of age-specific ewe survival rates, and \( r_t \) is a system-wide random year effect on adult survival. Counts for each age-class in the first timestep were taken as draws from a \( \text{Pois}(10) \) distribution. The number of lambs counted in each spring is equal to the number of lambs that survived the previous summer and also overwinter. As such, \( N_{\text{Lamb},j,t} \) is the sum over all age-classes of the number of lambs weaned in each age-class (dependent upon last summer’s disease status, \( d[j,t] \)) times the probability of overwinter lamb survival in a year with disease condition \( d[j,t] \) (here denoted \( \eta_{d[j,t]} \)). Specifically

\[
N_{\text{Lamb},j,t} = \sum_{a=2}^{A} F_{a,j,t} \times \eta_{d[j,t]} \quad (B.11)
\]

where \( F_{a,j,t} \) is the probability that a ewe in age-class \( a \) successfully weans a lamb during a year with disease conditions specified by \( d[j,t] \). Formally,

\[
F_{a,j,t} \sim \text{Binomial} \left( \frac{\exp[a\omega_{a,d[j,t]} + c_t]}{1 + \exp[a\omega_{a,d[j,t]} + c_t]} \right) \quad (B.12)
\]

and \( \omega_{a,d[j,t]} \) is the appropriate element from the 5 x 2 matrix of age-specific recruitment rates and \( c_t \) is a system-wide random effect on recruitment. In full, the likelihood of the state-space model for ewe and juvenile counts \( (\mathcal{L}_{SS}) \) is
\[
LSS \left( O_{\text{ewe},j,t}, O_{\text{Lamb},j,t} \mid N_{\text{ewe},j,t}, N_{\text{Lamb},j,t}, \sigma, \omega, \tau_r^2, \tau_c^2 \right) = \\
LO \left( O_{\text{ewe},j,t}, O_{\text{Lamb},j,t} \mid N_{\text{ewe},j,t}, N_{\text{Lamb},j,t} \right) \times LS \left( N_{\text{ewe},j,t}, N_{\text{Lamb},j,t} \mid \sigma, \omega, \bar{\mathcal{C}}_{\text{r},r}, \tau_c^2 \right)
\]

where \(LO\) is the likelihood of the observation process and \(LS\) is the likelihood of the biological process.

**Age-specific survival**

We estimated age-specific survival and observed recruitment using data from known-age ewes. A collared ewe’s survival through each year was modeled as a Bernoulli random variable in which the probability of survival was a function of age and population disease status, with a hierarchical effect to account for variability between different population-years. Summer lamb survival was similarly modeled as Bernoulli, with the probability of successfully weaning a lamb again treated as a function of ewe age and disease status in the local population, plus a hierarchical population-year effect. Due to the fact that all ewes were marked with radio-collars paired with the intensity of field monitoring, we assumed that both ewe survival and lamb weaning status were observed without error. For each individual \(i\) in \(1, \ldots, I\) residing in population \(j\) during year \(t\), we modeled individual survival status \(s_{i,j,t}\) as Bernoulli:

\[
s_{i,j,t} \sim Bernoulli \left( \frac{\exp(\sigma_{a[i],d[j,t]} + r_t)}{1 + \exp(\sigma_{a[i],d[j,t]} + r_t)} \right)
\]

where \(r_t\) is the same random year effect as specified for adult survival in the state-space model above.

**Age-specific weaning**

We recorded summer lamb survival status (weaned a lamb/did not wean a lamb) during each year of monitoring for all known-age ewes. Sample sizes for recruitment from ewes in each class by disease status are shown in Table 2.1. We modeled recruitment for individual ewes similar to survival, so that for ewe \(i\) residing in
population-year \(j, t\), recruitment \((f_{i,j,t})\) was treated as a Bernoulli trial:

\[
f_{i,j,t} \sim \text{Bernoulli} \left( \frac{\exp[\omega_{a[i],d[j,t]} + c_t]}{1 + \exp[\omega_{a[i],d[j,t]} + c_t]} \right)
\]  

(B.15)

Throughout the IPM, we assume that survival and reproduction are independent, so the full likelihood for individual data \((L_I)\) is the production of the likelihoods for individual ewe survival \((L_{Is})\) and reproduction \((L_{If})\), and can be written:

\[
L_I = L_{Is}(s_{i,j,t}|\sigma, \tau^2_r) \times L_{If}(f_{i,j,t}|\omega, \tau^2_c)
\]  

(B.16)

We placed diffuse Gaussian priors \((N(0, 100))\) on all elements of \(\sigma\) and \(\omega\). Random effects were drawn from \(N(0, \tau^2_r)\) and \(N(0, \tau^2_c)\), respectively. \(\tau^2_r\) and \(\tau^2_c\) both had \(\text{Unif}(0, 10)\) hyperpriors.

In its entirety, the IPM estimates 26 parameters, 12 each for healthy and diseased years, and two random year effect variances for adult survival and reproduction. We fit a null demographic model with a random year effect, but no fixed effects for disease status to be sure that our inclusion of disease status was justified. Models were compared using DIC.

**B.3.2 IPM fitting and posterior estimates**

The IPM converged slowly relative to the rest of the models presented here.

In addition to the posterior estimates presented in the main text, we also examined posterior distributions of vital rate elasticities and projected age structures under each disease status. These are shown in Figure B.1.

**B.3.3 IPM Caveats**

While this analysis rests on a very rich disease dataset, our study is still limited by available age-specific survival and reproduction information, particularly for animals in the oldest and youngest age classes, and animals living in the absence of disease (see Table 2.1). The weaknesses associated with limited data are largely adjusted for through the Bayesian estimation approaches which hinges on uninformative priors. The posteriors in healthy years are much wider than in disease years, which directly reflects the larger sampling variation associated with the smaller healthy
sample sizes. Fortunately, the very limited data on individuals in the oldest age class has little bearing on our overall findings, since population projections were much less sensitive to vital rates in the oldest age groups than they were to lamb survival and survival of prime-aged ewes (elasticity of survival in the oldest age class was between 1.0 and 7% of overall adult survival elasticity in persistently infected years, and 0.05 and 1% of overall adult survival elasticity in healthy years).

### B.4 Population projection models

#### Projection matrix structure

We used a 19x19 age-structured Leslie matrix to link population vital rates in Disease-free, Disease Introduction, and Disease persistence years.

Leslie matrices were of this form:

\[
L = \begin{pmatrix}
0 & \phi_1 & \phi_2 & \phi_3 & \phi_4 & \phi_5 & \phi_6 & \phi_7 & \phi_8 & \phi_9 & \phi_{10} & \phi_{11} & \phi_{12} & \phi_{13} & \phi_{14} & \phi_{15} & \phi_{16} & \phi_{17} & \phi_{18} \\
1 & \eta & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
2 & 0 & \sigma_1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
3 & 0 & 0 & \sigma_1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
4 & 0 & 0 & 0 & \sigma_2 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
5 & 0 & 0 & 0 & 0 & \sigma_2 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
6 & 0 & 0 & 0 & 0 & 0 & \sigma_2 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
7 & 0 & 0 & 0 & 0 & 0 & 0 & \sigma_2 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
8 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \sigma_2 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
9 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \sigma_3 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
10 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \sigma_3 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
11 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \sigma_3 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
12 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \sigma_3 & 0 & 0 & 0 & 0 & 0 & 0 \\
13 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \sigma_3 & 0 & 0 & 0 & 0 & 0 \\
14 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \sigma_4 & 0 & 0 & 0 & 0 \\
15 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \sigma_4 & 0 & 0 & 0 \\
16 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \sigma_4 & 0 & 0 \\
17 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \sigma_4 & 0 \\
18 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \sigma_4 \\
\end{pmatrix}
\]

Births are pulsed (Caswell pg. 25), with the consequence that all individuals in an age-class assumed to be born on the same day. This is a post-breeding model (e.g., the fraction of the year that passes between reproduction and census is close to one; Caswell pg. 25). Typically in post-breeding models, survival for year one includes first year mortality (Caswell pg. 27). However, in our case, fecundity estimates include both summer and overwinter survival, therefore we take the probability of survival for the first age class to be one.

We examined the sensitivity of population growth rate (e.g., \( \lambda \), the dominant eigenvalue) to changes in vital rates. Let \( \mathbf{w}_1 \) and \( \mathbf{v}_1^* \) be right and left eigenvectors...
of the population projection matrix $L$, such that

$$Lw_i = \lambda_i w_i$$  \hspace{1cm} (B.18)

$$v_i^* L = \lambda_i v_i^*$$  \hspace{1cm} (B.19)

Here, $v_i^*$ is the complex conjugate transpose of $v_i$ (Caswell pg. 209). Then, for each vital rate, $a_{ij}$, the sensitivity of population growth to that vital rate is (Caswell pg. 209):

$$\frac{\partial \lambda}{\partial a_{ij}} = \frac{\bar{v}_i \omega_j}{\langle w, v \rangle}.$$  \hspace{1cm} (B.20)

### B.4.1 Markov model projection outputs: Impact of age structure

We explored the role of age-structured disease impacts by simulating disease that impacts all age classes homogeneously every year (i.e., every year looks like a spillover) vs. a model in which post-invasion year mortality followed the empirical vital rates from Hells Canyon, as shown in Figure 2.3 of the main text. Simulated outputs from this experiment are shown in Figure B.1 above.

### B.4.2 Population projection model caveats

Our simulation results assume that future conditions and vital rates will match those exhibited in the past. However, host vital rates in this system are likely to be under heavy selective pressure from disease, and traits that mitigate disease costs should increase in frequency. Additionally, closure of domestic grazing allotments over the last fifteen years has likely changed domestic/bighorn contact patterns in the Hells Canyon region, and by proxy, pathogen introduction rates. Our models predict that this reduction in spillover risk should shift population dynamics from a regime driven by all-age outbreaks (as shown in the extreme in the bottom row of Fig. 5 in the main text) to the more gradual declines or apparent stagnation associated with fewer disease introduction events. However, if pathogens persist locally within bighorn populations themselves – as is likely in Hells Canyon – then reservoir management alone may be insufficient to spur system-wide population
Figure B.1: Projected population sizes for 100 simulations of 60 years each under varying disease introduction and persistence patterns. Simulations in the left column are governed by the Markov transition matrix shown in equation (1). The initial disease introduction event occurs at year 30 in all simulations, prior to which populations undergo healthy growth. After introduction, populations experience persistent disease under the specified persistence period (1, 5, or 20 years). They are also subject to additional disease introduction events at rates specified on the left margin. In the center column, populations experience exactly the same number of health, disease invasion, and disease persistence years, but disease status is distributed randomly through time. In the right column, populations only experience disease introduction events without any disease persistence in subsequent years. Black lines highlight the 2.5th and 97.5th simulated population size quantiles at each timestep; red lines highlight the median simulated population size.
Figure B.2: Impact of post-invasion age-structure on population projections. Grey regions show projections under models with different disease impacts in disease introduction years and persistence years (grey) as compared to models that apply the same demographic rates to disease introduction and disease persistence years (red). As persistence declines, a higher proportion of diseased years are introduction years, driving the age-structured and homogenous impacts models toward convergence.

growth. An open research task is to identify the individual-level processes that allow for local pathogen persistence. This effort will hinge on understanding risk factors and mechanisms associated with chronic M.ovi carriage in some bighorn adults.
Figure B.3: Shaded regions show the middle 50% of simulated population sizes at each timestep. Simulated populations underwent continuous healthy growth until year 0, and experience their first disease event between years 0 and 1. Expected time between introduction events was five years, and expected time to recovery was 20 years in the temporally structured models. The cost of all-age disease on adult survival was a 30% reduction in all adult survival probabilities. (a) Simulated population growth trajectories under a model with die-offs only (blue); spillovers followed by persistent juvenile disease (grey); and spillovers followed by persistent all-age disease (red). (b) Simulated population trajectories under a model with temporally random age-structured disease (blue); temporal structure, age structured disease (grey); and temporally random all-age disease (red). Simulations were controlled so that the same number of disease years occurs in simulations under each set of disease conditions.
Table B.6: Posterior credible intervals for vital rates estimated in the integrated population model. Parameters denoted “σ” refer to ewe survival; parameters denoted “ω” refer to probability of weaning; parameters denoted “η” refer to probability a lamb survives overwinter, given it survived until weaning. Credible intervals are presented on the probability scale.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Posterior median</th>
<th>95% Posterior Credible Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>σ_{healthy,2–3}</td>
<td>.99</td>
<td>.98, .99</td>
</tr>
<tr>
<td>σ_{infected,2–3}</td>
<td>.99</td>
<td>.98, .99</td>
</tr>
<tr>
<td>σ_{healthy,4–7}</td>
<td>.90</td>
<td>.82, .95</td>
</tr>
<tr>
<td>σ_{infected,4–7}</td>
<td>.98</td>
<td>.95, .99</td>
</tr>
<tr>
<td>σ_{healthy,8–13}</td>
<td>.84</td>
<td>.71, .93</td>
</tr>
<tr>
<td>σ_{infected,8–13}</td>
<td>.92</td>
<td>.87, .96</td>
</tr>
<tr>
<td>σ_{healthy,14–20}</td>
<td>.85</td>
<td>.71, .94</td>
</tr>
<tr>
<td>σ_{infected,14–20}</td>
<td>.87</td>
<td>.65, .96</td>
</tr>
<tr>
<td>ω_{healthy,2–3}</td>
<td>.59</td>
<td>.25, .90</td>
</tr>
<tr>
<td>ω_{infected,2–3}</td>
<td>.22</td>
<td>.08, .47</td>
</tr>
<tr>
<td>ω_{healthy,4–7}</td>
<td>.80</td>
<td>.67, .90</td>
</tr>
<tr>
<td>ω_{infected,4–7}</td>
<td>.22</td>
<td>.15, .30</td>
</tr>
<tr>
<td>ω_{healthy,8–13}</td>
<td>.75</td>
<td>.59, .87</td>
</tr>
<tr>
<td>ω_{infected,8–13}</td>
<td>.29</td>
<td>.21, .37</td>
</tr>
<tr>
<td>ω_{healthy,14–20}</td>
<td>.54</td>
<td>.18, .88</td>
</tr>
<tr>
<td>ω_{infected,14–20}</td>
<td>.16</td>
<td>.07, .29</td>
</tr>
<tr>
<td>η_{healthy}</td>
<td>.58</td>
<td>.48, .71</td>
</tr>
<tr>
<td>η_{infected}</td>
<td>.70</td>
<td>.58, .84</td>
</tr>
</tbody>
</table>
Appendix C
Supplementary Information for Chapter 3: Costs and Benefits

C.1 Ewe social network construction and structures

Ewes were located at least biweekly on the ground or from a fixed wing aircraft, and locations were usually accompanied by visual observation. The median time lag between locations for an individual was six days in the cohorts used in this analysis (electronic supplementary material Table C.1). We defined “summer” as the period from May 01 to September 30. This window ranges from birth to weaning,

![Bar chart](image)

Figure C.1: Distribution of number of ewe-subpopulations (“components”) observed for each population included in this study.
Figure C.2: Variance components when ewe-group was excluded from the model. Note that variance previously attributed to ewe-group is now attributed to population-year ("Year") in diseased years.

and is the interval during which most disease-induced lamb mortality is detected [1]. We constructed a social contact network of radiocollared individuals for each summer by considering each marked ewe to be a node, and allowing an edge to exist between every pair of marked individuals. We calculated pairwise association indices between all marked animals in the population, and used these association indices as edgeweights linking pairs of marked ewes. This had the consequence of removing edges between pairs of animals that were never observed together. We then used these networks to identify the ewe-subpopulation (Figure 3.1c) to which each radiocollared ewe belonged. We defined ewe-subpopulations to be sets of individuals observed at least once together, but never observed with individuals from other subpopulations (i.e., network “components”; Figure 2.1c). This definition does not require ewes within a subpopulation to be located with all other ewes in their subpopulation, rather that they be located with at least one other member of their subpopulation at least once. Our weekly sampling intensity allows for the presence of some undetected bridges linking ewe-subpopulations to one another. However, the fact that these bridges were never observed suggests that they were subject to limited activity, lowering their potential impact on disease transmission. The entities that we refer to as “ewe-subpopulations” in the main body of the text are in fact K2-clusters (i.e., open triplets) in each population-summer’s ewe social networks.
C.2 Bootstrap tests for relationship between number of collars and network structure

We used bootstrap-based approach to test for relationships between number of radiocollared individuals and network structure. Bootstrap-based approaches were chosen since they do not make assumptions about the underlying distributional form of the data, and are therefore robust to departures from normality. To determine whether the number of ewe-subpopulations differed significantly between healthy and diseased years, we sampled with replacement from the full distribution of observed number of components across all population-years. Resampled values were bound to the population-years recorded in the order observed in empirical the dataset, to generate resampled blocks of numbers of components for each population. In each resampled block, we recorded the resampled number of components and the empirically observed population disease status. This comprised a resampled dataset. We then calculated the (resampled) mean number of observed components for each population, and calculated the sum of squared error residuals and sum of squared treatment (e.g., disease status) effects in each bootstrapped dataset. In this way, we created a bootstrapped distribution of sum of squared treatment effects divided by the sum of squared residuals. We replicated this process 1000 times to generate a bootstrapped distribution that we then used as a null distribution in which disease status is independent of number of ewe-subpopulations detected. The final step was to compare the empirically observed sum of squared treatment effects over sum of squared residuals to the null distribution, with the proportion of bootstrapped test statistics in excess of the empirically observed test statistic providing a bootstrapped p-value. We used an identical bootstrap approach to determine whether the number of ewe-subpopulations differed significantly between populations, except in this case treatments were taken to be populations.

C.3 Temporal autocorrelation in ewe-subpopulation membership

To measure stability of ewe-subpopulation membership across years, we followed the approach taken by Palla et al. [2]. First, we determined groups in consecutive years
that had the maximum group membership overlap. For two ewe-subpopulations, A in year \( t \) and B in year \( t + 1 \), we defined “overlap” as

\[
C(A, B) = \frac{A \cap B}{A \cup B} \tag{C.1}
\]

where \( A \cup B \) represents the ewes contained in either group A or B, and \( A \cap B \) is the ewes present in both groups A and B. A ewe-subpopulation A in year \( t \) was “matched” with a ewe-subpopulation B in year \( t + 1 \) when the overlap between subpopulations A and B was greater than the overlap between subpopulation A and any other subpopulation in year \( t + 1 \). We considered “matched” subpopulations to be essentially the same, with marginal changes through time. We quantified year-to-year subpopulation stability using an approximate autocorrelation \([2]\) defined as

\[
C(t) = \frac{|A(t_0) \cup A(t_0 + t)|}{|A(t_0) \cap A(t_0 + t)|} \tag{C.2}
\]

where \( |A(t_0) \cup A(t_0 + t)| \) is the number of ewes present in both subpopulation A at time \( t_0 \) and in ewe-subpopulation A at time \( A(t_0 + t) \) and \( |A(t_0) \cup A(t_0 + t)| \) is the set of all ewes present in either \( A(t_0) \), \( A(t_0 + t) \), or both \([2]\). High autocorrelation suggested consistent year-to-year ewe-subpopulation membership, whereas low autocorrelation suggests reshuffling of subpopulation membership between years.

All study populations displayed temporal autocorrelation in ewe-subpopulation membership across years (shaded region of Fig. S5), suggesting that ewe social bonds generally persist or are re-established consistently. Although autocorrelation decay rates over time varied among populations, all populations displayed the similar qualitative trend, suggesting that temporal patterns in subpopulation stability do not vary substantially between populations. Perceived changes in ewe-subpopulation membership occurred primarily when new individuals were collared or died, and occasionally when individuals switched ewe-subpopulations between years.
C.4 Multilevel Poisson Model

After identifying distinct ewe-subpopulations within each cohort, we examined relationships between number and size of ewe-subpopulations, the daily observed group size within which ewes occurred, and population size. We tested whether the observed number of ewe-subpopulations during the $L^{th}$ study year in the $m^{th}$ population ($\lambda_l$) depended on population size (as measured by total number of ewes counted in annual aerial surveys) with a hierarchical Poisson model containing a fixed effect for total ewes counted (“TotEwes”), and a random intercept for each population (“pop”). We included an overdispersion term, $\psi$, to capture extra-Poisson variation [97]. Formally, this model was

$$
\log(\lambda_l) = \beta_0 + \beta_1 \text{TotEwes}_{m[l]} + \text{pop}_{m[l]} + \psi_l 
$$

(C.3)

$$
\text{pop}_m \sim N(\mu_{\text{pop}}, \tau_{\text{pop}}) 
$$

(C.4)

In this model, our inferential focus was on the posterior density associated with the parameter $\beta_1$, which links total counted ewes to the observed number of ewe groups in the population.

C.5 Piecewise Regression

We fit a piecewise linear regression model to describe how the number of ewe-subpopulations related to median group size. Piecewise regression allows a process to abruptly change forms at some (model-estimated) point along a covariate axis. In this case, we fit median daily group sizes as a function of the number of subpopulations present in each population, and allowed for a possible changepoint in the relationship between daily group size and number of ewe-subpopulations. In this model, $\lambda_k$ represents the median daily group size observed for individuals in the $k^{th}$ subpopulation in the $j^{th}$ year. Let $\beta_1$ be the linear relationship between number of ewe-subpopulations (“NumSubPops”) and median daily group size prior to the changepoint; let $U$ be the changepoint, and let $\gamma$ be the adjustment to
Table C.1: Interval estimates for all piecewise regression model coefficients.

<table>
<thead>
<tr>
<th>Changepoint</th>
<th>Initial slope</th>
<th>Slope change</th>
<th>Second Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5%</td>
<td>1.541</td>
<td>-14.176</td>
<td>0.110</td>
</tr>
<tr>
<td>97.5%</td>
<td>7.111</td>
<td>-0.228</td>
<td>14.636</td>
</tr>
</tbody>
</table>

the relationship between number of groups and group size for numbers of groups exceeding the changepoint. Then the changepoint model is

\[
\log(\lambda_k) = \beta_0 + \beta_1 \text{NumSubPops}_{j[k]} + \\
\gamma(\text{NumSupPops}_{j[k]} - U) \times \text{step}(\text{NumSupPops}_{j[k]} - u) \tag{C.5}
\]

### C.6 Group size by population size

We tested whether the median observed ewe group size during the \(l^{th}\) study year in the \(m^{th}\) population (\(\mu_i\)) depended on population size (as measured by total number of ewes counted in annual aerial surveys) with a hierarchical linear model containing a fixed effect for total ewes counted ("TotEwes"), and a random intercept for each population ("pop"). Formally, this model was

\[
\mu_i = \beta_0 + \beta_1 \text{TotEwes}_{m[j]} + \text{pop}_{m[j]} \tag{C.6}
\]

\[
\text{pop}_{m} \sim N(\mu_{pop}, \tau_{pop}) \tag{C.7}
\]

In this model, our inferential focus was on estimates associated with the \(\beta_1\) parameter, which links total counted ewes to the observed median group size during that population-summer.

### C.7 Variance Decomposition

To determine an organizational level of transmission most consistent with observed lamb mortality patterns in Hells Canyon, we decomposed the variance in the proportion of lambs that survived through September 30th. We compared variation in lamb mortality outcomes at four organizational levels: populations, years within
populations ("years"), ewe-subpopulations, and ewes. We took years to be nested in populations because disease severity was not temporally synchronized across all Hells Canyon populations. A nested structure allowed different populations to experience different disease statuses in the same year. Comparisons were based on a variance decomposition performed using a multilevel logistic regression model with random effects for ewes, ewe-subpopulations, years, and populations. Ewe effects were estimated across all years during which a ewe reproduced, regardless of that year’s disease status. For the $i^{th}$ lamb born to the $j^{th}$ ewe in the $k^{th}$ ewe-subpopulation during the $l^{th}$ year in the $m^{th}$ population, this corresponded to the following multilevel model:

$$ \log \left( \frac{p_i}{1 - p_i} \right) = \text{group}_{k[l]} + \text{ewe}_{j[k]} $$  \hfill (C.8)

$$ \text{group}_{k} \sim N(\text{year}_{l[k]}, \tau_{\text{group},PN} \times I(\text{PNStatus})_k + \tau_{\text{group,Healthy}} \times (1 - I(\text{PNStatus})_k)) \hfill (C.9)$$

$$ \text{year}_{l} \sim N(\text{pop}_{m[l]} + \delta I(\text{PNStatus})_l, \tau_{\text{year},PN} \times I(\text{PNStatus})_l + \tau_{\text{year,Healthy}} \times (1 - I(\text{PNStatus})_l)) \hfill (C.10)$$

$$ \text{ewe}_{j} \sim N(0, \tau_{\text{ewe}}) \hfill (C.11)$$

$I(\text{PNStatus})$ terms take on the value 0 for years classified as healthy, and 1 otherwise. In general, these indicator terms control the variance estimate to which each observation contributes. Indicator terms were generated separately for each level of the model, but always retain the same meaning: any ewe-group present in a year classified as having pneumonia is assigned an $I(\text{PNStatus})$ term of 1, as is any year classified as having pneumonia.

For our purposes, the critical attributes of this model are the precision pa-
rameters, $\tau_{\text{group,Healthy}}$, $\tau_{\text{group,PN}}$, $\tau_{\text{year,Healthy}}$, $\tau_{\text{year,PN}}$, and $\tau_{\text{ewe}}$, which we inverted to variances following model fitting. Our focus was on the relative size of each component. High variance at a particular level indicated that the proportion of lambs surviving differed between observations at that level, whereas low variance meant that lamb survival proportions were similar for all units at that level (e.g., Figure 3.1d). For example, high variance at the ewe-group level meant that lamb survival in some ewe groups was very different than in other groups, whereas low variance suggested all groups experienced similar within-group mortality rates.
Table D.1: Fits to logistic growth curve in degree accumulation for dams, dry ewes, and yearlings.

<table>
<thead>
<tr>
<th>Demographic group</th>
<th>Parameter</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dams</td>
<td>Asymptote</td>
<td>0.943</td>
<td>0.014</td>
<td>≤ 0.0001</td>
</tr>
<tr>
<td></td>
<td>Xmin</td>
<td>137.395</td>
<td>0.847</td>
<td>≤ 0.0001</td>
</tr>
<tr>
<td></td>
<td>Scal</td>
<td>23.695</td>
<td>0.908</td>
<td>≤ 0.0001</td>
</tr>
<tr>
<td>Dry Ewes</td>
<td>Asymptote</td>
<td>1.00</td>
<td>0.077</td>
<td>≤ 0.0001</td>
</tr>
<tr>
<td></td>
<td>Xmin</td>
<td>146.173</td>
<td>5.129</td>
<td>≤ 0.0001</td>
</tr>
<tr>
<td></td>
<td>Scal</td>
<td>29.307</td>
<td>3.859</td>
<td>≤ 0.0001</td>
</tr>
<tr>
<td>Yearlings</td>
<td>Asymptote</td>
<td>2.421</td>
<td>4.103</td>
<td>0.055</td>
</tr>
<tr>
<td></td>
<td>Xmin</td>
<td>268.408</td>
<td>256.554</td>
<td>0.296</td>
</tr>
<tr>
<td></td>
<td>Scal</td>
<td>90.337</td>
<td>46.858</td>
<td>0.054</td>
</tr>
</tbody>
</table>
Figure D.1: Pairwise correlations between predictors in the lamb survival model. Gold points indicate lambs born in Black Butte; green points indicate lambs born in Mountain View; blue points indicate lambs born in Asotin. Solid circles indicate covariate data for lambs born to 2-year-old dams.

Table D.2: Generalized additive model fits for cumulative node strength in each demographic groups. Each model contains population-year specific fixed effect terms, plus a smoother over time.

<table>
<thead>
<tr>
<th>Demographic group</th>
<th>EDF</th>
<th>F (p-value)</th>
<th>Deviance explained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reproductive ewes</td>
<td>8.33</td>
<td>8.87</td>
<td>201 (≤ 0.0001)</td>
</tr>
<tr>
<td>Dry ewes</td>
<td>7.74</td>
<td>8.58</td>
<td>74.2 (≤ 0.0001)</td>
</tr>
<tr>
<td>Yearlings</td>
<td>6.08</td>
<td>7.24</td>
<td>55.91 (≤ 0.0001)</td>
</tr>
</tbody>
</table>
Figure D.2: Relationship between interaction rate and eigencentrality.

Figure D.3: Node strength and degree for each population-year. Individual degrees and node strengths are relatively consistent within population-years. Note that these degree distributions show no power-law (or even linearly declining) trends in frequency with increasing degree.
Table D.3: Contact rate estimates for dyads of various demographic groups. “Dam” here refers to Reproductive ewes in general.

<table>
<thead>
<tr>
<th>Dyad type</th>
<th>Tot contact events</th>
<th>Tot minutes observed</th>
<th>Tot contact duration</th>
<th>Avg. contact duration</th>
<th>Contacts per day</th>
<th>Contact duration per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dam-dam</td>
<td>43</td>
<td>135330.1</td>
<td>106</td>
<td>2.47</td>
<td>0.45</td>
<td>1.11</td>
</tr>
<tr>
<td>Dam-dry</td>
<td>18</td>
<td>69998.35</td>
<td>54</td>
<td>3</td>
<td>0.37</td>
<td>1.11</td>
</tr>
<tr>
<td>Dam-lamb</td>
<td>635 (her lamb) + 796 (unknown lamb)</td>
<td>132494</td>
<td>75440</td>
<td>52.72</td>
<td>15.55</td>
<td>819.8</td>
</tr>
<tr>
<td>Dam-yr</td>
<td>29</td>
<td>41077</td>
<td>171</td>
<td>5.9</td>
<td>1.02</td>
<td>6.02</td>
</tr>
<tr>
<td>Dry-lamb</td>
<td>334 (her lamb) + 112 (unknown lamb)</td>
<td>34266</td>
<td>15185</td>
<td>34.05</td>
<td>18.74</td>
<td>638.1</td>
</tr>
<tr>
<td>Dry-yr</td>
<td>13</td>
<td>10623</td>
<td>34</td>
<td>2.61</td>
<td>1.76</td>
<td>4.59</td>
</tr>
<tr>
<td>Lamb-lamb</td>
<td>1086</td>
<td>126860</td>
<td>3788</td>
<td>3.49</td>
<td>12.33</td>
<td>40.57</td>
</tr>
<tr>
<td>Lamb-yr</td>
<td>17</td>
<td>26490</td>
<td>42</td>
<td>2.47</td>
<td>0.92</td>
<td>2.27</td>
</tr>
<tr>
<td>Yr-yr</td>
<td>0</td>
<td>26340</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Appendix E
Supplementary Information for Chapter 6: “One Health” or Three?

E.1 Search Methods

Search terms (Table S1) were initially applied to the 25 best-ranked journals in each designated category (Table S2) in the Scientific Journal Rankings from 2013 as determined by SciImago (http://www.scimagojr.com/journalrank.php?country=US). The initial search (run Nov. 4, 2014) returned 1651 papers, which we used to build a database consisting of citations from those 1651 papers. We augmented our journal list to include any journal excluded from the original journal list, but referenced more than 250 times by papers selected under the initial search (added journals listed in Table S3). This augmentation allowed us to incorporate lower-impact journals that still publish extensively on infectious disease transmission.

We reran the original search on the augmented journal list to achieve our final paper list on Nov. 4, 2014. This search returned 2258 papers. A single reader read abstracts of all returned papers and excluded papers with no direct or indirect reference to disease transmission in the title or abstract. A second reader read abstracts from all papers excluded by the first reader to confirm exclusion. Following abstract review, the paper list was reduced to 1605 papers, which formed our paper bank and the basis for the descriptions below.
Table E.1: Search terms, term content, and term objectives.

<table>
<thead>
<tr>
<th>Name</th>
<th>Content</th>
<th>Objective</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>(disease AND propogat*) OR (disease AND persist*) OR (disease AND infect*) OR (disease AND transmi*) OR (disease AND force of infect*) OR (disease AND R0) OR (disease AND R-naught) OR (disease AND R-0)</td>
<td>Capture references to disease transmission</td>
</tr>
<tr>
<td>T2</td>
<td>AND (dynam* OR simulat*)</td>
<td>Limit to papers emphasizing dynamic process</td>
</tr>
<tr>
<td>T3</td>
<td>AND (mechanis* OR stochastic* OR determinis* OR compartment* OR sensitivit* OR statistic* OR estimat*)</td>
<td>Limit to papers emphasizing dynamic process</td>
</tr>
<tr>
<td>T4</td>
<td>NOT (mouse model OR protease OR drug-drug interaction OR neutraliz* OR inhibitor* OR unfold* OR variant OR homolog* OR &quot;in vivo&quot; OR &quot;in vitro&quot; OR CD4 OR deep sequenc* OR enzyme OR &quot;infected cell&quot;)</td>
<td>Eliminate within-host models</td>
</tr>
<tr>
<td>T5</td>
<td>NOT (HIV OR AIDS OR human immunodeficiency virus)</td>
<td>Eliminate HIV/AIDS models</td>
</tr>
</tbody>
</table>
Table E.2: SciImago journal categories included.

<table>
<thead>
<tr>
<th>SciMago General Category</th>
<th>Subcategory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agricultural and Biological Sciences</td>
<td>Animal Science and Zoology</td>
</tr>
<tr>
<td></td>
<td>EEBS</td>
</tr>
<tr>
<td></td>
<td>Agricultural and Biological Sciences Miscellaneous</td>
</tr>
<tr>
<td>Environmental Science</td>
<td>Ecological Modeling</td>
</tr>
<tr>
<td></td>
<td>Ecology</td>
</tr>
<tr>
<td></td>
<td>Nature and Landscape Conservation</td>
</tr>
<tr>
<td>Medicine</td>
<td>Epidemiology</td>
</tr>
<tr>
<td></td>
<td>Infectious Disease</td>
</tr>
<tr>
<td></td>
<td>All Categories</td>
</tr>
</tbody>
</table>

Table E.3: List of journals added to initial search because they were cited 250 or more times by papers returned in the initial search of highly ranked journals.

<table>
<thead>
<tr>
<th>Journal Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>American Journal of Human Genetics</td>
</tr>
<tr>
<td>American Journal of Tropical Medicine and Hygiene</td>
</tr>
<tr>
<td>Epidemiology and Infection</td>
</tr>
<tr>
<td>Evolution</td>
</tr>
<tr>
<td>Genetics</td>
</tr>
<tr>
<td>Journal of Clinical Microbiology</td>
</tr>
<tr>
<td>Journal of the Royal Society Interface</td>
</tr>
<tr>
<td>Journal of Theoretical Biology</td>
</tr>
<tr>
<td>Journal of Virology</td>
</tr>
<tr>
<td>Journal of Wildlife Diseases</td>
</tr>
<tr>
<td>Mathematical Biosciences</td>
</tr>
<tr>
<td>New England Journal of Medicine</td>
</tr>
<tr>
<td>Phytopathology</td>
</tr>
<tr>
<td>PLoS Medicine</td>
</tr>
<tr>
<td>Theoretical population biology</td>
</tr>
<tr>
<td>Transactions of the Royal Society of Tropical Medicine and Hygiene</td>
</tr>
<tr>
<td>Veterinary Record</td>
</tr>
</tbody>
</table>
Table E.4: Number of papers from our search contained in the three dominant journal communities during three temporal blocks.

<table>
<thead>
<tr>
<th></th>
<th>Biological journals</th>
<th>Ecological journals</th>
<th>Vet journals</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998-2002</td>
<td>81</td>
<td>40</td>
<td>23</td>
<td>144</td>
</tr>
<tr>
<td>2003-2007</td>
<td>188</td>
<td>67</td>
<td>44</td>
<td>299</td>
</tr>
<tr>
<td>2008-2012</td>
<td>453</td>
<td>118</td>
<td>81</td>
<td>652</td>
</tr>
<tr>
<td>Total</td>
<td>1043</td>
<td>310</td>
<td>198</td>
<td></td>
</tr>
</tbody>
</table>
Table E.5: Metrics for coauthor, paper, and journal networks. Nodes are the number of entities in the network and edges represent coauthors (Authors) cited papers (Papers) and citations between journals (Journals). “Undirected” networks have symmetric edges linking nodes (e.g., network has an identical strength connecting node A to B and node B to A). Size of the largest component is measured in number of nodes. Isolated nodes are completely unconnected from the rest of the network. Mean degree is the average number of edges connected to each node, diameter is the maximum distance between any pair of connected nodes, and mean path length is the average minimum distance between any pair of connected nodes.

<table>
<thead>
<tr>
<th>Network</th>
<th>Number nodes/edges</th>
<th>Directed/undirected</th>
<th>Size largest component (2nd largest)</th>
<th>Mean degree</th>
<th>Diameter</th>
<th>Mean path length</th>
</tr>
</thead>
</table>

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Table E.6: Journal community attributes associated with the 1551 papers in journals from the three major journal communities. “Directed” networks are those in which edges need not be symmetric in weight (i.e., one paper can cite another without the reciprocal occurring, so the paper network is directed; co-authorship roles, however, are reciprocal, so the author network is undirected). Lead author affiliation was designated based on author institution as listed in the paper bank metadata. Total and percent citations from papers in each community to papers in the other communities are shown in the final three columns. Within-community citations are bolded.

<table>
<thead>
<tr>
<th>Community</th>
<th>Number journals</th>
<th>Number papers</th>
<th>Median number authors (2.5th, 97.5th)</th>
<th>Most common lead author affiliation</th>
<th>Citations to human-focused epi</th>
<th>Citations to ecology</th>
<th>Citations to vet</th>
</tr>
</thead>
</table>


E.2 Network construction and descriptions

E.2.1 Journals

Journals identified by journal name Nodes = journals that published papers included in search; Edges link journals whose papers cite one another (asymmetric edges weighted by frequency of citations from Journal A of papers in Journal B)

E.2.2 Papers

Papers identified by DOI (when available), or title and year published (when DOI not available) Nodes = all papers included in reduced paper bank; Edges link papers that cite each other in search (directed, binary edges – in only instance did two papers reciprocally cite each other).

E.2.3 Authors

Authors identified by last name + first initial Nodes = all authors who wrote papers included in the reduced paper bank Edges link authors who coauthored a manuscript (symmetric and weighted by frequency of coauthorship)

E.3 Supplementary Methods Text

The search was limited to the 25 highest-ranked journals identified by SciMago (1) in nine different subdisciplines related to infectious diseases (listed in Table S2). We extracted all references from papers captured in a first-round search of Web of Science, and expanded the search to include any additional journals that were cited more than 250 times in the preliminary search, but that were not ranked in the top 25 of their particular subdisciplines (see added journals in Table S3). We reran the search on this expanded journal list in Web of Science. A primary reader (KM) read abstracts from all papers in the initial complete paper list. Papers that did not directly reference disease transmission in the abstract or title were eliminated. A second reader (JGW) reviewed all eliminated abstracts to confirm that elimination was appropriate. This list of papers (n = 1628) constituted our paper bank.
We extracted Digital Object Identifiers (DOIs), lead author surname and first initial, and year of publication for each reference cited by each paper in our paper bank. Then, we searched the paper bank for papers that matched cited DOIs (when available) or lead author/year combinations. When matches were discovered, we assigned a directed edge linking the citing paper to the paper cited. Next, we built a coauthorship network linking all authors (nodes) in the paper bank along edges defined by coauthorship. A third network of journal communities described citations (edges) between the journals (nodes) included in the paper bank.

E.3.1 Network topology

The journal and paper networks were directed, so relationships between nodes were not usually symmetric (for example, Paper A can cite Paper B without B citing A); the author network had symmetric edges. The author and journal networks both had weighted edges reflecting the frequency of coauthorship or citations, respectively, but edges in the paper network were binary.

We first identified (weakly) connected components, sets of nodes that can be accessed from one another, in each network. We also characterized each network in terms of its average degree (the mean number of edges attached to each node), diameter (the length of the longest non-looping path between connected nodes), and average path length (the mean number of steps separating any pair of nodes in the network). Average degree describes how many connections each node has on average. Diameter reflects how compactly a network is structured. Low values mean that all nodes are closely tied to one another. Average path length describes the average number of steps separating a pair of nodes. All network analyses were conducted using the igraph package (2) in R (3).

E.3.2 Author affiliations and research discipline diversity

For authors that contributed multiple papers to the paper bank or had multiple affiliations, we relied on the oldest affiliation or first-listed, which we presumed best-reflected the author’s primary training. Lead author affiliations and corresponding discipline assignments are included in Supplementary Dataset 1. We also used paper metadata to assign disciplines to each co-author. Specifically, we conducted a text search of author affiliations for the following strings: “stat”, “math”, “eco”, “evo”,

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“biol”, “vet”, “med”, and “epi”. We reviewed all lead-author affiliations, and added the following domain categories: animal science/animal health; health informatics; governmental domains; other academic research disciplines (anthropology, chemistry, economics, psychology, sociology); and pharmacy. Authors with multiple domain affiliations were counted under each identifiable discipline, and papers whose lead author affiliation could not be clearly assigned to a particular discipline were excluded from author-affiliation analyses (true for 58 of the 1628 papers, or 3.5%).

**E.3.3 Diversity models**

We measured author disciplinary diversity within particular papers, and lead author disciplinary diversity within journal communities using Shannon’s diversity index, $H'$, as implemented through the diversity function in the R package vegan (4). Shannon’s diversity was used to measure author diversity in two ways. First, we measured author diversity on particular papers through time, and second, we measured lead author diversity within each journal community through time. Shannon’s diversity is calculated as follows. If a group has I distinct categories, each represented at proportion $p_i$, then $H' = \sum_{i=1}^{I} p_i \log(p_i)$. We distinguished between lead-author diversity within a journal community, $H'_{Li}$, and diversity among co-authors on each paper, $H'_{Ci}$. We examined patterns between each measure of author diversity and number of authors on the paper (for $H'_{Ci}$) or papers in the community (for $H'_{Li}$) to determine whether trends were driven by increasing numbers of authors or numbers of papers through time (Fig. S5).

We used AIC to compare models that allowed different year-diversity relationships and different baseline diversities for each community to models that assumed common baselines and year-diversity relationships. The second model characterized how citations between journal communities and citations within journal communities vary between communities and over time. In both models, the primary predictor, year of publication, was incorporated through smoothing splines. In each case, the model included an additive shift due to membership of individual $i$ in the $j^{th}$ community ($\alpha_{ij}^{[i]}$), a spline function describing changes over years ($s_1$), and a spline term capturing interaction between year and community ($s_2$), where community is denoted by indicator variables $Comm_1$, $Comm_2$, and $Comm_3$. In full, the author diversity of the $i^{th}$ observation ($H'_{Li}$ and $H'_{Ci}$) within journal community
\( j \) was modeled as

\[
H'_i = \alpha_{j[i]} + s_1(Year_i) + s_2(Year_i) \times Comm_{j[i]} + \epsilon_i
\]

where \( \epsilon_i \sim N(0, \sigma^2) \). We compared models that fit separate splines for each journal community to a model that only allowed community intercepts to vary. Models were fit in the \texttt{mgcv} package in R.

### E.3.4 Citation rate model

We identified factors leading to high citation rates by modeling the total number of times each of the 1628 papers in the full paper bank was cited (denoted \( Y_i \), such that \( Y_i \sim \text{Poisson}(\lambda_i) \)), as a function of that paper’s within-paper authorship diversity (\( H'_C \)), citation diversity (\( R \), the ratio of between-community citations to total citations), and publication year (\( Year \)). The model allowed for community-specific intercepts (\( \alpha_{j[i]} \)), slopes associated with author diversity (\( \delta_{j[i]} \)), and slopes associated with citation diversity (\( \phi_{j[i]} \)). The model had a log-link and an offset term for years since publication.

\[
\lambda_i = \exp \left( \alpha_{j[i]} + \beta Year_i + \delta_{j[i]} H'_C + \phi_{j[i]} R_i + \epsilon_i \right)
\]

Models were fitted using the \texttt{glm} function from R’s \texttt{stats} package.

### E.3.5 Community identification and stratified sampling

Strata were defined by journal community, annual paper citation rate (lowest 20% within community, 40th to 60th percentile within community, highest 20% within community), and year of publication in detail. We binned year of publication into three four-year blocks (1998-2002; 2003-2007; 2008-2012). Years were chosen to capture long-term trends in collaboration, while still incorporating/including a sufficient number of papers within each journal community. See Table S4 for the number of papers in each stratum. A team of 10 readers read 20 articles each within these nine strata and extracted information on 25 different variables (listed in Table S5). When extracted information was ambiguous, papers were read and independently scored by a second reader from the team. To compare inter-reader variation, a pre-selected subsample of 35 papers was read by
Table E.7: Publication growth rate model output.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate</th>
<th>Standard error</th>
<th>t-value (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1.790</td>
<td>0.134</td>
<td>13.38 (&lt;0.0001)</td>
</tr>
<tr>
<td>Year</td>
<td>0.146</td>
<td>0.008</td>
<td>1.31 (p&lt;0.0001)</td>
</tr>
</tbody>
</table>

two readers. We estimated binomial confidence intervals and used them to compare features of the literature from the three journal communities through time.

Finally, we identified highly cross-disciplinary journals in dynamic modeling of infectious disease by comparing the composition of lead author affiliations across all papers in our paper bank to the composition of lead author affiliations within paper bank papers from each particular journal. We calculated the deviance of lead author affiliations within each journal from the overall distribution of lead authors to identify journals whose author affiliation frequencies reflected frequencies over the entire paper bank.

E.4 Specific effect sizes from Figure 4

Effect of increasing author diversity from 0 to 1 in the ecology community was \( \exp(\beta) = 1.64; \ SE = 0.27 \). The same effect in the human-focused epidemiology community was \( \exp(\beta) = 1.08; \ SE = 0.05 \); and in the veterinary community it was \( \exp(\beta) = 1.26; \ SE = 0.08 \). All three effects were highly significant (\( p < 0.001 \) in all cases). The effect of increasing citations from entirely internal to entirely external in the ecology community was \( \exp(\beta) = 1.03; \ SE = 0.03; \ p = 0.371 \); in the veterinary community it was \( \exp(\beta) = 0.654; \ SE = 0.10; \ p\text{-value of interaction term} < 0.001 \); in Group 3, this effect was \( \exp(\beta) = 0.95; \ SE = 0.07; \ p\text{-value of interaction term} = 0.072 \).
Figure E.1: Connected journals from the journal network, color-coded by journal community membership. Journals assigned by the walk-trap algorithm to the ecology community are in gold ("Ecol"); journals in the veterinary community are in blue ("Vet"); journals in "Group 3" are in red; and green nodes reflect outlying journals that were not explicitly assigned to any one community.
Figure E.2: Journal community structure. (a) Within- and between-journal-community citation frequencies. (b) Edge width scales with the number of cross-community citations. Node size scales with the number of papers from each community included in our paper bank (ranging from 198 in the veterinary community to 1043 in Group 3).

Figure E.3: Comparison of how communities test and evaluate models based on the sample of 236 read papers. (a) Data incorporation by community. (b) Incorporation of new data in modeling papers by community. (c) Assessment of model fit by community. Error bars show 95% binomial confidence limits.
Figure E.4: Modeling approach comparison across communities. (a) Stochastic or deterministic modeling approaches; (b) Papers incorporating simulation; (c) Papers incorporating sensitivity analyses; (d) Papers incorporating other methods of mathematical evaluation including mathematical proof, equilibrium analyses, derivation of new theoretical relationships, asymptotic conditions, etc. Error bars depict 95% binomial confidence bounds.

Figure E.5: Number of papers and journals returned by our search through time. The left panel shows papers and journals summed across all journal communities; the right panel shows papers and journals within each community through time.
Figure E.6: (a) Author domain diversity (measured using Shannon’s diversity index, $H'$) within papers through time in each community. (b) Proportion of papers with author diversity equal to zero through time in each journal community.

Table E.8: Model output for parametric terms from the within-paper author diversity GAM.

<table>
<thead>
<tr>
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<th>t-value (p)</th>
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<td>Veterinary</td>
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<td>0.043</td>
<td>-0.505 (0.614)</td>
</tr>
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</table>
Appendix F
Spatio-temporal dynamics of pneumonia in bighorn sheep
Spatio-temporal dynamics of pneumonia in bighorn sheep

E. Frances Cassirer¹*, Raina K. Plowright², Kezia R. Manlove², Paul C. Cross³, Andrew P. Dobson⁴, Kathleen A. Potter⁵ and Peter J. Hudson²

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Summary

1. Bighorn sheep mortality related to pneumonia is a primary factor limiting population recovery across western North America, but management has been constrained by an incomplete understanding of the disease. We analysed patterns of pneumonia-caused mortality over 14 years in 16 interconnected bighorn sheep populations to gain insights into underlying disease processes.

2. We observed four age-structured classes of annual pneumonia mortality patterns: all-age, lamb-only, secondary all-age and adult-only. Although there was considerable variability within classes, overall they differed in persistence within and impact on populations. Years with pneumonia-induced mortality occurring simultaneously across age classes (i.e. all-age) appeared to be a consequence of pathogen invasion into a naïve population and resulted in immediate population declines. Subsequently, low recruitment due to frequent high mortality outbreaks in lambs, probably due to association with chronically infected ewes, posed a significant obstacle to population recovery. Secondary all-age events occurred in previously exposed populations when outbreaks in lambs were followed by lower rates of pneumonia-induced mortality in adults. Infrequent pneumonia events restricted to adults were usually of short duration with low mortality.

3. Acute pneumonia-induced mortality in adults was concentrated in fall and early winter around the breeding season when rams are more mobile and the sexes commingle. In contrast, mortality restricted to lambs peaked in summer when ewes and lambs were concentrated in nursery groups.

4. We detected weak synchrony in adult pneumonia between adjacent populations, but found no evidence for landscape-scale extrinsic variables as drivers of disease.

5. We demonstrate that there was a >60% probability of a disease event each year following pneumonia invasion into bighorn sheep populations. Healthy years also occurred periodically, and understanding the factors driving these apparent fade-out events may be the key to managing this disease. Our data and modelling indicate that pneumonia can have greater impacts on bighorn sheep populations than previously reported, and we present hypotheses about processes involved for testing in future investigations and management.

Key-words: bacterial pneumonia, livestock-wildlife interface, Markov model, time series

Introduction

Over the past 20 years, considerable advances have been made in understanding the spatio-temporal patterns of disease persistence and fade-out following invasion into susceptible host populations. Infections that generate
rapid mortality such as Ebola virus, burn through susceptible populations until there are no more hosts and effectively die out (Sanchez et al. 2001). Infections with a strong immunizing effect, such as measles in England and Wales, persist in populations and exhibit biannual epidemic peaks that coincide with the birth and aggregation of sufficient susceptibles (Bjørnstad & Grenfell 2008). The dynamics of strong immunizing or fatal infections can leave a distinct spatio-temporal signature, although an infection that results in predictable disease in one instance, may appear almost chaotic in another setting; for example, contrast the dynamics of measles in the UK and Niger (Ferrari et al. 2008). Describing these spatio-temporal patterns can reveal underlying processes and this approach can be especially important in understanding infections that have recently invaded a population where the transmission routes or aetiological agents are not clear (Cleaveland et al. 2007). In this article, we examine the spatio-temporal dynamics of pneumonia in bighorn sheep, where the disease has been described for at least 80 years (Rush 1927), but debate continues about the identities and roles of causal agents, and disease remains an important factor limiting recovery of populations.

Bighorn sheep (Ovis canadensis) are social, sexually dimorphic ungulates. The species commonly occurs in spatially structured, demographically independent, interconnected populations in steep, rugged terrain. Males and females pursue different life-history strategies (Bleich et al. 1996; Rubin, Boyce & Caswell-Chen 2002). Interactions between the sexes are concentrated around the breeding season which is relatively short in northern latitudes and high altitudes (Bunnell 1982; Thompson & Turner 1982; Bleich, Bowyer & Wehausen 1997; Valdez & Krausman 1999). Seasonal breeding also governs contact patterns between age classes, and each year a pulse of neonates is reared in female-juvenile nursery groups. Outside the breeding season, mature males and females generally occur in male-only, female-only or female-offspring associations. Males are more mobile and more likely than females to contact conspecific hosts in adjacent populations, or potential disease reservoirs such as domestic sheep (Bleich, Bowyer & Wehausen 1997; Rubin et al. 1998; DeCesare & Pletscher 2006).

Pneumonia is a significant factor limiting the distribution and abundance of bighorn sheep (Gross, Singer & Moses 2000; Cassirer & Sinclair 2007; Boyce et al. 2011). The disease is associated with infection by directly transmitted bacteria, principally thought to be Mycoplasma ovipneumoniae and Mannheimia haemolytica, but, as is often the case with the pneumonia, the precise aetiology remains unclear (Foreyt, Snipes & Kasten 1994; Beser et al. 2008, 2012b; Dassanayake et al. 2009, 2010). Initially, infection probably originates in domestic sheep, but once it has spilled over into bighorn sheep populations it is most likely maintained in the population and spread by bighorn sheep. Bighorn sheep appear highly susceptible to infection from domestic sheep: nearly all (98%) of a total of 90 bighorn sheep that were co-pastured with domestic sheep in 11 experimental commingling studies conducted between 1979 and 2009 died of pneumonia within 100 days, while the domestic sheep remained healthy (summarized in Beser et al. (2012a)). Although these captive experimental results support field observations by naturalists and field biologists (Grinnell 1928; Shillenger 1937; Goodson 1982; George et al. 2008), they do not replicate the range of demographic variation in pneumonia events observed under natural conditions. Pneumonia described in free-ranging bighorn sheep populations includes acute die-offs with wide ranges in all-age mortality (10–90%), chronic or sporadic low levels of adult mortality, and annual or sporadic epizootics with high mortality rates restricted to juveniles from 1 to many (>20) years following all-age outbreaks (Rush 1927; Jorgenson et al. 1997; Aune et al. 1998; Enk, Picton & Williams 2001; Hnilicka et al. 2002). The aim of this paper was to use empirical data to describe these mortality patterns in detail and to develop hypotheses about the underlying processes involved. Indeed, a lack of data has so far constrained models of pneumonia dynamics in bighorn sheep (Hobbs & Miller 1992; Gross, Singer & Moses 2000; Clifford et al. 2009; Cahn et al. 2011). Our objective was to develop an understanding of the disease that will ultimately aid in identifying and assessing intervention options.

Materials and methods

STUDY AREA

We studied bighorn sheep in a 22 732 km² area encompassing Hells Canyon of the Snake River in the Blue Mountain and Columbia Plateau ecoregions of Idaho, Oregon and Washington (−117.875°, 46.500° to −116.250°, 44.750°; Fig. 1). Bighorn sheep occupy three climate zones within this diverse area from lowest to highest elevation: Snake River, Blue Mountains and Wallowa Mountains. The low elevation Snake River canyon is warm and dry with temperatures averaging 17.6 °C at Lewiston, ID. Average annual precipitation of 31.4 cm occurs fairly evenly year-round except during the months of July and August. The adjacent uplands including the Blue Mountains in Washington, are cooler and wetter with average temperatures of 10 °C in Pomeroy, Washington (WA) and average annual precipitation of 61 cm at Asotin, WA and 66 cm in Pomeroy. The upper elevations in the Wallowa and Seven Devils mountains receive annual precipitation of up to 205 cm, over two-thirds of which occurs as snow. Temperature averages 7 °C at the base of the Wallowa Mountains in Enterprise, OR and annual precipitation averages 76 cm. Seasonal temperature patterns in all three climate zones are similar, with highs in July and August and lows in December and January (Johnson & Simon 1987; Western 2008).

Bighorn sheep are native to Hells Canyon, but were extirpated by 1945, probably through a combination of unregulated hunting, competition with livestock for forage and diseases introduced from domestic sheep (Smith 1954; Johnson 1980; Coggins & Matthews 1996). From 1971 to 1995, wildlife agencies in Idaho, Oregon and Washington translocated a total
of 329 bighorn sheep into Hells Canyon and moved 79 within
the metapopulation, establishing 12 interconnected...

Fig. 1. Distribution of the 16 bighorn sheep populations in the
Hells Canyon metapopulation, Idaho, Oregon and Washington.

of 329 bighorn sheep into Hells Canyon and moved 79 within
the metapopulation, establishing 12 interconnected populations
prior to our study (Figure S1). Another four populations were
established and one population supplemented with transloca-
tions 1997–2005, during our study. Populations were delineated
by movement patterns of females (Rubin et al. 1998). Females
rarely move between populations whereas males may move
seasonally or disperse among populations. Periodic pneumonia
outbreaks were documented prior to this study, although
monitoring was sporadic and most pneumonia events were doc-
umented following reports of sick and dying sheep. Over the
same time period, domestic sheep grazing declined dramatically.
However, reduced numbers of domestic sheep and goats con-
tinue to graze intermittently on public and private lands. Active
management is ongoing to prevent contact between species: 22
bighorn sheep, five domestic goats and three domestic sheep
were removed from areas where there was risk of contact during
the study, nonetheless, some potential for disease transmission
from domestic sheep and goats existed for all bighorn sheep
populations throughout the study.

We calculated annual adult survival by sex as the proportion
alive in May that survived to the following May in populations
with at least five radio-collared animals. Summer lamb survival
was the proportion of known offspring of radio-collared ewes that
survived until October (approximately to weaning). We classified
a female as having a lamb when she was observed alone with, or
nursing a lamb. We assumed lambs were dead when the female was
no longer associating with a lamb. We located dead lambs through
visual observation. We defined recruitment as the ratio of lambs to
ewes recorded in the annual February–April surveys.

We located radio-collared sheep at least bi-weekly from the
ground or from fixed-wing aircraft. We located females up to se-
veral times per week during lamb-rearing to monitor productivity
and lamb survival. Radiocollars were equipped with a motion-
sensitive switch. When no movement was detected for 4 h, the
switch was activated and we conducted an investigation on site
and collected the entire carcass or tissue samples for analysis at the
Washington Animal Disease and Diagnostic Laboratory (WADDL), Washington State University, Pullman. On the basis
of site investigations and necropsy results, we classified causes of
death as disease, predation, accident or injury, human-caused or
unknown. We censored animals that died within 30 days of cap-
ture and animals translocated to Hells Canyon did not enter the
study until the start of the biological year following translocation
(2–4 months following release).

We based diagnoses of pneumonia on gross and histological
examination of lung tissue at WADDL. Gross features used to
diagnose pneumonia included consolidation, presence of lung
adhesions, abscesses, bronchiecstasis or pleuritis. Affected areas of
the lung were characterized by tissue colour, consistency and
ability to float in formalin. Histological features of acute
pneumonia included fibrin and oedema, increased presence of
pulmonary macrophages, neutrophils, necrotic neutrophils,
necrosis, haemorrhage and bacterial colonies in lung tissue. Chronic pneumonia was characterized by the presence of fibrosis, abscesses or bronchiectasis. Bronchiolar epithelial hyperplasia and peribronchiolar lymphocytic infiltrates in the absence of fibrosis or abscessation was designated as subacute pneumonia. Severity (mild, moderate or severe) was based primarily on the percentage of both right and left lung fields affected on gross examination. Severity assessed by histopathology was based on the total percentage of affected tissue on individual sections of lung. Five to 15% total affected lung or tissue was considered mild, >15–50% was moderate and > 50% was severe.

HEALTH STATUS

We used confirmed and suspected (for lambs) pneumonia-caused mortalities to characterize the seasonality, duration and intensity of four types of pneumonia events by population and year: (i) all-age pneumonia, (ii) secondary all-age pneumonia, (ii) adults only, and (iv) lambs only. We classified a population-year as healthy if animals were radiocollared in the population, but we did not detect any pneumonia in adults or detect or suspect pneumonia in lambs as described in the results.

ANALYSIS

We used Mann–Whitney’s \( U \) test and Wilcoxon’s Rank Sum (Siegel & Castellan 1988) to compare median survival rates of adults and juveniles and population growth by health class due to lack of normality in the data (Shapiro-Wilks test \( P < 0.0001 \)). We analysed seasonal patterns in lamb survival to weaning by a piecewise-constant hazard approach where the instantaneous daily mortality hazard, \( h(a) \), was assumed to be constant for each day. Daily hazard estimates were smoothed using a first order conditional autoregressive approach, \( h(a) = \exp(\beta + y(a)) \), where \( \beta \) is a global intercept with an improper flat prior distribution and \( y(a) \) was specified using the \texttt{car.normal} function in \texttt{WinBUGS} assuming a Uniform(0,10) hyperprior on \( \sigma \) and \( \tau \), the \texttt{car.normal} precision parameter, set equal to \( \frac{1}{2} \) (Besag, York & Mollie 1991; Helsey et al. 2010). We used Markov chain Monte Carlo methods to generate separate posterior distributions for daily mortality hazards by health class (pneumonia or healthy). We ran three Markov chains for 100 000 iterations, discarded the first 50 000 steps, and thinned the remaining steps so that our posterior included every 10th iteration. The Markov chains readily converged (Gelman-Rubin statistic \( \leq 1.13 \) for healthy years, and \( \leq 1.02 \) for pneumonia-years). Further details are provided in Appendix S1.

To identify significant seasonal clustering in adult pneumonia mortalities, we fit a logistic regression model to a series of seasons. The response was a binomial equal to the proportion of adult pneumonia mortalities occurring in that season weighted by month, and the predictor was a binary season indicator for ‘summer’ or ‘winter’. We varied the months categorized as summer by starting with the lamb-rearing months, May-August, and classifying all other months as ‘winter’ and systematically extended the endpoints of the summer season. We present the grouping that showed the greatest difference between seasons.

In populations where we documented pneumonia during the study (we excluded the healthy Asotin and Upper Saddle Creek populations), we used health status in the current year (a categorical predictor taking on separate values for all-age pneumonia, adult-only pneumonia, lamb-only pneumonia or healthy, with healthy as the baseline) as a predictor for future pneumonia (coded as 0 if the next year was healthy, and 1 otherwise). To test for differences among translocated and resident populations, logistic regression models were of the form, 
\[
\frac{e^{\hat{b}Y}}{1 + e^{\hat{b}Y}} = e^{\hat{a} + \hat{b}Xi}
\]
where \( e^{\hat{a}} \) is the odds of pneumonia this year given last year’s health status and \( e^{\hat{b}Xi} \) is the multiplicative adjustment to these odds accounting for the population’s translocation status, \( Xi \) (an indicator taking on the value 0 for resident populations and 1 for translocated populations). We used Firth’s bias-reduction technique for complete separation (Firth 1993) because we always observed pneumonia the year following all-age pneumonia.

We estimated annual transition probabilities between pneumonia classes for populations that had experienced epizootics by building a matrix from the frequency of transitions between classes during the study. Since the transition matrix was regular and irreducible (any state could potentially transition to any other state), we derived the stationary distribution by repeatedly multiplying the probability transition matrix by itself until row values converged (c. 15 iterations) (Taylor & Karlin 1998).

To assess the evidence for spatial synchrony of pneumonia, we used logistic regression to evaluate the influence of pneumonia status in neighbouring populations on a population’s odds of pneumonia. We calculated centroids of 95% contours of fixed kernel home ranges of radiolocations of resident animals by population in Hawth’s Tools (Beyer 2004) and ArcMap 9.3 (ESRI 2008). We defined a population’s neighbours to be all populations with centroids within a designated Euclidean distance (from 10 to 70 km) of the population of interest. Pneumonia in neighbours was a categorical predictor that took on the value 1 if any neighbouring population had pneumonia in the year of interest, and 0 otherwise. We included years when pneumonia was known to be present in the neighbourhood, even if some neighbours were not sampled. We recognize that our probability of detecting pneumonia was less than 1, so we excluded data points (range from 26 to 53% of points at each distance category) where no pneumonia was detected in neighbours, but not all neighbours were sampled. Since a population’s pneumonia status in year t-1 altered its pneumonia odds in year t, we included last year’s pneumonia status in both the population of interest and the neighbouring populations as predictors in the models. To evaluate the effect of translocations, we added an indicator variable for translocated populations in the neighbourhood.

Data were analysed in the R statistical computing environment (R Development Core Team, 2008) through the \texttt{lme4} (Bates, Maechler & Dai 2008) and \texttt{logistf} (Pioner et al. 2006) packages. The lamb mortality hazard model was fit in \texttt{WinBUGS} version 1.4 (Lunn et al. 2000) through \texttt{R} version 2.13.0 using the \texttt{R2WinBUGS} package (Sturtz, Ligges & Gelman 2005).

Results

PNEUMONIA IN ADULTS

Between 1997 and 2010, 477 bighorn sheep were radiocollared (313F, 164M) in 14 populations (Fig. 1) and monitored for a total of 141 population-years (1–14 years...
per population). On average, 117 radio-collared adults (range 35–146) were monitored each year, with a median of 24% (range 5–100%) of adults collared in each study population (Table S1). This included 339 resident sheep monitored for 1220 sheep-years. Another 104 sheep translocated to Hells Canyon from presumably healthy populations in British Columbia, Alberta and Montana 1997–2002, and 34 sheep that were moved within the Hells Canyon metapopulation 1999–2005 were monitored for a total of 459 sheep-years. The translocations established the Big Canyon, Muir Creek, and Myers Creek and Saddle Creek populations, and supplemented existing populations at Asotin, Upper Hells Canyon Oregon, Lostine and Bear Creek (Table S1 and Figure S1).

We determined a cause of death for 179 of 264 radio-collared bighorn sheep (94M, 170F) that died and 53 (30%) were diagnosed with bacterial pneumonia (17M, 36F). We also found 12 (8M, 4F) unmarked dead adult sheep that were diagnosed with bacterial pneumonia. Pneumonia-caused mortality of radio-collared sheep was 27% (28 of 104) of translocated animals and 7% of radio-collared resident animals (25 of 339, $\chi^2 = 28.87$, 1 d.f., $P < 0.01$).

**PNEUMONIA IN LAMBS**

We submitted 129 unmarked dead lambs from 14 populations for necropsy and euthanized 11 live lambs in four populations. We determined a cause of death for 104 lambs and 92 (88%) were diagnosed with pneumonia including 9 of 11 euthanized lambs (Besser et al. 2008). Although juveniles of all ages died from pneumonia, most mortality was prior to weaning, between 4 and 14 weeks of age (Fig. 2). We found no differences in the summer survival distribution functions of lambs in years with pneumonia among the Snake River, Blue Mountains and Wallowa Mountains climate zones ($\chi^2 = 0.1$, 2 d.f., $P = 0.97$) or between lambs of translocated and resident ewes ($\chi^2 = 1.5$, 1 d.f., $P = 0.23$).

Due to the difficulty of detecting freshly dead unmarked lambs in a large, relatively inaccessible and rugged landscape, we assigned a class of 'suspected pneumonia' in lambs based on (1) the distinct temporal signature of documented pneumonia-induced mortality in 37 lamb-only or secondary all-age population-years (Fig. 2); and (2) observations of clinical signs of pneumonia including lethargy, coughing, nasal discharge and discovery of intact dead lambs that were too autolysed for diagnosis. We were conservative in assigning the suspected class of pneumonia to lambs. Median summer lamb survival and recruitment (lamb : ewe ratio) were higher or did not differ in population-years with documented vs. suspected pneumonia (Fig. 3).

**HISTOPATHOLOGY**

Lung lesions observed at necropsy included acute fibrinous bronchopneumonia and pleuritis, sub-acute broncho-interstitial pneumonia with lymphocytic cuffing of airways and bronchiolar hyperplasia, and chronic pneumonia with fibrosis and abscessation. Acute lesions were observed in approximately half of the mortalities regardless of age class (30 of 65 adults and 33 of 66 lambs). Chronic lesions were present in about half (33) of the adult mortalities compared with about a quarter of the lambs (15). Sub-acute lesions were more common in lambs ($n = 18$, 27%) than in adults ($n = 2$, 3%).

**SEASONAL PATTERNS**

There was no difference between sexes in monthly patterns of pneumonia-caused adult mortality ($\chi^2 = 6.77$, d.f. = 11, $P = 0.82$). In both sexes, the odds of pneumonia-caused mortalities were almost three times higher between October and February than during the rest of the year (odds ratio 2.85, 95% CI 1.7–4.8, $P < 0.0001$). The seasonal pattern was driven by mortalities with acute lesions (odds ratio 4.29, 95% CI 1.7–10.9, $P = 0.002$). Deaths of animals with chronic lesions were more evenly distributed across seasons (odds ratio 1.9, 95% CI 1.0–4.0, $P = 0.05$). No acute pneumonia was detected in adults between May and July, the period when most (80%) pneumonia mortalities were detected in lambs. Peak pneumonia mortalities in lambs at 1–3 months of age corresponded to the period when ewes congregated in nursery groups and mortalities associated with pneumonia in adults peaked during the breeding season when mixed sex group sizes were largest (Fig. 4).

**TEMPORAL AND SPATIAL PATTERNS**

Pneumonia was detected or suspected in 33–77% of the study populations each year. Two populations remained healthy throughout the study: Asotin and Saddle...
Differed significantly among age-structured health classes, indicating that pneumonia was a dominant and additive source of mortality (Table 1). Pneumonia restricted to lambs (lamb-only) was the most frequent class of pneumonia observed, and populations usually remained stable (Table 1). Pneumonia in both adults and lambs simultaneously (all-age) occurred in translocated populations in biological years 2000, 2002 and 2003. This accounted for 68% (19 of 28) of the pneumonia mortalities in translocated animals and resulted in immediate population declines. Secondary all-age pneumonia events occurred in both resident and translocated sheep in populations that had previously experienced all-age outbreaks. These events were characterized by summer pneumonia outbreaks in lambs followed by lower rates of pneumonia-induced mortality in adults. Pneumonia in adults only was an infrequent, usually low mortality event (Table 1).

We observed high survival and stable to increasing populations in population-years classified as healthy, even in populations with a previous history of pneumonia. However, once pneumonia invaded a population, healthy periods were usually of short duration (median 1 year, range 1–3 years, Table 1, Fig. 5).

Median Euclidian distance between population centroids was 67 km with a range from 1 (populations separated by the Snake River) to 156 km (Fig. 5). We detected no significant differences in probability of pneumonia relative to distance to neighbouring populations with pneumonia. There was a slight, but insignificant increase in probability of adult or all-age pneumonia-years in populations centred 20 km or less apart (β_{NeighborPN} = 0.97, SE = 0.76, P = 0.20) and no spatial correlation of pneumonia in lambs (Figure S2). Adding a 1-year lag or an indicator for the presence of translocated populations in the neighbourhood did not alter this result (P > 0.32).

We found a significant predictive effect of current pneumonia class on health status of the population the follow-
Continued pneumonia, usually in lambs, was most likely following all-age and secondary all-age (98%) or lamb-only pneumonia-years (83%). The probability of a pneumonia-year following adult-only and healthy years was similar (63% and 62%, respectively, \( P = 0.98 \)), and pneumonia was significantly less likely after healthy years than all-age or lamb pneumonia-years (\( P < 0.05 \), Table 2).

We used the observed frequency of transitions between health classes to develop a transition matrix (Table 2) with Markov properties: there were a finite number of health classes (or states), health class in the current year was dependent on health in the previous year, and any health class could transition to any other health class. Thus, we could predict the stationary distribution of health classes. Assuming transition probabilities among health classes remain constant, pneumonia is predicted in 81% of populations annually; lamb-only pneumonia 57%, all-age and secondary all-age pneumonia combined 17%, adult-only pneumonia 7%. To further illustrate the dynamics of pneumonia-induced mortality, we combined the stationary distribution with mortality and transition rates (Tables 1 and 2) for a visual representation of the impact of disease over time (Fig 6).

Discussion

Analysis of a 14-year time series of pneumonia in 16 interconnected bighorn sheep populations revealed that age-structured classes of pneumonia and healthy years had markedly different demographic impacts on populations. All-age pneumonia was consistently associated with population declines, but ultimately, lambs carried the greatest burden of disease. Rates of pneumonia-induced mortality in lambs can vary significantly by population and year, but on average, pneumonia in lambs had an even greater impact than previously reported (Clifford et al. 2009; Cahn et al. 2011). Recurring annual pneumonia epizootics in lambs may pose the greatest threat to population recovery, and when accompanied by high adult survival, the true consequences of disease may not be realized until senescent adults die and are not replaced.

While pathogen invasion, reinvasion, persistence and fade-out can’t be confirmed in the absence of known disease agents, we can evaluate evidence for these processes to develop hypotheses for future investigation. High initial all-age mortality, when compared with subsequent adult mortality in translocated and resident populations is consistent with invasion of pathogens into groups of apparently naive individuals. Pneumonia in lambs after all-age events must be due to infection from carrier ewes as lambs have little contact with other potential sources of pathogens prior to weaning (Festa-Bianchet 1991; Bleich, Bowyer & Wehausen 1997). Lamb pneumonia outbreaks have also been described in captivity with similar conclusions (Foreyt 1990; Ward et al. 1992; Cassirer et al. 2001). Pneumonia in lambs is thus a good indication of
infection and pathogen shedding in ewes. The absence of pneumonia-induced mortality or clear symptoms in these ewes during outbreaks in lambs confirms that they have either developed resistance or perhaps tolerance of the pathogens(s) that are lethal to their offspring (Rábeg, Graham & Read 2009). Reasons for more frequent fade-out following years with pneumonia restricted to adults remains unclear, but could be explained by differences in pathogens, host immunity or transmission rates.

Our study confirms previously reported accounts of seasonality of pneumonia deaths in bighorn sheep, a pattern commonly observed in infectious diseases of humans and wildlife (Spraker et al. 1984; Aune et al. 1998; Enk, Picton & Williams 2001; Altizer et al. 2006; Cassirer & Sinclair 2007). Age-specific seasonal patterns in pneumonia mortality corresponded to breeding and lamb-rearing: life-history events that are accompanied by especially intensive and concentrated social interactions. The distinct seasonality of adult pneumonia mortality observed in wild populations is not observed in captive experimental bighorn and domestic sheep commingling trials where bighorn sheep die of pneumonia regardless of season. Seasonal physiological or environmental factors are therefore probably less important in precipitating pneumonia epizootics than the timing of pathogen introduction, pathogen virulence and exposure to infections (contact rates). The lack of synchrony of disease events across populations and the absence of an effect of climate on lamb survival during pneumonia years also suggest that weather or other landscape-scale extrinsic variables (Grenfell et al. 1998; Cattadori, Haydon & Hudson 2005), are unlikely to be important drivers of pneumonia in Hells Canyon.

In lambs, most pneumonia-induced mortality occurred between 1 and 3 months of age, a period that coincided with aggregation in nursery groups. Lamb-to-lamb contact may be an important route of infection as happens in many directly transmitted human ‘childhood diseases’; thus, the synchrony in parturition and subsequent concentration of ewes during lamb-rearing which is typical in northern latitudes, could contribute to the timing and high rates of mortality. This period also coincides with the age when passively acquired immunity is probably waning in lambs (Rajala & Castrén 1995), which would further promote transmission and mortality.

By analysing long-term monitoring data to elucidate disease processes from patterns of mortality, we have diverged from studies of bighorn sheep pneumonia that focus on identifying the primary causal agent. The benefits of such a study were that we were able to examine demographic patterns at comparatively large spatial and temporal scales, allowing us to make inferences about processes such as disease introduction, persistence and fade-out. However, the weakness in our approach is an inability to track a known pathogen and directly measure transmission (i.e. infection may occur long before mortality); no opportunity to verify pathogen absence during healthy years;

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**Table 1.** Demographic characteristics of health classes in 14 Hells Canyon bighorn sheep populations, 1997–2010. Data reported as median (range). Years where no adults were radiocollared were excluded from analyses.

<table>
<thead>
<tr>
<th>Outbreak class</th>
<th>Consecutive years</th>
<th>Population growth (R)</th>
<th>Ewe survival</th>
<th>Ram survival</th>
<th>Summer lamb survival</th>
<th>Spring lamb:ewe ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>All-age</td>
<td>3</td>
<td>0.34 (−0.32 to 0.28)</td>
<td>0.03 (−0.17 to 0.63)</td>
<td>0.01 (−0.17 to 0.16)</td>
<td>0.01 (−0.17 to 0.16)</td>
<td>1 (1, 1)</td>
</tr>
<tr>
<td>Secondary all-age</td>
<td>16</td>
<td>0.03 (−0.17 to 0.63)</td>
<td>0.01 (−0.17 to 0.16)</td>
<td>0.01 (−0.17 to 0.16)</td>
<td>0.01 (−0.17 to 0.16)</td>
<td>1 (1, 1)</td>
</tr>
<tr>
<td>Adult only</td>
<td>11</td>
<td>0.01 (−0.17 to 0.16)</td>
<td>0.01 (−0.17 to 0.16)</td>
<td>0.01 (−0.17 to 0.16)</td>
<td>0.01 (−0.17 to 0.16)</td>
<td>1 (1, 1)</td>
</tr>
<tr>
<td>Lamb only</td>
<td>62</td>
<td>0.01 (−0.17 to 0.16)</td>
<td>0.01 (−0.17 to 0.16)</td>
<td>0.01 (−0.17 to 0.16)</td>
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</tr>
<tr>
<td>Healthy</td>
<td>49</td>
<td>0.01 (−0.17 to 0.16)</td>
<td>0.01 (−0.17 to 0.16)</td>
<td>0.01 (−0.17 to 0.16)</td>
<td>0.01 (−0.17 to 0.16)</td>
<td>1 (1, 1)</td>
</tr>
</tbody>
</table>

*Survival of radio-collared ewes in years where n ≥ 33 lamb-only. n = 8 secondary all-age; n = 33 lamb-only.*

<p><strong>Abbreviations</strong></p>

- R: Population growth rate
- R0: Basic reproductive number
- T: Threshold for pathogen transmission
- T* : Threshold for pathogen transmission with density-dependent effects
- n: Number of cases
- p: Proportion of cases

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and no possibility to monitor genetic variation in the pathogen over time. Given these limitations, as well as the usual constraints of marking and monitoring animals in the field, a primary concern is an imperfect detection probability for pneumonia, which could lead to overestimating healthy population-years. However, the likelihood of detecting pneumonia was not correlated with the intensity of monitoring as measured by the proportion of the population that was radiocollared (median in suspected and detected pneumonia-years = 0.22; in healthy years = 0.28, U = 3331.5, 1 d.f., P = 0.09, Tables S1 and S2), or the frequency of locations (median locations per animal per year in suspected and detected pneumonia-years = 30; in healthy years = 32; U = 2427.5, 1 d.f., P = 0.45). Therefore, there was no bias towards monitoring populations with pneumonia and, despite potentially misclassifying some lower mortality pneumonia events, we still detected significant differences in population dynamics between several different classes of pneumonia and healthy years. Survival and population growth were also similar in years classified as healthy in populations with and without a history of pneumonia, suggesting that healthy years, with true absence of disease-related mortality (but not necessarily true absence of infection), did occur, even in populations with previous pneumonia, and these classifications are useful and appropriate for describing the system.

Our observations concur with many of the results of previous studies, but also raise questions about disease models that assume all-age pneumonia outbreaks followed by lamb mortality at a constant or declining rate for a period of usually 1–6 years (Gross, Singer & Moses 2000; Clifford et al. 2009; Cahn et al. 2011). We observed that pneumonia persisted within populations (or was periodically reintroduced) consistently longer than previous models have assumed, and, as indicated by the Markov model stationary frequency distribution, continued to affect all-age classes, not just lambs. The consequence is that all-age pneumonia events can result in sporadic or chronic, long-term reduction of survival of both adult and juvenile age classes. The disparity between our findings and previous studies may be due to the greater sampling intensity, duration and spatial scale of our study. Furthermore, whereas initial invasion associated with high rates of mortality is fairly easy to detect, the end of an epizootic is not always clear. Previously published models assume that low mortality or healthy years represent the pathogen extinction and the end of the epizootic. However, if disease in a long-lived animal like bighorn sheep is accompanied by latent periods and low rates of mortality in chronically infected animals, absence of mortality may not reflect absence of pathogens. Long-term dynamics could be a function of changes in immune status in individuals and include stochastic events common to small populations, such as dispersal, colonization, recruitment, death, intermittent pathogen shedding or lambing status of asymptomatic carriers.

By analysing long-term patterns, we have generated hypotheses about the disease processes associated with pneumonia epizootics in bighorn sheep. As with other diseases with high levels of heterogeneity, these processes are probably affected by a number of factors, including previous exposure of hosts, pathogen dose or virulence, and spatial structuring and contact rates in host populations.
tions (Grassly & Fraser 2008; Salkeld et al. 2010; Wendland et al. 2010; Jesse & Heesterbeek 2011). On the basis of the patterns we observed, the disease appears to be an infection that, in some ways is similar to measles and other immunizing diseases in humans in that it spreads through all-age classes during invasion, but subsequently mainly affects susceptible juveniles. However, in contrast with measles, pathogens apparently persist, occasionally causing fatal pneumonia in previously exposed adults, and the variable lung lesions and associated bacteriology suggest a polymicrobial aetiology, thus secondary pathogens may play a role in severity and recurrence (Besser et al. 2012b). The course of the disease may also be affected by the timing of pathogen invasions relative to contact rates associated with seasonal breeding and parturition. The importance of between-population transmission and recurrent infection from domestic sheep deserves additional investigation as do the conditions that lead to disease and pathogen fade-out.

Acknowledgements

This project would not have been possible without the support of many Hells Canyon Initiative Bighorn Sheep Restoration Committee members and collaborators. We are especially indebted to wildlife managers in Oregon, Washington and Idaho who re-established bighorn sheep in Hells Canyon and funded, coordinated and conducted monitoring and management before and during this project. Many thanks to P. Zager and to field personnel including W. Lammers, J. Ratiff, C. Strobøl, M. Vekasy, M. Hansen, C. Killstrom, D. Gadwa, J. Myers, J. Spence, R. Ward and others. Funding was provided by Idaho Department of Fish and Game (IDFG), Oregon Department of Fish and Wildlife, Washington Department of Fish and Wildlife, Bureau of Land Management, U.S. Forest Service, Wild Sheep Foundation National, chapters and affiliates, Shikari Safari Club International, Idaho Wildlife Disease Research Oversight Committee, Morris Animal Foundation and Federal Aid to Wildlife Restoration Project W-160-R administered through IDFG. RKP was supported by the Cedar Tree Foundation through the Smith Fellows Restoration Project W-160-R administered through IDFG. RKP was supported by the NIH RAPIDD initiative. Any use of trade, product or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

References


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**Supporting Information**

Additional Supporting Information may be found in the online version of this article.

**Appendix S1.** Lamb mortality hazard analysis.

**Figure S1.** Demographic histories of Hells Canyon bighorn sheep populations.

**Figure S2.** Spatial synchrony of pneumonia-years across populations.

**Table S1.** Number and proportion of adult bighorn sheep radio-collared in each population by year and translocation status.

**Table S2.** Health classification of population-years (141) used to estimate demographic characteristics and transition probabilities of pneumonia and healthy years in Hells Canyon, 1997–2010.
Appendix G
Use of Exposure History to Identify Patterns of Immunity to Pneumonia in Bighorn Sheep (Ovis Canadensis)
Use of Exposure History to Identify Patterns of Immunity to Pneumonia in Bighorn Sheep (Ovis canadensis)

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Abstract

Individual host immune responses to infectious agents drive epidemic behavior and are therefore central to understanding and controlling infectious diseases. However, important features of individual immune responses, such as the strength and longevity of immunity, can be challenging to characterize, particularly if they cannot be replicated or controlled in captive environments. Our research on bighorn sheep pneumonia elucidates how individual bighorn sheep respond to infection with pneumonia pathogens by examining the relationship between exposure history and survival in situ. Pneumonia is a poorly understood disease that has impeded the recovery of bighorn sheep (Ovis canadensis) following their widespread extinction in the 1900s. We analyzed the effects of pneumonia-exposure history on survival of 388 radio-collared adults and 753 ewe-lamb pairs. Results from Cox proportional hazards models suggested that surviving ewes develop protective immunity after exposure, but previous exposure in ewes does not protect their lambs during pneumonia outbreaks. Paradoxically, multiple exposures of ewes to pneumonia were associated with diminished survival of their offspring during pneumonia outbreaks. Although there was support for waning and boosting immunity in ewes, models with consistent immunizing exposure were similarly supported. Translocated animals that had not previously been exposed were more likely to die of pneumonia than residents. These results suggest that pneumonia in bighorn sheep can lead to aging populations of immune adults with limited recruitment. Recovery is unlikely to be enhanced by translocating naïve healthy animals into or near populations infected with pneumonia pathogens.

Introduction

The population-level dynamics of infectious diseases in both time and space are shaped by individual-level responses to infection: how long an individual is infectious, how many individuals she or he infects, and how that host develops resistance to subsequent exposures. For example, a high $R_0$ (basic reproductive rate of a disease) [1] coupled with lifelong immunity drives diseases like measles to become so-called childhood diseases characterized by an early age of infection and an adult population mostly resistant to infection but with a small proportion of susceptible individuals protected by herd immunity [2]. Infections with these characteristics can persist within populations larger than a critical community size, where births introduce a sufficient number of susceptible hosts to keep the effective $R_0$ above unity [3], or by reinvasion of smaller populations within a metapopulation [4]. On the other hand, waning immunity, as observed with diseases such as whooping cough [3,6], results in the reemergence of infections in older age cohorts [7], which in turn increases the likelihood of disease persistence and reduces the critical community size. If the immune response of the host is weak, then infections may persist within individuals, reducing condition and fitness; for example, helminths can produce persistent infections that reduce fecundity and generate oscillations in abundances of both parasites and hosts [8,9].

Clearly, how the average individual responds to infection, and the variation in this response across the population, shapes population-level dynamics, and knowledge of these relations is essential for understanding and controlling infectious diseases [10]. However, elucidating individual-level responses to infection can be challenging, particularly when systems cannot be replicated in the laboratory and results of diagnostic tests are not correlated with resistance to infection or disease. Laboratory investigation of pneumonia in bighorn sheep (Ovis canadensis) has been challenging because secondary bacterial pneumonia masks the identity of the...
primary pathogen [11]. Recently, the bacterial pathogen *Mycoplasma ovipneumoniae* was identified as the most likely primary infectious agent [11–13]. *M. ovipneumoniae* was identified as the most likely primary pneumonia agent [11–13].

Confusion about the causative agent of pneumonia has constrained research on disease in bighorn sheep. Pneumonia in bighorn sheep continues to be one of the most poorly understood and intractable of the diseases that threaten wildlife in the United States and Canada. Moreover, despite substantial management efforts, ongoing mortality from pneumonia continues to impede the recovery of bighorn sheep since regional extirpation in many areas of the United States in the 1900s [14–16]. The effect of the disease during invasion (the first colonization of a population with pneumonia pathogens) is highly variable; infections of individuals in all age cohorts with up to 90% mortality are sometimes reported [17]; and events ranging from 30–50% mortality are commonly observed (Fig. 1a,b) [18–20]. Disease invasion frequently occurs during the breeding season (rut) in autumn and is followed by high adult mortality in autumn and winter [18–21]. After invasion, epidemics, manifested as summer pneumonias outbreaks in lambs prior to weaning, endure for a year to over a decade, whereas adult mortality from pneumonia is absent or low and sporadic (Fig. 1a,b) [14,18,20–23]. Bighorn sheep are spatially segregated by sex for most of the year [24]; ewes and lambs do not interact with mature rams or other sources of pathogens during summer. Moreover, candidate pneumonia agents are obligate parasites that do not persist in the environment; therefore, the assumption is that outbreaks in lambs originate from asymptomatic chronic carrier ewes [25–28]. Our premise was that the pattern of individual resistance to infection would reveal drivers of the population-level dynamics of pneumonia. Even in the absence of experimental immunological data, identifying these drivers could inform the development of management strategies to control the disease.

We examined individual-level responses to infection by analyzing disease-exposure history and pneumonia-induced mortality in 388 radio-collared bighorn sheep and 753 lambs born to 223 radio-collared ewes (Fig. 2a; Fig. S1) in 12 connected populations. At least 34 pneumonia epidemics occurred in these populations over a 14-year period, including invasion events that caused high mortality in all age cohorts and mortality events primarily restricted to lambs.

We developed alternative, but not mutually exclusive, hypotheses about the relationship between host immune response to infection and survival during subsequent exposures (Table 1). Each hypothesis was consistent with the observed dynamics: high adult mortality during pneumonia invasion, followed by low, sporadic adult mortality and frequent outbreaks of pneumonia in lambs. First, we hypothesized that a single exposure to pneumonia immunizes individuals against pneumonia during all subsequent exposures. Second, we hypothesized that immunity wanes in the absence of reexposure to disease. Third, we hypothesized that immunity is boosted by each exposure, so that the risk of dying decreases with increasing past exposures. Finally, we predicted that lambs born to previously exposed ewes are protected by maternally derived passive immunity.

We assessed the relationship between previous exposure and survival by analyzing the relative risk of dying of pneumonia, conditional on an individual's pneumonia exposure history, including time since last exposure and number of past exposures (Fig. 2b; Fig. S1). We also analyzed the relationship between a ewe's exposure history and her lamb's survival. Our objectives were to obtain insights into responses of bighorn sheep to pneumonia, understand how resistance to infection affects population-level disease dynamics, and inform the assessment of management strategies such as supplementing populations with translocated animals and culling symptomatic individuals.

**Materials and Methods**

**Study system and data**

The Hells Canyon bighorn sheep study system includes 16 interconnected bighorn sheep populations containing approximately 800 animals. The populations occur over 23 thousand square kilometers in Idaho, Oregon, and Washington (U.S.A.; Fig. 2a). We report data from 388 radio-collared adults and 753 lambs born to 223 radio-collared ewes (Table 2) within 12 populations that were monitored through pneumonia epidemics from 1997 through 2010. Three of these populations were started with translocations from outside Hells Canyon during this study. The radio-collared animals represent a median of 24% of the adults in populations that range in size from less than 10 to more than 240 animals. We do not report data for radio-collared animals in populations that did not experience pneumonia epidemics (n = 51), or for animals for which we could not extrapolate an exposure history such as individuals translocated...
within Hells Canyon (n = 27), or animals from populations not regularly monitored during the study period (n = 11). Animals were located at least every two weeks from the ground or air, and most of the more than 60,000 locations were visual observations. Survival, causes of mortality, movement, productivity, and whether a ewe’s lamb survived to weaning were recorded for each radio-collared animal. Collared ewes and rams were followed for a maximum of 14.3 and 9.4 years, respectively. Data on population size and composition were collected in annual surveys. All animal capture and handling were conducted and coordinated by state wildlife agencies in accordance with accepted animal welfare protocols [29] (see Cassirer and Sinclair [14] and Cassirer et al. [20] for detailed field methodology).

Disease diagnoses were based on necropsies conducted at the Washington Animal Disease and Diagnostic Laboratory. A cause of death was determined for 173 radio-collared adults and 104 lambs that died during the study. Bacterial pneumonia was diagnosed in 47 (27%) of the adults and 92 (88%) of the lambs [20]. Difficulty finding freshly deceased unmarked lambs in relatively inaccessible terrain meant that some pneumonia outbreaks in lambs were inferred from observations of clinical signs and the distinct temporal signature of mortality associated with lamb pneumonia outbreaks [20]. Mortality from pneumonia occurred in at least one population every year during the study period, including at least three invasion events in populations of naïve translocated animals. Four populations had experienced

Table 1. Potential relationships between past exposure of bighorn sheep to pneumonia and mortality during subsequent pneumonia epidemics.

<table>
<thead>
<tr>
<th>Hypothesized relationship between infection (exposure) and immunity to disease</th>
<th>Predictions tested with models that included age as a baseline hazard and translocation status as a covariate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure confers consistent long-term immunity</td>
<td>Risk of dying from pneumonia is highest during the first exposure and consistently low during subsequent exposures</td>
</tr>
<tr>
<td>Exposure confers immunity that wanes over time</td>
<td>Risk of dying from pneumonia is highest during the first exposure, surviving animals are protected for a short period of time and then their risk of dying when reexposed increases</td>
</tr>
<tr>
<td>Cumulative exposures strengthen immune response</td>
<td>Risk of dying from pneumonia decreases as number of exposures (Count) increases</td>
</tr>
<tr>
<td>Cumulative exposures strengthen immune response but immunity wanes between exposures</td>
<td>Risk of dying from pneumonia decreases as number of exposures (Count) increases but increases as time since exposure (Lag) increases</td>
</tr>
<tr>
<td>Exposure does not confer immunity</td>
<td>No relationship between risk of dying from pneumonia and any measures of past exposure</td>
</tr>
<tr>
<td>Exposure results in long-term infection</td>
<td>No relationship between risk of dying of pneumonia and measures of past exposure. Mortality is associated with specific risk factors for mortality in chronic carriers</td>
</tr>
<tr>
<td>Multiple exposures appear to strengthen immune response because weak or &quot;frail&quot; individuals are most likely to die first</td>
<td>Risk of dying from pneumonia decreases as number of exposures (Count) increases</td>
</tr>
<tr>
<td>Ewes with more exposures transfer higher concentrations of immunoglobulins to lambs</td>
<td>Risk of lamb mortality decreases as maternal exposure increases</td>
</tr>
</tbody>
</table>

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invasion events in 1995 and 1996 before animals were radio-collared. Post-invasion dynamics were characterized by frequent outbreaks of pneumonia in lambs and sporadic low-level adult pneumonia mortality. Some populations experienced infrequent pulses of substantial adult pneumonia mortality, and all populations, excluding Sheep Mountain, experienced occasional healthy years (no pneumonia detected or suspected in adults or lambs) [20].

We used the results of the necropsies of adults and lambs, and field observations of pneumonia outbreaks in lambs, to classify the pneumonia status (healthy or pneumonic) of three classes of individuals, based on age and sex, within each population: ewes, rams, and lambs. We classified the pneumonia status of each of these classes within each population once each biological year, (defined as May 1–April 30, because most lambing occurs in May). We considered it reasonable to assign an annual disease status to each class because disease-related mortality is highly seasonal. Most lambs died June through August and most adults died October through February [14,20]. Each class’s annual pneumonia exposure status was binary: positive if there were pneumonia mortalities in that age or sex class within the biological year, and negative if there were no pneumonia mortalities within that class. Each individual’s exposure history was then derived from the exposure history of its age or sex class within its population. For example, if ram(s) experienced pneumonia mortalities, we assumed that all surviving rams within that population were exposed. This assumption, that pneumonia mortality within a sex class results in exposure of all other animals of that sex, was based on the common observation that most adult mortality (sometimes up to 90% [17]) occurs in the year of pathogen invasion. Therefore, most, if not all, members of a population must be exposed within that first year.

We differentiated pneumonia status by sex because sometimes mortalities occurred after the sexes had separated. We do not report results of models in which the sexes were aggregated, which yielded the same inferences as models in which the sexes were differentiated.

We also considered summer pneumonia events restricted to lambs as exposure events for ewes within that population. Lamb mortality rates were high (median 80%; putatively driven by high contact rates among lambs [20]). Intense lamb-ewe interaction likely exposes ewes to pneumonia-causing pathogens. Ewes never died of pneumonia during outbreaks in lambs from May through July [20], indicating protection (presumably immunity) from disease that probably was derived from previous exposure. Lamb-only pneumonia was not considered an exposure for rams because they have little-to-no contact with lambs or ewes during the summer pneumonia outbreaks in lambs [24].

We constructed a pneumonia-exposure history for each radio-collared adult on the basis of the pneumonia history of the age and sex classes within the population(s) of which it was a member (as described above; Fig. 2b, Fig. S1). We assumed that the population in which each animal occurred at the time of collaring was its natal population; if marked animals permanently dispersed to another population (rare within the data set) we adjusted their exposure status to reflect their known residence history. We based estimates of age, and thus exposure, prior to radio-collaring on horn annuli for rams [30], and on tooth eruption for ewes less than four years of age [30,31]. We estimated the ages of ewes that died during the study on the basis of incisor cementum analysis [31] (n = 115). We assumed ewes with full adult dentition at capture were four years old when no incisors were available for aging (either the ewe did not die, or no incisors were collected at mortality). The longest exposure history (including the period from birth to radio-collaring) we constructed for a ewe was 19 years and for a ram was 13 years.

**Mortality hazard model construction**

We characterized the relationship between pneumonia mortality and previous pneumonia exposure by fitting proportional hazards and logistic regression models implemented in the `survival` [32], `coxme` [33] and `lme4` [34] packages in R [35].

We used semi-parametric Cox proportional hazards models in which an individual’s covariates changed over time [36] to assess whether previous exposure events changed an individual’s relative risk of dying of pneumonia during an epidemic. These models estimate the effects of predictor variables on the response variable by comparing values of variables associated with individuals who died versus other individuals of the same sex and cohort (“risk set”). We grouped individuals into risk sets using two survival time-scales. First, we used a study-based timescale so that individuals were grouped by year, regardless of age. Second, we grouped individuals of the same age across years (Fig. S1) [37]. The former risk sets were small, especially early in the study, and we had limited power to detect trends in relative risk of mortality that were associated with any covariate except age. Furthermore, grouping individuals by age allowed us to incorporate age into the baseline hazard of dying while explicitly estimating the effects of other covariates; we therefore report results from the age scale.

Our models had four fixed effects: translocation status (`Source`, a binary variable set at 1 if an individual was translocated and 0 if it was resident); whether an individual previously was exposed to pneumonia (`IPrevious`; a binary variable); the number of previous exposure events (`Count`; the number of biological years with confirmed pneumonia within the individuals’ population of residence); and the number of years since the most recent exposure event (`Lag`). Our sample size was insufficient to examine interaction effects.

The saturated model of the $i$th individual’s hazard of dying of pneumonia at age $a_i$, $h(a_i|\beta)$, was a function of the baseline hazard at age $a$, $h_0(a)$, as well as a linear combination of the covariates:

$$h(a|\beta) = h_0(a) \exp(\beta'\mathbf{x})$$

### Table 2. Number of animals included in the analysis.

<table>
<thead>
<tr>
<th>Radio-collared adults</th>
<th>Ewes</th>
<th>Rams</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residents</td>
<td>196</td>
<td>110</td>
<td>306</td>
</tr>
<tr>
<td>Translocated</td>
<td>66</td>
<td>16</td>
<td>82</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>388</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Outcomes and pneumonia-years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Died of pneumonia</td>
</tr>
<tr>
<td>Died of other causes or cause not determined</td>
</tr>
<tr>
<td>Censured</td>
</tr>
<tr>
<td>Still alive</td>
</tr>
<tr>
<td>Sheep-years</td>
</tr>
<tr>
<td>Sheep-pneumonia-years</td>
</tr>
<tr>
<td>Sheep-healthy-years</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lambs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Lambs*</td>
</tr>
<tr>
<td>Lambs that died during lamb pneumonia outbreaks</td>
</tr>
</tbody>
</table>

*lambs born to radio-collared ewes with a known fate by October 1.

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We did not include population as a source of shared frailty because in some populations Count or Lag was identical for all individuals within a risk set over successive years, preventing within-population estimation of the covariate effects. For a subset of ewes born during the study period that were aged by cementum analysis, we also examined the effect of pneumonia status of lambs during their birth year on probability of mortality.

We evaluated models that included all combinations of the covariates described above, with the exception of Previous and Lag, which were identical for individuals with no past exposure to pneumonia. We examined scaled Schoenfeld residuals as a function of time for all fitted models to assess whether the proportional hazards assumption was met. We used standard metrics to examine whether the models included overly influential points, and assessed Martingale residuals to check whether variance was constant across values of all covariates. Models without higher-order terms or shared frailty components had no overly influential points and met the proportional hazards assumption of consistent relative risk across time. Statistical significance was assessed at $\alpha = 0.05$.

Maternal analysis

We fit both proportional hazards and logistic regression models to examine whether maternal exposure history was associated with either the timing or the rate of lamb mortality prior to weaning. To monitor lamb survival to weaning we identified lambs born to radio-collared ewes through observations of close association and suckling. Ewes were observed weekly during lambing to determine whether or not they produced a lamb. We attempted to locate ewes with lambs at least weekly during lactation and all radio-collared ewes were observed a minimum of every two weeks during this period. We assumed lamb mortality had occurred if the radio-collared ewe was no longer associating with the lamb prior to the expected date of weaning (October 1) [20,38]. We examined data from 753 lambs born to 223 radio-collared ewes (ewes almost always give birth to a single lamb) over 14 years. Of these lambs, 432 were born in years with pneumonia outbreaks in lambs and 321 were born in years without pneumonia (detected or suspected).

Within the proportional hazards models of lamb mortality, we accounted for the effect of a given ewe on the relative risk of dying by including a shared frailty term ($Ewe\_i$) for all lambs born to the same ewe [39]. We also included four fixed effects: count of ewe’s previous exposure events ($Count\_i$); ewe translocation status ($Trans\_i$); estimated ewe age ($Ewe\_Age\_i$); and whether the lamb was born in a pneumonia year ($PneuYear\_j$).

The saturated model of the mortality risk for the $j^{th}$ lamb born to the $i^{th}$ ewe at time $t$, $h_{ij}(t_j | \beta_i)$, relative to the baseline hazard ($h_{0}(t_j)$), is:

$$h_{ij}(t_j | \beta_i) = h_{0}(t_j) \exp(\beta_0 + \beta_1 \text{PneuYear}_j + \beta_2 \text{Ewe} \_\text{Age}_i + \beta_3 \text{Source}_i + \beta_4 \text{Count}_i + \beta_5 \text{Ewe}_i).$$

We did not include Lag in the lamb-mortality models because this would require that some ewes have Lag $>0$. This would conflict with our assumption that carrier ewes are the source of outbreaks in lambs: we assume that the ewe population must be infected immediately before the lamb population, because ewes serve as the source of lamb infection.

We used the same ewe-level covariates defined above, including the random effect Ewe, in logistic regression models to assess the effect of ewe-exposure history on lamb mortality through October 1st. In addition, we investigated trade-offs between reproduction and immunity with a Cox proportional hazards model to assess the risk of ewe pneumonia mortality given the survival or death of her previous year’s lamb. We hypothesized that death of a lamb in year $t$-1 could increase the probability that its mother survived a pneumonia epidemic in year $t$.

Results

Ewes

Our analyses showed that translocation status was the covariate most strongly associated with the probability of dying of pneumonia. Translocated ewes’ risk of dying of pneumonia was about three times greater than that of residents’ (Fig. 3; Table 3). Translocation did not have a statistically significant effect on mortality risk in years without pneumonia epidemics (Table 4). The statistical significance and relative change in risk associated with translocation was similar among all ewe models.

Past exposure was significantly associated with a decrease in relative risk of pneumonia, as were the number of previous exposure events ($Count\_i$). The relative risk of pneumonia mortality increased with time since last exposure ($Lag\_i$). The direction of these covariates suggests that previous exposure confers immunity. The change in values and statistical significance of Count and Lag beyond the first year suggests that waning and boosting may modulate the level of immunity. AIC values based on partial likelihoods were similar among all multivariate models (Table 3). Even if all hypotheses were correct, individuals’ immunity could both decrease and increase over their lifetimes with waning and boosting, respectively. However, the comparable level of support for all three models indicated that none of our hypotheses could be rejected (Table 1). As previously noted, the hypotheses are not mutually exclusive, and the data are not likely to fully discriminate between them. Thus, we did not focus on the relative support for the various hypotheses. Instead, we relied on the estimated effect of each covariate on an individual’s hazard of dying of pneumonia to gain insight into individuals’ immune responses.

The negative coefficient on Count suggests that a ewe’s relative risk of pneumonia mortality decreases slightly as the number of past exposure events increases (Figs. 3,4a). Time since previous exposure ($Lag\_i$) was statistically significantly associated with changing mortality risk. The risk that previously exposed ewes would die from pneumonia was approximately 22% (95% confidence interval 0.09, 0.38) of that of naïve (unexposed) ewes for two years after exposure. The risk of dying of pneumonia three or more years after exposure was not significantly different from the risk of a naïve individual, suggesting that protective immunity may wane after two-to-three years (Table 3). However, sample size of ewes with $Lag\_i$$>2$ was very small. Ewes of known age (<cementum-aged>) that were born during an outbreak of pneumonia in lambs and survived did not have higher or lower probability of dying compared to ewes of known age born in a year with no pneumonia detected (Table S1). None of the models explained mortality risk in years without pneumonia (Table 4).

Rams

Translocation status was the only covariate with a significant effect on mortality risk in rams. The risk that translocated animals
would die of pneumonia was around 4 (95% confidence interval 1.10, 15.14) times that of resident rams in a model including Lag (Fig. 3; Table 5). Count and Lag had negative coefficients, but were not statistically significant. Support for all multivariate models was similar on the basis of AIC values (Fig. 3; Table 5).

Lambs

In all models, the relative risk of lamb death prior to weaning in years with pneumonia outbreaks was approximately four times that in years without outbreaks (Fig. 3; Table 6). The exponentiated ewe-frailty terms ranged from 0.83 to 1.16, suggesting that the median probability of lamb mortality increased by a maximum of 16% for the worst-performing ewe and decreased by a maximum of 17% for the best-performing ewe (in a model including pneumonia years and healthy years). Ewe-translocation status was not reliably associated with altered lamb mortality risk (Fig. 3; Table 6).

Paradoxically, a lamb’s risk of dying significantly increased with its mother’s previous exposures during years with pneumonia outbreaks (Fig. 4b; Table 6), but not during years without outbreaks (Table 6). The number of previous exposures and ewe age were collinear; however, number of previous exposures (but not age) was statistically significantly associated with risk of mortality in a model that included both covariates (Table 6).

There were no naïve dams (mothers) during lamb epidemics. Hence, we compared each value of Count to a baseline of Count = 1 (one previous exposure to pneumonia); a greater effect might be expected if Count = 0 was the baseline. We did not find a significant relation between the mortality risk of a ewe during exposure to pneumonia in year t and the survival or mortality of her lamb in the year t-1. Results from the logistic regression models were consistent with the results from the proportional hazard models (Table S2).

Discussion

We used data on host survival to draw inferences about immunological processes in a system where the etiological agent is unknown and thus serology-based inferences are not feasible. We examined whether previous exposure protects bighorn sheep from pneumonia and whether the strength of the response (presumably immunity) is a positive function of the number of previous exposures to pneumonia and a negative function of time since exposure. We also explored whether passively acquired immunity protects offspring during lamb pneumonia outbreaks. Our results indicate that past exposure decreases ewes’ risk of dying from pneumonia. More-frequent exposure of ewes to pneumonia was associated with higher offspring mortality during outbreaks of pneumonia. We were unable to discern the specific dynamics of immunity in ewes because models with waning, boosting, or consistent immunity were similarly supported. Furthermore, epidemiological processes such as herd immunity and individual frailty may generate patterns analogous to waning and boosting immunity, respectively.

Time since exposure (Lag) and number of exposures (Count)

Waning immune responses are consistent with our understanding of upper- and lower-respiratory tract immunity. Waning immunity may be a consequence of antigenic variation, immune system hyporesponsiveness (induced by commensal flora involved in secondary pneumonia) [40,41], or immune exclusion (secretory IgA binding to bacterial pathogens and preventing development of adaptive immunity) [41]. However, two aspects of the data prevented us from differentiating waning immunity from consis-
First, we documented few fade-out events (years without observed pneumonia); and, second, fade-out events were of short duration. Therefore, sample sizes for investigating waning immunity were limited and immune boosting from frequent exposure likely masked waning. Furthermore, herd immunity (proportion of immune animals in the population) inherently confounds the effects of time since exposure on immunity; while disease is absent, recruited susceptible juveniles gradually dilute the pool of immune animals, hence herd immunity declines even if individual immunity remains constant. Therefore, the risk of exposure (and subsequent disease) increases with time since an epidemic. Finally, survival probability declines in older animals [19] and therefore age may confound the relationship between survival and time since exposure, particularly if we underestimated the ages incorporated into the baseline hazard.

The data suggest a trend of decreasing mortality with increasing exposures (\(\text{Count}\)) to pneumonia. At least two phenomena may

### Table 3. Ewes: results from Cox proportional hazards model of ewe relative risk of dying from pneumonia given covariates over the age-based timescale.

<table>
<thead>
<tr>
<th>Model</th>
<th>Covariate</th>
<th>Beta</th>
<th>Exp. Beta (95% CI)</th>
<th>SE</th>
<th>P-value</th>
<th>AIC</th>
<th>Delta AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Count &amp; translocation</td>
<td>Count</td>
<td>−0.36</td>
<td>0.70 (0.55, 0.89)</td>
<td>0.12</td>
<td>0.002</td>
<td>245.15</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>Translocated</td>
<td>1.19</td>
<td>3.32 (1.54, 7.13)</td>
<td>0.39</td>
<td>0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Past exposure &amp; translocation</td>
<td>Exposed</td>
<td>−1.43</td>
<td>0.26 (0.10, 0.58)</td>
<td>0.45</td>
<td>0.002</td>
<td>244.98</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Translocated</td>
<td>1.32</td>
<td>3.76 (1.76, 7.95)</td>
<td>0.38</td>
<td>0.0005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lag &amp; translocation</td>
<td>Lag &gt;2 Yr</td>
<td>−1.16</td>
<td>0.31 (0.10, 0.96)</td>
<td>0.57</td>
<td>0.041</td>
<td>1.47</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Translocated</td>
<td>1.31</td>
<td>3.72 (1.76, 7.86)</td>
<td>0.38</td>
<td>0.0006</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lag (4 categories) &amp; translocation</td>
<td>Lag 1 Yr</td>
<td>−1.49</td>
<td>0.22 (0.09, 0.58)</td>
<td>0.48</td>
<td>0.0003</td>
<td>247.43</td>
<td>2.45</td>
</tr>
<tr>
<td></td>
<td>Lag 2 Yr</td>
<td>−1.46</td>
<td>0.23 (0.06, 0.59)</td>
<td>0.70</td>
<td>0.034</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lag &gt;3 Yr</td>
<td>−0.60</td>
<td>0.55 (0.13, 2.35)</td>
<td>0.74</td>
<td>0.417</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Translocated</td>
<td>1.38</td>
<td>3.98 (1.85, 8.57)</td>
<td>0.39</td>
<td>0.0004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Translocation</td>
<td>Translocated</td>
<td>1.67</td>
<td>5.30 (2.62, 10.73)</td>
<td>0.36</td>
<td>&lt;.0001</td>
<td>252.18</td>
<td>7.2</td>
</tr>
<tr>
<td>Count</td>
<td>Count</td>
<td>−0.48</td>
<td>0.62 (0.49, 0.77)</td>
<td>0.11</td>
<td>&lt;.0001</td>
<td>252.45</td>
<td>7.46</td>
</tr>
<tr>
<td>Past exposure</td>
<td>Exposed</td>
<td>−1.95</td>
<td>0.14 (0.06, 0.32)</td>
<td>0.43</td>
<td>&lt;.0001</td>
<td>253.56</td>
<td>9.58</td>
</tr>
<tr>
<td>Lag</td>
<td>Lag &gt;2 Yr</td>
<td>−2.08</td>
<td>0.13 (0.05, 0.31)</td>
<td>0.46</td>
<td>&lt;.0001</td>
<td>255.83</td>
<td>10.85</td>
</tr>
<tr>
<td></td>
<td>Translocated</td>
<td>1.64</td>
<td>0.19 (0.07, 0.56)</td>
<td>0.54</td>
<td>0.0026</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SE = Standard error.

doi:10.1371/journal.pone.0061919.t003

### Table 4. Ewes in non-pneumonia (healthy) years: impact of covariates on the relative risk of dying (of causes other than pneumonia) outside of pneumonia epidemics.

<table>
<thead>
<tr>
<th>Model</th>
<th>Covariate</th>
<th>Beta</th>
<th>Exp. Beta (95% CI)</th>
<th>SE</th>
<th>P-value</th>
<th>AIC</th>
<th>Delta AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Count &amp; translocation</td>
<td>Count</td>
<td>−0.042</td>
<td>0.95 (0.76, 1.21)</td>
<td>0.12</td>
<td>0.62</td>
<td>217.64</td>
<td>1.76</td>
</tr>
<tr>
<td></td>
<td>Translocated</td>
<td>0.225</td>
<td>1.25 (0.51, 3.06)</td>
<td>0.46</td>
<td>0.72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Past exposure &amp; translocation</td>
<td>Exposed</td>
<td>0.35</td>
<td>1.42 (0.51, 3.92)</td>
<td>0.52</td>
<td>0.50</td>
<td>217.30</td>
<td>1.42</td>
</tr>
<tr>
<td></td>
<td>Translocated</td>
<td>0.39</td>
<td>1.48 (0.60, 3.63)</td>
<td>0.46</td>
<td>0.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lag &amp; translocation*</td>
<td>Lag 1 Yr</td>
<td>0.39</td>
<td>1.47 (0.52, 4.17)</td>
<td>0.53</td>
<td>0.46</td>
<td>219.59</td>
<td>3.71</td>
</tr>
<tr>
<td></td>
<td>Lag 2 Yrs</td>
<td>0.64</td>
<td>1.89 (0.53, 6.70)</td>
<td>0.64</td>
<td>0.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lag &gt;3 Yrs</td>
<td>−0.62</td>
<td>0.54 (0.06, 4.85)</td>
<td>1.12</td>
<td>0.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Translocated</td>
<td>0.39</td>
<td>1.47 (0.60, 3.64)</td>
<td>0.46</td>
<td>0.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Count</td>
<td>Count</td>
<td>−0.06</td>
<td>0.94 (0.76, 1.17)</td>
<td>0.11</td>
<td>0.58</td>
<td>215.88</td>
<td>0</td>
</tr>
<tr>
<td>Past exposure</td>
<td>Exposed</td>
<td>0.21</td>
<td>1.23 (0.48, 3.17)</td>
<td>0.48</td>
<td>0.67</td>
<td>216.00</td>
<td>0.12</td>
</tr>
<tr>
<td>Lag</td>
<td>Lag 1 Yr</td>
<td>0.26</td>
<td>1.29 (0.48, 3.46)</td>
<td>0.50</td>
<td>0.61</td>
<td>218.27</td>
<td>2.39</td>
</tr>
<tr>
<td></td>
<td>Lag 2 Yrs</td>
<td>0.47</td>
<td>1.60 (0.48, 5.25)</td>
<td>0.61</td>
<td>0.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lag &gt;3 Yrs</td>
<td>−0.78</td>
<td>0.46 (0.05, 3.92)</td>
<td>1.10</td>
<td>0.47</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SE = Standard error.

*Model was inestimable, since all pneumonia-year mortalities among individuals with lags of 2 or more occurred among residents; the translocation coefficient could not be calculated.

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account for this effect. First, immunity may be dose-dependent: each successive exposure may strengthen the anamnestic immune response (immune memory) to a particular agent, or diversify exposure to multiple primary and secondary agents. Second, inherently weak or high-risk individuals (for example, individuals with weaker innate immune responses or highly social individuals) are more likely to be the first (individual frailty) [42,43] when their exposure counts are, coincidently, lower. Increasing proportions of stronger individuals remaining in the population are exposed repeatedly, generating an apparent relationship between number of exposures and risk of mortality (Fig. 4c). The removal of age through its incorporation into the baseline hazard, and the observed relationship of increasing mortality as a function of age (Fig. S2), suggest that age is not driving this relationship. The data did not allow an examination of cumulative exposure within each level of Lag.

Lamb survival and maternal immunity

We had hypothesized that ewes with more exposures would transfer higher concentrations of passively acquired immunoglobulins to their lambs, resulting in lower lamb mortality. By contrast, the data showed that increasing ewe exposures were weakly associated with earlier and higher lamb mortality. This relation was opposite to the relationship between number of exposures of ewes and ewe mortality. The earlier timing of lamb death for ewes with more exposures suggests that the force of infection to lambs varies among mothers with differing exposure histories. One potential explanation is that ewes with more exposures are more likely to be infectious carriers (perhaps either cumulative exposures or age increase the risk of becoming a carrier), providing direct and early exposure to their lambs.

We considered reproductive senescence as an alternative explanation, because number of exposures and age are inherently collinear. However, the relationship between number of ewe exposures and earlier or higher lamb mortality was only observed during pneumonia epidemics (although a paucity of ewes with high numbers of exposures in years without pneumonia made assessment difficult); furthermore, Festa-Bianchet and King [38] showed no difference in lamb mortality between prime-age and older bighorn ewes in pneumonia-free populations. Variations in pathogen virulence or the number of carrier ewes over time are alternative explanations.

We assumed all ewes that gave birth to lambs during pneumonia epidemics had prior exposure to pneumonia; therefore, we could not examine the effect of presence versus absence of passively transferred maternal immunity on lamb mortality. Given the extremely high lamb mortality rates during pneumonia outbreaks among lambs in populations with previous exposures, it appears that passive immunity transferred from the ewe does not prevent lamb mortality. Besser et al.’s [13] detection of pulmonary *M. ovipneumoniae* infection in asymptomatic lambs as young as four days old, and the development of bronchopneumonia in (passively) seropositive 10 day old lambs, similarly suggests that passive immunity has little effect in delaying progression of pneumonia disease. Given the inverse relationship between number of previous exposures of ewes and timing of lamb death discussed above, ewe infection status (leading to early lamb exposure) may be a better predictor of lamb mortality than the ewe’s maternal antibody concentration (presuming that multiple exposures increase maternal antibody concentration).

Rams

We did not find an association between past exposure to pneumonia and ram mortality. However, the few rams in this
study limited our ability to examine the association of exposure covariates with mortality. Furthermore, ram exposure is difficult to monitor. Rams are more likely to be exposed through unobserved interactions with other bighorn sheep populations and domestic sheep populations, particularly during the rut. Rams are also spatially separated from ewe-lamb groups so lamb pneumonia cannot be used as a sentinel of disease transmission, potentially leading to underestimation of exposure. On the other hand, the lack of recirculation opportunities, due to separation from summer lamb pneumonia outbreaks, could drive real differences in exposure patterns between rams and ewes. Also, sexual dimorphism in immune function is well documented in some species [44,45] and factors such as the immunosuppressive effects of testosterone, and life history differences between sexes, could be responsible for different responses to disease exposure.

Translocation

Even when we accounted for previous exposure, number of previous exposures, time since previous exposures, and age and sex, translocated animals had three-to-four times the risk of dying of pneumonia of resident animals, a result consistent with previous studies [26,46]. Translocated animals did not enter the study until the biological year following translocation (2–4 months after release), pneumonia deaths occurred from 2–5 years after release, and animals translocated into populations without pneumonia did not die of pneumonia. Therefore, it is unlikely that the act of translocating, or short-term post-release effects such as stress, contributed to the higher risk of dying of pneumonia. We suspect that two issues account for the difference between translocated and resident animals. First, these data did not capture the major invasion events, and associated high mortality, that occurred in naive resident populations prior to this study. Invasion events in this study only occurred in populations of naive translocated animals. Second, because resident populations had been exposed to pneumonia prior to radio-collaring, resident animals could only be categorized as naive if born into a population during a healthy year. Animals remained naive for each subsequent year that the population remained healthy. Inaccurate age estimates and failure to detect pneumonia within infected populations were likely to lead to misclassification of residents as naive when in fact they were exposed. For these reasons, and the small sample size of translocated and resident animals that died of pneumonia in this study, the relationship between translocation and pneumonia risk seems to warrant further exploration.

Limitations

Ideally, one would examine the relationship between exposure and immunity experimentally, by inoculating animals repeatedly at various intervals and following their fate, or by documenting individuals’ serological status before and after pneumonia epidemics. However, the identity of the pathogen that causes pneumonia remains controversial, which poses a challenge for studies based on inoculation and serology. Given that field conditions such as weather and nutritional stress cannot be replicated in captivity [47]; that serological status is not necessarily correlated with protective immunity [48]; and that long-term re-exposure experiments are rarely feasible, we relied on population-level data to describe effects of exposure on individuals. As a result, a potential limitation of this study is misclassification. For example, we may have overestimated exposure if population substructuring (behavioral or spatial), or low transmission rates, led to incomplete exposure. Alternatively, we may have underestimated exposure if we failed to detect pneumonia outbreaks (a less likely scenario for ewes than rams, given that lambs provide a sentinel for pneumonia transmission in ewes). Assuming that some ewes were four years of age at capture also may have led to underestimation of exposure. Regardless of the direction of misclassification, in most cases the effect would be to increase similarity between the exposure history of the individual dying of pneumonia and the risk-set, therefore contributing to our inability to distinguish among hypotheses. A larger sample size of known-age adults, and adults that died of pneumonia, would have strengthened our analyses.

Another limitation of this study is difficulty differentiating between resistance to disease and resistance to infection. Animals that do not get sick may still be infected, or re-infected in subsequent epidemics (defined as ‘tolerant’ in some ecological literature) [49]. This distinction is important because chronically infectious animals, which are resistant to disease, will have profoundly different effects on the epidemiology of pneumonia than individuals that are resistant to infection and not infectious.

Table 5. Rams: results from Cox proportional hazards model of ram hazard of dying from pneumonia given covariates over the age-based timescale.

<table>
<thead>
<tr>
<th>Model</th>
<th>Covariate</th>
<th>Beta</th>
<th>Exp. Beta (95% CI)</th>
<th>SE</th>
<th>P-value</th>
<th>AIC</th>
<th>Delta AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Translocation</td>
<td>Translocated</td>
<td>1.04</td>
<td>2.83 (0.83, 9.60)</td>
<td>0.62</td>
<td>0.10</td>
<td>78.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Count &amp; Translocation</td>
<td>Count</td>
<td>−0.67</td>
<td>0.51 (0.22, 1.21)</td>
<td>0.44</td>
<td>0.13</td>
<td>77.9</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Translocated</td>
<td>1.40</td>
<td>4.04 (1.10, 5.14)</td>
<td>0.67</td>
<td>0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Past exposure &amp; Translocation</td>
<td>Exposed</td>
<td>−0.72</td>
<td>0.49 (0.15, 1.57)</td>
<td>0.60</td>
<td>0.23</td>
<td>79.1</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>Translocated</td>
<td>1.21</td>
<td>3.36 (0.94, 11.99)</td>
<td>0.65</td>
<td>0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lag &amp; Translocation</td>
<td>Lag 1 Yr</td>
<td>−1.40</td>
<td>0.25 (0.04,1.37)</td>
<td>0.87</td>
<td>0.11</td>
<td>79.4</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>Lag ≥/= 2 Yr</td>
<td>−0.16</td>
<td>0.85 (0.21, 3.44)</td>
<td>0.71</td>
<td>0.83</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Translocated</td>
<td>1.50</td>
<td>4.50 (1.20, 17.19)</td>
<td>0.68</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Count</td>
<td>Count</td>
<td>−0.44</td>
<td>0.65 (0.28, 1.48)</td>
<td>0.42</td>
<td>0.30</td>
<td>80.0</td>
<td>2.1</td>
</tr>
<tr>
<td>Past exposure</td>
<td>Exposed</td>
<td>−0.49</td>
<td>0.61 (0.20, 1.85)</td>
<td>0.57</td>
<td>0.38</td>
<td>80.4</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>Lag</td>
<td>Lag 1 Yr</td>
<td>−0.82</td>
<td>0.44 (0.09, 2.13)</td>
<td>0.81</td>
<td>0.31</td>
<td>82.0</td>
</tr>
<tr>
<td></td>
<td>Lag ≥/= 2 Yr</td>
<td>−0.24</td>
<td>0.79 (0.21, 2.96)</td>
<td>0.67</td>
<td>0.73</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SE = Standard error.

doi:10.1371/journal.pone.0061919.t005
The simultaneous presence of animals resistant to infection and animals that are carriers but protected from disease is consistent with observations of pneumonia in bighorn sheep in the wild and in captivity [25–28]. Carrier ewes within resistant populations are necessary to explain annual outbreaks in lambs in the absence of ewe mortality because lambs rarely contact other sources of pathogens (rams or domestic sheep) prior to weaning [24,50]. Difficulty in differentiating between resistant and tolerant individuals may be common when using survival data to infer resistance to infection and is an important limitation of our study, given that a few tolerant individuals may be responsible for most disease transmission [51].

Conclusions

By defining individuals’ pneumonia-exposure histories, we tested hypotheses about immune response in a system for which immunological data are absent, the causative agent is unknown, and experimental approaches are not feasible. Without directly identifying the pathogen, we found that ewes develop some level of protective immunity following exposure; protection may wane in the absence of exposure or be boosted by repeated exposures; protective immunity is not effectively transferred from ewes to lambs; and unexposed animals translocated near infected populations have a high risk of developing pneumonia. Our results explain the high mortality during pathogen invasion and low adult mortality after invasion. The lack of protection via passive immunity in lambs suggests that pneumonia in bighorn sheep will lead to aging populations with limited recruitment. Although a larger sample size of animals that died of pneumonia would be desirable, most limitations stemmed from our inability to directly track a pathogen and therefore our inability to discriminate between resistant and tolerant (carrier) animals or to distinguish among epidemiological processes that might explain our findings. The recent discovery of *M. ovipneumoniae* as the probable primary pathogen provides further opportunities to test our hypotheses with additional field, laboratory, and dynamic modeling studies. We hope these studies will eventually inform development of management strategies that can break the cycle of prolonged

<p>| Table 6. Lambs: results from Cox proportional hazards models of lamb hazard of dying given dam (ewe) covariates in all years (pneumonia and healthy years; top), years without pneumonia (pneumonia years excluded; middle) and years with pneumonia (healthy years excluded; bottom). |</p>
<table>
<thead>
<tr>
<th>Model</th>
<th>Covariate</th>
<th>Beta</th>
<th>Exp. Beta (95% CI)</th>
<th>SE</th>
<th>P-value</th>
<th>SD of ewe shared frailties</th>
<th>AIC</th>
<th>Delta AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lambs in all years</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ewe Only</td>
<td>PN Year</td>
<td>1.46</td>
<td>4.30 (3.27, 5.64)</td>
<td>0.14</td>
<td>&lt;.0001</td>
<td>0.21</td>
<td>4411.43</td>
<td>18.7</td>
</tr>
<tr>
<td>Count</td>
<td>PN Year</td>
<td>1.27</td>
<td>4.17 (2.68, 4.73)</td>
<td>0.15</td>
<td>&lt;.0001</td>
<td>0.21</td>
<td>4392.73</td>
<td>0</td>
</tr>
<tr>
<td>Translocation</td>
<td>PN Year</td>
<td>1.46</td>
<td>4.32 (3.26, 5.71)</td>
<td>0.14</td>
<td>&lt;.0001</td>
<td>0.21</td>
<td>4431.43</td>
<td>20.68</td>
</tr>
<tr>
<td>Age</td>
<td>PN Year</td>
<td>1.42</td>
<td>4.15 (3.15, 5.46)</td>
<td>0.14</td>
<td>&lt;.0001</td>
<td>0.24</td>
<td>4405.60</td>
<td>12.87</td>
</tr>
<tr>
<td>Age &amp; Count</td>
<td>PN Year</td>
<td>1.28</td>
<td>3.59 (2.70, 4.78)</td>
<td>0.15</td>
<td>&lt;.0001</td>
<td>0.22</td>
<td>4394.44</td>
<td>1.71</td>
</tr>
<tr>
<td>Trans &amp; Count</td>
<td>PN Year</td>
<td>1.28</td>
<td>3.66 (2.75, 4.88)</td>
<td>0.15</td>
<td>&lt;.0001</td>
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SE = Standard error; SD = standard deviation; PN Year = years with outbreak of pneumonia in lambs.

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pneumonia epidemics and aid recovery of bighorn sheep across their range.

Supporting Information

Figure S1 Data collection and pneumonia histories within Hells Canyon populations of bighorn sheep. A. Individual pneumonia histories of 15 ewes within the Wenaha (population 2 in Fig. 2). Top panel: annual pneumonia status of the population based on a study-based time-scale. Bottom panel: annual pneumonia status of the population on an age-based time-scale. Red indicates years when adults and/or lambs died of pneumonia, green are years when no pneumonia mortality was detected (or suspected in lambs; see [20]); x’s represent death or censoring. B. Annual pneumonia status in the 16 Hells Canyon bighorn sheep populations, 1994–2010 (see map in Fig. 2). 1 = Asotin, 2 = Wenaha, 3 = Mountain View, 4 = Black Butte, 5 = Redbird, 6 = Lower Hells Canyon, 7 = Imnaha, 8 = Big Canyon, 9 = Muir Creek, 10 = Meyers Creek, 11 = Saddle Creek, 12 = Upper Hells Canyon Oregon, 13 = Upper Hells Canyon Idaho, 14 = Sheep Mountain, 15 = Lostine, 16 = Bear Creek. (PDF)

Figure S2 Ewe pneumonia survival probability as a function of age. The shaded area represents the 95% confidence bounds for the probability that a ewe of a given age died of pneumonia, using data included in the age-based proportional hazards models of ewe pneumonia mortality. Inclusion of an individual in each category of age is conditional on its survival up until that age, and each individual contributed as many data points as its age at last observation. The points are the proportion of ewes that survived to a given age-class and experienced a pneumonia epidemic that died of pneumonia during that epidemic. (PDF)

Table S1 Ewe relative risk of dying as a function of birth year pneumonia status for cementum-aged ewes. (DOCX)

Table S2 Lambs: logistic regression results. (DOCX)

Acknowledgments

We thank Mike Ehinger for help with the figures; Marco Festa-Bianchet, Erica Fleishman, the Cross lab, Leslie Bienen, and two anonymous reviewers for comments that improved this manuscript. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Author Contributions

Conceived and designed the experiments: RKP KRM EFC TEB PJH. Performed the experiments: RKP KRM. Analyzed the data: RKP KRM. Contributed reagents/materials/analysis tools: EFC TEB. Wrote the paper: RKP KRM EFC TEB PJH. Collected field data: EFC.
Appendix H
Female elk contacts are neither frequency nor density dependent
Female elk contacts are neither frequency nor density dependent


Abstract. Identifying drivers of contact rates among individuals is critical to understanding disease dynamics and implementing targeted control measures. We studied the interaction patterns of 149 female elk (Cervus canadensis) distributed across five different regions of western Wyoming over three years, defining a contact as an approach within one body length (~2 m). Using hierarchical models that account for correlations within individuals, pairs, and groups, we found that pairwise contact rates within a group declined by a factor of three as group sizes increased 33-fold. Per capita contact rates, however, increased with group size according to a power function, such that female elk contact rates fell in between the predictions of density- or frequency-dependent disease models. We found similar patterns for the duration of contacts. Our results suggest that larger elk groups are likely to play a disproportionately role in the disease dynamics of directly transmitted infections in elk. Supplemental feeding of elk had a limited impact on pairwise interaction rates and durations, but per capita rates were more than two times higher on feeding grounds. Our statistical approach decomposes the variation in contact rate into individual, dyadic, and environmental effects, and provides insight into factors that may be targeted by disease control programs. In particular, female elk contact patterns were driven more by environmental factors such as group size than by either individual or dyad effects.

Key words: brucellosis; Cervus canadensis; contact rate; disease models; elk; Greater Yellowstone Ecosystem, Wyoming, USA; hierarchical models; proximity loggers; super-spreading events; supplemental feeding.

INTRODUCTION

The relationship between host density and parasite transmission is fundamental to understanding infectious disease dynamics and implementing effective control strategies (Anderson and May 1991, McCallum et al. 2001). Models predict that when transmission is correlated with host density, parasites will be unable to persist when the host density is reduced below some threshold (Kermack and McKendrick 1927, Getz and Pickering 1983). In addition, culling is only expected to reduce disease prevalence when transmission is density dependent, which has important management implications for several wildlife diseases (Schauber and Woolf 2003, Lloyd-Smith et al. 2005a, Conner et al. 2007, Cross et al. 2010b). Defining the relationship between disease transmission and host density has been hampered by a paucity of data on host interaction rates across a range of densities and spatial scales.

In disease models, density-dependent (DD) transmission can be expressed as $\beta_\pi SN$, where $\beta$ is the transmission coefficient, $\pi$ is the disease prevalence, $S$ is the density of susceptible individuals, and $N$ is the total population density. On the other hand, frequency-dependent (FD) transmission is modeled as $\beta_\pi S$. There are many alternatives to these two formulations (McCallum et al. 2001), but these two create a useful context in which we can place our empirical results. More generally, one could model transmission as power function $\beta_\pi S N^{\kappa}$, where the force of infection is $\beta_\pi N^{\kappa}$ for a given susceptible individual and $\kappa$ can be used to transition between DD and FD models. De Jong et al. (1995) pointed out that the origin of the term “mass action” assumes that the units of $S$ and $I$ are densities rather than numbers, and they introduced the term pseudo-mass action to refer to $\beta SI$ where $S$ and $I$ are numbers of individuals. In this paper, we use group sizes and number of individuals rather than densities, and are somewhat cavalier in our semantics referring to density-
Interactions among individuals are necessary pair-wise in contrast to most dynamic disease models that are parameterized on a per capita basis (but see Keeling 1999, Lloyd-Smith et al. 2004). In addition, non-directional interaction data are more naturally analyzed on the basis of pairs rather than individuals because the same interaction would appear twice in an individual-level data set. We can write the per capita encounter rate, irrespective of whether individuals are infectious (i.e., \( \pi = 1 \)), as \( eN^\gamma \), where \( e \) is a contact coefficient that does not account for the probability of infection, given contact. However, the per capita contact rate is not as easily observed and measured as the pairwise encounter rate, \( \lambda \), which is \( eN^\gamma \) divided by the number of potential pairs an individual has, \( N - 1 \), where \( N \) is now the population or group size rather than density. Thus \( \lambda \) is approximately \( eN^\gamma \), where \( \gamma = \kappa - 1 \) for large \( N \). When \( \gamma \) equals zero or \(-1\), we recover the pairwise equivalents of the DD and FD transmission functions, respectively. In this study we illustrate how to directly estimate \( \gamma \), and hence \( \kappa \), from empirical interaction data while accounting for the repeated sampling of individuals and pairs over time and the correlation among pairs within a group.

There has been an extended debate about which disease transmission models are useful approximations of particular wildlife disease systems (McCallum et al. 2001, Begon et al. 2002, Schauber and Woolf 2003, Lloyd-Smith et al. 2005a). One challenge is that the adequacy of the model often depends upon spatial scale. Transmission may be density dependent at a local scale, but appear frequency dependent at a broad spatial scale (Turner et al. 2003, Cross et al. 2013). For socially aggregated species, this is likely to be true whenever disease transmission is closely related to local group size, but the frequency distribution of group size does not change with population size (Cross et al. 2009). Further, host density is a challenging variable to measure because it is often unclear what area should be in the denominator, and density measurements will therefore depend upon the spatial scale. For these reasons, we believe that models of interactions and transmission for socially aggregated species can be more easily connected to empirical data on group size, rather than densities. In this study, we measured how elk contact rates were affected by local group size and discuss our results in the broader context of density- and frequency-dependent transmission.

Few studies have directly estimated interaction rates across a range of host densities or population sizes, although several studies have related host density to some disease-related variable (for review see Ferrari et al. 2011) or have used indirect measures of contact from a coarse spatial or temporal scale, which may not correlate well with disease transmission (for a review see Cross et al. 2012). Ramsey et al. (2002) and Vander Wal et al. (2012) are two noteworthy exceptions. Ramsey et al. used radiotelemetry locations of brushtail possums (Trichosurus vulpecula) before and after density reductions to show that interaction rates were positively associated with host density, but that male–female interaction rates did not decrease in proportion to the decrease in density during the breeding season. Vander Wal et al. (2012) assessed elk interaction rates in enclosures of different sizes using proximity loggers, and found that male interaction rates increased with density, but interactions among females were unrelated to density. This experimental study controlled group size while modifying the area of the enclosure; however, it is not clear how to relate the results to a field setting where the area a group occupies is unconstrained and groups vary in size by more than an order of magnitude. In this study, we measured interaction rates at a fine spatial scale, \( \sim 2 \) m, in a field setting with 149 collared elk distributed across five different sites over three years with group sizes ranging from 10 to 336 (Fig. 1).

Elk in the Greater Yellowstone Ecosystem (GYE) are aggregated into groups from two to over 2000 individuals, which vary in size seasonally and spatially (Cross et al. 2010a). Within a season, factors that influence herd size include habitat type, habitat openness, and exposure to predation risk (Creel and Winnie 2005). In addition, many GYE elk are supplementally fed during winter at 22 feeding grounds in Wyoming, which affects aggregation patterns (Cross et al. 2007). In this region, elk are a reservoir host for brucellosis, a bacterial disease caused by B. abortus, which is a political and economic issue due to the potential transmission of brucellosis to cattle (Bienen and Tabor 2006). Our past work shows that recent increases in brucellosis seroprevalence among elk are correlated with increased elk density in many areas of the GYE (Cross et al. 2010a,b). However, the functional form of the relationship between seroprevalence and density was not well defined and the analyses were conducted at a relatively broad spatial scale.

Here we address one underlying mechanism that affects transmission and prevalence: elk interaction rates within groups. Disease ecologists often refer to contacts as interactions among individuals where pathogen transmission may occur even without physical contact. We refer to interactions and contacts interchangeably, but note that contacts do not necessarily imply physical touch. We use DD and FD models as two contrasting hypotheses about how pairwise and per capita interaction rates will correlate with group size. We also hypothesized that pairwise contact rates (interactions within \( \sim 2 \) m) may be higher during times when elk are supplementally fed, because artificial feeding on hay lines can cause elk to tightly aggregate in comparison to typical winter foraging behavior (Creech et al. 2012, Forristal et al. 2012). Finally, using a hierarchical approach, we illustrate how to partition the variation in the expected contact rates among individual, dyad, and environmental effects, which has important impli-
cations for designing control efforts that attempt to
target super-spreading events.

METHODS

We conducted our study in the Wyoming and Wind River mountain ranges in the southern portion of the GYE (Fig. 1). In January and February of 2009, 2010, and 2011, we captured a total of 167 female elk (≥1.5 yr old) at five sites and fitted them with Sirtrack proximity logger collars (Sirtrack, Hawkes Bay, New Zealand). Eighteen loggers were either not recovered or the data were corrupted, resulting in an average of 30 usable loggers at each site (Appendix A). Four sites had supplemental feeding during the winter (Soda Lake, Alpine, Muddy, and Fall Creek), and elk population sizes attending these feeding grounds ranged from 550 to 700 individuals. We captured elk at the site without supplemental feeding (elk hunt area 99) for two consecutive years (2010 and 2011; Fig. 1). Totals of 379 and 506 elk were counted during helicopter elk classifications conducted on winter ranges in hunt area 99 (1782 km²) during February in 2010 and 2011, respectively. We collared only adult female elk (≤2 yr old) because of our interest in brucellosis, which is thought to be primarily transmitted by abortion events from February to June (Cheville et al. 1998). We captured elk by chemical immobilization, helicopter net-gun, and corral traps. All captures were performed in accordance with approved Montana State University Animal Care and Use Protocol (no. 2010-2002).

It is difficult to interpret the mechanisms driving a lack of interaction among individuals using only the proximity logger data. In particular, one does not know whether noninteracting dyads from a given site and time period were separated by a vast distance and thus had no opportunity for contact, or if the dyad was in the same social group but remained outside of the distance required to log an interaction (Cross et al. 2012). Elk
tend to aggregate in winter and disaggregate into smaller groups in summer, so fewer recorded interactions during summer may be the result of having only a few proximity loggers in smaller groups rather than a change in the within-group behavior (Fig. 2). Therefore, we limited our analysis to days when group membership was known, which reduced the amount of data used in the analysis, but allowed us to interpret zero interactions as a failure to interact despite being in the same group. When we directly observed elk groups containing two or more proximity-collared individuals, we recorded the time, identity of collared individuals, and group size for each observation. At sites with supplemental feeding, we used contact data from January to March when all the elk with loggers were known to be present, based upon GPS collar data as well as visual inspection of pairwise contacts. This showed that all possible pairs of individuals were contacting one another almost every day during the feeding season (Appendix B; P. C. Cross and B. M. Scurlock, unpublished data).

We used a single count of the elk population at each feeding ground conducted in February, when the attendance at the feeding ground was highest. Daily counts of elk attending feeding grounds did not vary much over the time span we used for the analysis. While not attending feeding grounds, we delineated elk groups based upon relatively consistent internal spacing, whereby individuals were generally only a few body lengths away from one another, and individuals were moving in roughly the same direction (Winnie and Creel 2007). These groups were counted by direct observation using spotting scopes, which limited our analyses to open sagebrush habitats where the elk were most visible. We monitored elk from January to July each year, at which point the collars were programmed to drop off.

We calibrated proximity loggers to record interactions within 2 m in the field using a modified version of the laboratory calibration procedure described in Prange et al. (2006); the receiving range of each logger was tested using five other loggers to transmit signals, and we adjusted power settings on the receiving logger until its mean receiving range in the laboratory setting was as close to 3.5 m as possible. Our field tests, conducted with horses, revealed that a 3.5-m laboratory receiving range was approximately equivalent to a 2-m receiving range on the animal. Interactions were considered separate events if separated by $\geq 90$ seconds. We removed all contacts with a duration $\leq 1$ second as potentially spurious (Prange et al. 2006).

Analyzing association patterns is statistically complicated because interactions may be correlated within and among individuals, pairs, groups, and regions. We used Bayesian hierarchical generalized linear models to assess what factors are correlated with number of contacts while accounting for the repeated observations of some individuals, pairs, and multiple pairs observed within a group of a given size (Cross et al. 2012). Let $y_{ik}$ represent the number of contacts between dyad $l$ for observation period $k$, where $l$ is the unique dyad for individuals $i$ and $j$. We used a Poisson-Gamma mixture model formulation of the negative binomial model because the variance of the means of the posterior predictive distributions was roughly a quadratic function of the mean contact...
rate (Ver Hoef and Boveng 2007, Cross et al. 2012). Our initial model can be written as follows:

\[ y_{lk} \sim \text{Poisson}(r_{lk} \lambda_{lk}) \]

\[ \lambda_{lk} \sim \exp(\alpha_l + \alpha_k + \delta_l + \rho_k) \]

\[ r_{lk} \sim \text{Gamma}(\theta, \theta) \]

\[ \alpha_l \sim \text{Normal}(0, \sigma^2_\alpha) \]

\[ \alpha_k \sim \text{Normal}(0, \sigma^2_\alpha) \]

\[ \delta_l \sim \text{Normal}(0, \sigma^2_\delta) \]

\[ \rho_k \sim \text{Normal}(0, \sigma^2_\rho) \]

where \( \alpha_l \) and \( \alpha_k \) are individual effects; \( \delta_l \) are dyad effects (i.e., an interaction term, whereby some pairs may make more or less contacts than expected given their individual effects); and \( \rho_k \) are effects associated with the observation period (e.g., group size), which we refer to as environmental effects. Each logger stores data on its own interaction history, so most contacts are recorded twice, once on each logger in the pair. We used data from the logger with the larger number of contacts recorded for the pair when loggers differed for a particular observation period. Each observation period \( k \) represented a single observation of a group and contacts were summed for the 12 h before and after this observation. Our choice of a 24-h interval was motivated by the frequent switching of elk among groups; we did not want to assign individuals to the wrong group (Cross et al. 2012). Potential dyads that were never observed in the same group were excluded from the data analysis. Meanwhile, we added zeros to the data set when dyads were known to be in the same group from radiotelemetry or direct observations, but had no recorded interactions.

Proximity logger data include both the number of contacts and the duration of each contact. Total contact duration incorporates both of these, but it is complicated to statistically analyze because the distribution is likely to be bimodal, with a peak at zero (for those dyads that did not contact one another) and another peak at some average duration of contact. Thus, we conducted separate analyses of the number of contacts as well as the duration, \( \tau_{g_k} \), given that the pair made contact. We modeled contact duration as \( \tau_{g_k} = \exp(\alpha_l + \alpha_k + \delta_l + \rho_k) \) because the residuals (i.e., means of the posterior predictive distributions) were approximately normally distributed on a log scale. To calculate the expected per capita contact rate and duration, we multiplied the pairwise posterior means \( \lambda_{lk} \) and \( \tau_{g_k} \) by \( g_k - 1 \), where \( g_k \) was the observed group size for observation period \( k \). This accounts for the fact that not all individuals in a group are sampled. By formulating the statistical model on a pairwise basis and then translating those results to the per capita scale, we avoid the statistical issue on the per capita scale of having group size on both sides of the equation (e.g., \( \lambda_{lk}[g_k - 1] = \exp(\gamma \log(g_k)) \)).

We were primarily interested in the effects of group size and supplemental feeding, and one potential hierarchical model is \( \rho_k \sim \text{Normal}(\phi_{site} + \phi_{fed} + \gamma \log(g_k), \sigma^2_\rho) \), where \( \phi_{site} \) and \( \phi_{fed} \) are the main effects of site and feeding, and \( \gamma \) is the effect of log group size. However, these predictors were correlated in our data set, such that during the feeding season, group sizes were larger and tended not to vary much over time. As a result, we could not assess the supplemental feeding effect independent of a group size effect. Thus we conducted one set of analyses using only observations collected after the feeding season when group sizes were known, and assumed that \( \rho_k \sim \text{Normal}(\phi_{site} + \gamma \log(g_k), \sigma^2_\rho) \). In a second analysis, we included data from during and after the supplemental feeding and used all observations where the group membership was known (by radiotelemetry) even when we did not have an estimate of the group size. For this data set, we estimated a parameter for each site both during and after the supplemental feeding season, \( \rho_k \sim \text{Normal}(\phi_{site} + \phi_{fed} + \gamma \log(g_k), \sigma^2_\rho) \), and then compared the site-level differences during and after feeding using posterior distributions of the linear contrasts.

Our statistical approach addresses several challenging problems that arise for valid inferences from dyadic data. First, each dyad involves two individuals, whose individual effect estimates may be assumed to derive from the same overall population (i.e., a single distribution). Second, the estimates of the variation associated with individuals, dyads, and environments are interesting in their own right; thus we would like to estimate the precision of those estimates. In particular, individual variation, \( \sigma^2_\alpha \), relates to the 20/80 rule of Woolhouse et al. (1997), who hypothesized that, in some cases, 20% of the individuals may be responsible for at least 80% of the infections. The estimate of \( \sigma^2_\delta \) is the predictable component of the variation among individuals; as \( \sigma^2_\delta \) increases, fewer individuals are involved in more of the interactions. Finally, because some of our individuals, dyads, or observation periods had only a few data points, the shrinkage toward the mean associated with our “random effects” produces better estimates with superior statistical properties than fixed effects (Efron and Morris 1977).

We used uninformative prior distributions on all parameters where possible. We assumed a diffuse normal prior distribution for site effects with a mean of 0 and a precision of 0.0001 (SD = 100). We assigned the random effects \( \alpha_l \), \( \delta_l \), and \( \rho_k \) normal prior distributions with a mean of 0 and a standard deviation with a hyperprior of Uniform(0, 10). The prior distribution for \( \phi_{site} \) was normal with a mean of 0 and a precision of 0.0001. In previous analyses, we tested other forms of uninformative prior distributions, and our estimates were nearly identical (Cross et al. 2012). All models were run for 600,000 iterations on three different platforms.
Markov chains and the first half of each chain was discarded. We assessed convergence using the Gelman-Rubin-Brooks statistic, where $\hat{R} < 1.1$ for all parameters indicated that relatively little variation was associated with a specific MCMC chain (Gelman and Hill 2007). All models were run using WinBUGS version 1.4.3 (Lunn et al. 2000) from R version 2.13.2 (R Development Core Team 2011). We were concerned that the sparse sampling of some pairs and groups may bias the group size parameter or the variance of the random effects. Therefore we repeated similar analyses using a Poisson linear mixed model using the lme4 package in R (Bates et al. 2011) and conducted simulations with known parameter values; we found no systematic bias for our sampling design (Appendix C).

After we recovered the proximity loggers from the field, we remeasured the distance at which they recorded contacts. Loggers, and pairs of loggers, differed from one another in the recording distance at the completion of the study, but this distance was not correlated with our estimated individual ($\gamma_i$) or dyadic random effects ($\delta_i$) in the contact analyses (data not shown). Further, our estimates of the differences among individuals, due to collar performance or other factors, were small; as a result, we ignored this complication. However, accounting for the performance of collars may be important to other studies interested in estimating the biological variation in the sociality of individuals.

**RESULTS**

The full data set of contacts per dyad per day shows dramatic site and seasonal differences (Fig. 2). If we assume that all collared females within a site are available for contact, then average contact rates went as high as six times per pair per day during winter to less than 0.5 in the summer (Fig. 2). Data from Alpine and Soda Lake lasted longer because the collars did not fall off the animals as planned and individuals were recaptured in 2010 for Alpine and 2011 for Soda Lake. Supplemental feeding at Soda Lake occurred during the 2009 and 2011 winters, but not in 2010, primarily due to a lack of snow, which coincided with lower contact rates during the 2010 winter compared to 2009 and 2011. It is not clear from Fig. 2 if the seasonal variation in contact was a function of how the proximity loggers are distributed among groups or of behavioral changes within a group of a given size.

In our statistical analyses, we limited the data set to include only those dyads that were present within the same social group. Excluding data during the supplemental feeding season, the within-group pairwise contact rate declined with group size across all four sites for which we had group size observations ($\gamma = -0.38$; using lme4: $\gamma = -0.37, \text{SE} = 0.14$). Pairwise contact rates declined by a factor of three, from about five to 1.4 contacts per day with a 33-fold increase in group size (Fig. 3A). However, the corresponding per capita contact rates, $\bar{r}_k(g_k - 1)$, increased with group size from 45 contacts per individual per day in a group of 10 to over 400 contacts per individual per day in groups of 300 or more (Fig. 3B). Thus, as group size increased, the decrease in pairwise contact rates was more than offset by the increase in the number of possible dyads within the group. Pairwise duration together per day, given contact, $\tau_{rk}$, followed a similar pattern, declining from ~7.4 min/day in the smallest groups to 1.2 min/day in the largest group, while the per capita contact duration increased from 1.2 h/d to 6.7 h/d (Fig. 3C, D). The 2.5th and 97.5th percentiles of the posterior distribution of $\gamma$ did not overlap either 0 or −1 for either the contact rate or contact duration analyses, suggesting that neither density-dependent (i.e., $\gamma = 0$) nor density-independent (i.e., $\gamma = -1$) models of per capita contact were supported (Table 1, Fig. 3).

We sampled at four sites (Alpine, Fall, Soda, and Muddy Creek) where elk were supplementally fed during winter, and we expected that the supplemental feeding would dramatically alter contact rates and duration of time spent together. This appears to be the case in Fig. 2, which implicitly assumes that all possible pairs at a given site could contact one another. On a pairwise basis, however, there was no clear pattern suggesting that supplemental feeding increases the per pair contact rate or duration (Fig. 4A, B). In particular, the unfed site (HA99), had contact rates and durations that were similar to those of feeding sites after the supplemental feeding had ended. Meanwhile, at Soda and Muddy Creek feeding grounds, the pairwise contact rates were slightly lower during the feeding season, while the opposite appears to be true for Fall Creek, although evidence for a significant statistical difference was not strong (Fig. 4). However, contacts and contact duration on the per capita scale were much higher during the supplemental feeding season (Fig. 4C, D). One might expect that more social individuals may be more likely to be exposed to infection. We found no relationship between brucellosis status and the estimate of that individuals’ overall sociality, $\alpha_i$, for either contact rate or duration (data not shown).

The variation in observation periods, as measured by $\sigma^2_\rho$, was larger than either the individual $\sigma^2_i$ or dyad $\sigma^2_{d_k}$ variation (Table 1). Our simulations of the statistical model suggest that these differences were not likely to be due to our observational sampling design (Appendix C). The variation among individuals, dyads, and observation periods was lower when we included data during the supplemental feeding season compared to after the feeding season (Table 1). As an example of how to interpret the estimated standard deviations in Table 1, consider just the estimates from the model of pairwise contact rate ($\gamma_{rk}$) using only the data after the supplemental feeding season. For this model, the average observation period effect ($\rho$) was 0.92, which we will use as the baseline. A dyad in which one elk had an individual effect ($\alpha_i$) one standard deviation higher than average would be expected to interact 3.5 times per
day \([\exp (0.92 + 0.33) = 3.5]\) compared to an average of 2.5 times per day. Meanwhile, a pair of female elk with a dyad effect \((\delta_d)\) one standard deviation higher would interact 3.9 times per day. Finally, for an observation period that was one standard deviation higher than average, we would expect all pairs to interact, on average, 4.8 times per day, or almost twice as often as the baseline. Using data after the feeding season, the proportion of the variation in log contact duration explained by the random effects was relatively small \((\frac{\sigma_z^2 + \sigma_l^2 + \sigma_p^2}{\sigma^2} = 0.14; \text{Table 1})\).

**Discussion**

Adult female elk interactions varied dramatically across sites, years, and seasons (Fig. 2). Each pair of individuals within a group was less likely to make contact in 24 hours, and the duration of contact decreased as group size increased (Fig. 3). These pairwise decreases were more than offset by the increasing number of pairs in larger groups, such that per capita contacts and durations increased with group size. Adult female elk interactions were intermediate to what might be expected for density- or frequency-dependent disease models, where per capita contacts increase linearly with group size or are constant, respectively (Fig. 3). To our knowledge, this is the first study to directly estimate per capita and pairwise contact rates, of any species, across a wide range of group sizes.

Woolhouse et al. (1997) proposed a “20/80 rule” whereby 20% of individuals are responsible for 80% of disease transmission. Lloyd-Smith et al. (2005b) related this variation to super-spreading events and assessed how heterogeneity in pathogen transmission affects disease dynamics and the efficacy of control efforts. The variability in disease transmission is the product of variation in infectiousness, susceptibility, and contact rates among individuals and environments. However, only a portion of the variation in transmission and contacts is predictable, and in some cases it is only the predictable variation that can be targeted by control efforts (e.g., limiting large aggregations or targeting more sexually active individuals for a sexually transmitted infection). Here we illustrated an approach to assessing behavioral factors that are likely to create super-spreading events, using proximity loggers to measure interaction rates. Our statistical approach accounts for the dyadic nature of interactions and allows us to partition the predictable variation in contact rate into individual, dyad, and environmental effects. In our data set, environmental effects accounted for more variation in pairwise female contact rates than did either individual or dyad effects (Table 1). This suggests that identifying highly social individuals is less important than identifying the environmental conditions associated with high contact rates for disease control. By applying the approach we present here, in combination with quantitative measures of susceptibility and infectiousness, we can determine the drivers of super-spreading events and develop targeted control measures even prior to a disease outbreak.

Proximity logger data are probably a useful surrogate for disease-relevant contacts for directly transmitted pathogens with limited survival in the environment. We focused our study on female elk over 1.5 years old, due to our interest in *Brucella abortus*, which is primarily transmitted by abortion events (Cheville et al. 1998). Placing a proximity logger under a fetus is a more direct measure in this host–parasite system (Creech et al. 2012). With the exception of the supplemental feeding grounds, however, it is difficult to place a fetus within free-ranging elk groups. We believe that elk-to-elk contact rates are a useful proxy for elk–fetus contacts within a group, particularly for retained placentas, which are periodically observed on the feeding grounds. Our focus on adult females, however, probably underestimates the total individual variation in contact rate for other diseases, such as tuberculosis, where males may play an important role and interact differently (Vander Wal et al. 2012). The amount of transmission between social groups due to the survival of *B. abortus* in the environment is unknown and not addressed in this study.

Contacts are, by definition, pairwise rather than individual events; however, disease models are typically formulated on a per capita basis. Translating between these two scales can result in counterintuitive results. For example, in our early statistical models we assumed a linear, rather than log-linear, effect of group size on pairwise interactions. If pairwise contact rates are statistically modeled as \(\exp (\phi + \gamma N)\), then the per capita contact rate is approximately \(N \exp (\phi + \gamma N)\). If \(\gamma\) is negative, then this function is nonlinear and unimodal, with a maximum at intermediate population sizes. It is unlikely that per capita contacts would decline, rather than saturate, in the largest groups, but this is an important consequence of how the pairwise contacts are modeled. On the other hand, a pairwise contact rate modeled as \(\exp (\phi + \gamma \log [N])\) becomes approximately \(\exp (\phi) N^{\gamma + 1}\) on the per capita scale. For our data set, linear or log-linear models of group size effects were not substantially different, but this may not be generally true.

Our results suggest that pairwise contact rates and durations were similar during and after the supplemental feeding season. On a per capita basis, however, contact rates were over two times greater during the feeding season. In this study we did not have data from unfed elk groups that were of equivalent size to the feeding ground populations, which creates potential confounding between group size and feeding effects. We addressed this by only estimating the group size effect using data after the supplemental feeding season had ended. The largest unfed groups had \(~300\) individuals, whereas the smallest feeding ground population was 420. The contact rate at that site (Muddy Creek) was roughly
830 contacts per individual per day (Fig. 4), which is at least double the contact rate in the largest groups of unfed elk (Fig. 3), but only a 40% increase in group size. This suggests that supplemental feeding may increase per capita contact rates beyond what might be expected from the group size alone.

By limiting our analyses to just those days when group membership was known, we dramatically reduced the data available. In addition, our group size observations were limited to open habitats where groups could be counted. Pairing proximity loggers with global positioning systems (GPS) would allow future studies to use all of the available data and assess contact rates in areas, and at times, when individuals are not directly observable. The inclusion of more habitat-related variables may help to explain more of the variation in contacts, which in this study was relatively modest at 14% of the total variation in log contact duration (Table 1). We used 24 hours as the period of time to sum contacts and contact duration because elk switched group membership every few days. Longer time periods would have increased the chances of assigning individuals to the wrong group, resulting in lower contact rates. Longer time periods will be less problematic in systems where group membership is less dynamic. If the empirical data are going to be used in disease models,
FIG. 4. (A, B) Comparisons of adult female elk pairwise (A) contact rates (\(k\)) and (B) durations (\(s\)) at each site during and after the supplemental feeding season. (C, D) Per capita (C) contacts and (D) durations, given contact, were calculated as \(k(1/g)\) and as \(s(1/g)\), respectively. Points represent means of the posterior predictive distributions. Thin and wide lines are the 95% and 50% credible intervals, respectively, some of which are hidden behind the points.

TABLE 1. Parameter estimates from statistical models of pairwise female elk (Cervus canadensis) contact rate and duration together per day.

<table>
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<tr>
<th>Dependent variable</th>
<th>Data set</th>
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<th>Mean</th>
<th>Median</th>
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<td></td>
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<tr>
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<tr>
<td></td>
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<tr>
<td>Duration per day ((\tau_{lk}))</td>
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<td>0.60</td>
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Notes: For the dependent variables, \(l\) and \(k\) are indices for the dyad and observation period, respectively. Parameters \(\sigma_x\), \(\sigma_\theta\), and \(\gamma\) are the standard deviations of the random effects of individuals, dyads, and observation periods, respectively; \(\gamma\) is the effect of \(\log(\text{group size})\); and \(\theta\) is the shape and scale of the Gamma distribution in the Poisson-Gamma mixture model.
the time period should be kept shorter than the infectious period.

Using time series data, Smith et al. (2009) found that cowpox prevalence in field voles (Microtus agrestis) was best fit by a function that was intermediate to density or frequency dependence. We expect this to be a general pattern for many host-pathogen systems because contacts, even nonsexual contacts, take time and time is limited (Antonovics et al. 1995). Our results suggest that, for a directly transmitted pathogen, we would expect the largest elk groups to play a disproportionate role in the disease dynamics for two reasons. First, by definition, more individuals will be in the largest groups. Secondly, on a per individual basis, contacts increase with group size, but at a decreasing rate. At broader spatial scales, brucellosis seroprevalence is potentially a nonlinear increasing function of elk density (Cross et al. 2010a, b).

There does not appear to be much hierarchical structure among adult female elk within a group, nor would we expect many predictable super-spreaders in this system, based on their contact patterns. In our analyses, a cohesive group-within-group structure would be evidenced by a strong dyadic interaction effect, suggesting that some pairs contact often whereas others do not, even though they are all in the same group. If we had proximity loggers on mother–calf pairs, we probably would have observed such hierarchical structure. Instead it appears that adult female elk may be more random with their contacts, but that they interfere with one another as groups get larger, such that the number and duration of contacts with particular individuals declines with increasing group size. We hypothesize that this may be a general pattern for social ruminants, but not primates (Nunn and Altizer 2006) or elephants (Wittemyer et al. 2005), where hierarchies within groups are likely (Whitehead 2008).

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Literature Cited


SUPPLEMENTAL MATERIAL

Appendix A

Table with covariate data on the female elk used in the analyses (Ecological Archives E094-189-A1).

Appendix B

Table with covariate data on the direct observations of elk groups used in the analyses (Ecological Archives E094-189-A2).

Appendix C

Details of a simulation study to assess the statistical properties of the hierarchical model with multiple random effects and sparse observational sampling (Ecological Archives E094-189-A3).
Appendix I
Heterologous Vaccination and Checkpoint Blockade Synergize to Induce Antileukemia Immunity
Heterologous Vaccination and Checkpoint Blockade Synergize To Induce Antileukemia Immunity


Checkpoint blockade-based immunotherapies are effective in cancers with high numbers of nonsynonymous mutations. In contrast, current paradigms suggest that such approaches will be ineffective in cancers with few nonsynonymous mutations. To examine this issue, we made use of a murine model of BCR-ABL+ B-lineage acute lymphoblastic leukemia. Using a principal component analysis, we found that robust MHC class II expression, coupled with appropriate costimulation, correlated with lower leukemic burden. We next assessed whether checkpoint blockade or therapeutic vaccination could improve survival in mice with pre-established leukemia. Consistent with the low mutation load in our leukemia model, we found that checkpoint blockade alone had only modest effects on survival. In contrast, robust heterologous vaccination with a peptide derived from the BCR-ABL fusion (BAp), a key driver mutation, generated a small population of mice that survived long-term. Checkpoint blockade synergized with heterologous vaccination to enhance overall survival in mice with leukemia. Enhanced survival did not correlate with numbers of BAp:I-Ab–specific T cells, but rather with increased expression of IL-10, IL-17, and granzyme B and decreased expression of programmed death 1 on these cells. Our findings demonstrate that vaccination to key driver mutations cooperates with checkpoint blockade and allows for immune control of cancers with low nonsynonymous mutation loads. The Journal of Immunology, 2016, 196: 4793–4804.

Patients with B cell acute lymphoblastic leukemia (B-ALL) harboring the BCR-ABL chromosomal translocation have very poor outcomes (1, 2). Current therapies for BCR-ABL+ B-ALL include cytotoxic chemotherapeutics, tyrosine kinase inhibitors, and bone marrow transplantation. These treatments are often transiently effective, indicating that new treatment options are urgently needed. One such option is immunotherapy. Recent work in cancers with frequent nonsynonymous mutations, such as melanomas, has demonstrated that immunotherapy involving neutralization of programmed death 1 (PD1) and CTLA4 (checkpoint blockade) is an effective treatment option (3, 4). It remains unclear whether immunotherapy involving checkpoint blockade strategies will also be effective in cancers with few nonsynonymous mutations, such as B-ALL (5).

To determine whether immunotherapy is an effective option for treating B-ALL, we used a syngeneic mouse model of BCR-ABL+ B-ALL to characterize the host immune response to this leukemia in immune-competent recipient animals (6–8). We previously demonstrated that the host adaptive immune system responds to BCR-ABL+ B-ALL (9). Although B-ALL cells have been shown to have low numbers of nonsynonymous mutations (5), the fusion between BCR and ABL does generate an MHC class II (MHC-II)–restricted peptide Ag that can be recognized by a small population of endogenous BCR-ABL peptide (BAp):I-Ab–specific T cells in mice (9). Transfer of BCR-ABL+ leukemic cells into C57BL/6 mice resulted in proliferation of BAp:I–Aβ-specific T cells, although 50% of these cells differentiated into FOXP3+ regulatory T cells (Tregs) (10). Thus, T cells do respond to BCR-ABL+ leukemia in this mouse model, but the response was immunosuppressive in nature and detrimental to host survival. In this study, we address whether the immune response to leukemia could be modulated, thus making BCR-ABL+ B-ALL malleable to checkpoint blockade–based T cell immunotherapy.

Materials and Methods

Mice

C57BL/6 mice and Cdx2a−/− (strain 01XF6, B6, 129-Cdx2a−/−(Cg)Nei) (11) mice came from the National Cancer Institute. Foxp3-GFP (stock no. 006772) and Ifng−/− (stock no. 002287) mice came from The Jackson Laboratory (Bar Harbor, ME). OT-I (10). Thus, T cells do respond to BCR-ABL+ leukemia in this mouse model, but the response was immunosuppressive in nature and detrimental to host survival. In this study, we address whether the immune response to leukemia could be modulated, thus making BCR-ABL+ B-ALL malleable to checkpoint blockade–based T cell immunotherapy.

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Abbreviations used in this article: B-ALL, B cell acute lymphoblastic leukemia; BAp, BCR-ABL peptide; IQR, interquartile range; LCMV, lymphocytic choriomeningitis virus; LCMV+BAp, lymphocytic choriomeningitis virus plus BCR-ABL peptide; LM+BAp, Listeria monocytogenes expressing BCR-ABL peptide; MHC-II, MHC class II; PC, principal component; PCA, principal component analysis; PD1, programmed death 1; PDL1, programmed death ligand 1; Treg, regulatory T cell; VSV, vesicular stomatitis virus; VSV+BAp, vesicular stomatitis virus plus BCR-ABL peptide.
Listeria monocytogenes generation

ActA–L. monocytogenes strain 1942 (from Dr. Sing Sing Way) expressing BAp from a plasmid was constructed as previously described (13–15).

Infections and immunizations

*L. monocytogenes* expressing BAp (LM+BAp) (10^7 CFU) was injected i.v. through the tail vein. Mice vaccinated with lymphocytic choriomeningitis virus (LCMV)–Armstrong received 2 × 10^5 PFU i.p. at day 0. Vescicular stomatitis virus (VSV)–Indiana was used at 5 × 10^5 PFU i.v. at day 0. At days 3 and 5, mice were injected i.v. with 200 μg BAp. Mice were harvested at indicated time points.

Leukemia model

Cd20α−/− mouse bone marrow cells were transduced with viral superantigen containing a BCR-ABL (P190)–IRES-GFP retrovirus (16) and cultured for adoptive transfer as previously described (7, 9).

In vivo Ab treatment

Unvaccinated mice were treated with 100 μg anti–PD ligand 1 (PD1L1) and anti-CTLA I.p. every other day. Vaccinated mice received 200 μg anti-PD1L1 and anti-CTLA I.p. twice per week. Mice treated with anti-CD40 received 200 μg I.p. every other day.

**Abs**

Abs for flow cytometry included CD3 PE, CD4 (RM4-5) PerCP-Cy5.5, CD8 (53-6.7) BV650, CD11c (N418) PE, FOXP3 (FJK16S) PE, CD80 (16-10A1) allophycocyanin, CD86 (GL1) PE-Cy7, CD19 BV605, B220 (RA3-6B2) Horizon V500, IFNγ (XMG1.2) BV650, LAP (TTW16B4) PE, TNFα (MP6-XT22) BV421, IL-17A (TC11-18H10) Alexa Fluor 488, and Pdgl1 (2PH1) BV421 purchased from BD Biosciences (San Jose, CA). CD27 (309) FITC, CD40 (L387.55) PE-Cy7, CD80 (16-10A1) PE, and F4/80 in allophycocyanin–eFluor 780, and PD1 (J43) FITC, CD73 eFluor 450, FR4 PE-Cy7, PD1 PerCP–eFluor 710, MHC-II I-A^b Fluor 450, IL-10 (JESS-16E3) PE, granocyte B (NZGB) PE-Cy7, GARP (YGC86) eFluor 450, and all ELISPOT Abs were purchased from eBioscience (San Diego, CA); and IgM F(ab')2 allophycocyanin was purchased from Jackson ImmunoResearch Laboratories (West Grove, PA). Rat IgG1 (HRPN) PerCP-Cy5.5 isotype and rat IgG2a (2A3) violet eFluor 450 isotype were purchased from Tonbo Biosciences (San Jose, CA). Cells from enriched fractions were analyzed on LSRII or Fortessa cytometers (BD Biosciences, San Jose, CA) and data were analyzed in FlowJo (Tree Star, Ashland, OR).

Statistics and principal component analysis description

Standard normality tests suggested departures from normality, and thus nonparametric tests (Mann–Whitney test for two groups, Kruskal–Wallis test for more than two groups) were used unless otherwise stated. Normality assessments, nonparametric tests, and survival analyses were done in GraphPad Prism (La Jolla, CA). For the Cox–Mantel tests, we report hazard ratios, which describe the multiplicative change in risk when moving from the baseline group to the treatment group. Principal component analysis (PCA) was conducted in R (prcomp function) (18). Linear regressions and correlation coefficients were estimated in GraphPad Prism and R.

Detailed descriptions of the PCA and corresponding linear regression are included below. We performed a PCA on the following five phenotype metrics collected on each mouse: PD1L1, MHC-II, CD40, CD80, CD86. We added one to each metric, and then log transformed the resulting value so that our data met the PCA assumption of joint normality. Components were estimated using the prcomp function in the stats package in R.

First, pair plots of the raw manifest variables and log-transformed manifest variables were created. PCA is a method for reducing the dimension of a dataset by transforming an initial set of possibly correlated manifest variables into a set of new orthogonal variables, which are referred to as PCs. These components describe the multivariate correlation in the dataset. Although the method generates as many components as there are measured variables in the dataset, most of the variation can usually be captured with only a few components. Each component consists of a value that describes the proportion of variation in the original dataset explained by the component, and a set of loadings that describe the extent to which each manifest variable correlates with that component. Let X be an n × m matrix containing measurements of k different manifest variables for n sampled individuals. Estimation is obtained through an eigen decomposition of the square matrix X’X. Eigenvalues correspond to proportions of variance in the original dataset captured by each component, and eigenvectors describe correlations between each manifest variable and each component. Although the method constructs as many PCs as there are manifest variables in the dataset, interpretation is limited to those components that explain the preponderance of variation. The number of components to interpret is often determined using a scree plot, showing the proportion of variance explained by each component.

A scree plot for the components identified for the log-transformed immunogenicity phenotype metrics is shown in Fig. 1D. Based on the scree plot, we interpreted the first two components. Loading of each manifest variable on each component is shown in Table I.

We extracted PC scores for PC1 and PC2 for each mouse in our dataset. We used a linear regression model to relate these PC scores to percentage leukemic burden measured on these same mice. The regression model consisted of four terms: an intercept (β0), main effects for each PC (β1 and β2), and an interaction term between the two PCs (β3). Let yi be the percentage leukemic burden in the ith studied mouse, let X1i be the ith mouse’s PC1 score, and let X2i be the ith mouse’s PC2 score. Then the regression model we fit can be written as: $y_i = \beta_0 + \beta_1X_{1i} + \beta_2X_{2i} + \beta_3(X_{1i}X_{2i}) + \epsilon_i$. The model was fit using the lm function in R (1). An overall F test clearly suggested that at least some of the coefficients in the model differed significantly from 0 (F statistic = 25.73 on 3 and 26 df; p < 0.0001). Specifically, the model detected strong relationships between the first two PCs and leukemic burden, as well as a marginal significant interaction effect in our dataset.

In general, the interpret term corresponds to expected leukemic burden for mice with average scores on both PC1 and PC2. Specifically, under this model we expect that mice with average PC1 and PC2 scores have an average leukemic burden of 62.64%. For mice with an average score of PC2, but who are 1 unit above average on PC1, we expect an average leukemic burden of 52.64% (62.64 − 10.00%). For mice with an average score on PC1, but who are 1 unit about average on PC2, we expect an average leukemic burden of 43.34% (62.64 − 19.30%). For mice that are 1 unit above average on both PC1 and PC2, we expect an average leukemic burden of 27.38% (62.64 − 10.00 − 19.30 = 9.56%).

**Results**

Adaptive immunity plays a role in the anti–BCR-ABL + B-ALL response

We previously showed that there was a higher fraction of live leukemic cells in the bone marrow and secondary lymphoid organs of OT-I × Rag2−/− mice than in C57BL/6 mice. Furthermore, the range of leukemic burdens was quite broad in the C57BL/6 hosts (interquartile range [IQR] = 11–69%) but less so in the OT-I × Rag2−/− hosts (IQR = 90–98%) (9). To understand why there was such a range in leukemic burden, we looked for characteristic differences in the leukemic cells from mice with low versus high leukemic burden. Because B cells can function as APCs, we examined the expression of MHC-II to stratify the leukemic burden based on expression of surface markers. MHC-II expression inversely correlated with leukemic burden (mice that had a low percentage of leukemic cells in the bone marrow had high MHC-II expression on the leukemic cells; Fig. 1A). We also examined the expression of the costimulatory molecules CD40, CD80, CD86, and PD1. None of these costimulatory molecules individually correlated with leukemic burden. Therefore, we used PCA to identify whether there was an ensemble of costimulatory molecules that correlated with leukemic burden (Fig. 1B). The first component described a positive correlation between MHC-II, CD80, CD86, and PD1. The second component was driven by a negative correlation between CD40 and PD1 (Fig. 1B). These first two components described 77% of the variation in leukemic burden that we observed in mice (Fig. 1C, Table I). Mice that had low leukemic burden tended to have high scores for both PC1 and PC2 (Fig. 1D). Therefore, we used linear regression to examine relationships between the first two PC scores and leukemic burden (Fig. 1E). Both high PC1 and high PC2 scores were associated with significantly decreased leukemic burden.
A low PC1 score (score of 2) was predictive of high leukemic burden (Fig. 1E, left panel) regardless of PC2 score. In contrast, leukemias with higher PC1 scores (score of 0–2) showed dependence on PC2 in predicting leukemic burden (Fig. 1E). Taken together, this analysis supports the conclusion that robust Ag presentation combined with CD80/86 costimulation (PC1), as well as a high ratio of CD40/PDL1 (PC2), correlates with improved disease outcome. Modulation of individual costimulatory molecules modestly improves survival of leukemic mice

Our PCA suggested that an ensemble of costimulatory molecules (CD80, CD86, PDL1, CD40) functioned as a cohesive unit to modulate antileukemia immunity. Nonetheless, it was possible that individual targeting of Ag presentation and costimulatory molecules might change the disease course. We tested whether Ab targeting of PDL1, CTLA4, or CD40 would be sufficient to change leukemia progression. Ab blockade of PDL1 and CTLA4 (either individually or in combination) led to a modest but significant increase in survival of leukemic mice (Fig. 2A–C). Additionally, treatment of leukemic mice with an anti-CD40 Ab that is characterized as an agonist also led to a modest yet significant increase in survival (19) (Fig. 2D). Thus, the components defined by our PCA do not individually identify very effective therapeutic targets in this model. These results support

FIGURE 1. Adaptive immunity plays a role in the anti-BCR-ABL° B-ALL response. (A) Representative dot plots and histograms from five mice with varying leukemic burden (gated as live, singlet, CD19°, B220low cells). Black curves are MHC-II, gray curves are isotype. Listed are the percentages of live singlet events that fall into the CD19°, B220low gate. (B) Correlations of measured variables with first two PCs. PDL1, MHC-II, CD80, and CD86 correlate positively with PC1; CD40 and (to a lesser extent) CD86 correlate positively, whereas PDL1 correlates negatively, with PC2. (C) Scree plots of PCs. The y-axis shows proportion of variance accounted for by each PC. (D) Distribution of individual mouse scores on PCs 1 and 2. Mouse leukemic burden is indicated by dot size and dot shade; larger white dots indicate mice with higher leukemic burden, and smaller black dots indicate mice with lower leukemic burden. (E) Predicted leukemic burden as a function of PC2 scores at three separate levels of PC1 (low, average, and high). Gray regions denote 95% confidence bounds. PCs were derived from 27 separate mice in three experiments.
the concept that the molecules identified by our PCA are best addressed as an ensemble.

**BAp-specific T cells can be primed by acute infection plus exogenous peptide**

None of the immune checkpoint modulations we tested substantially improved survival of leukemic mice. However, we have previously shown that Ly6C<sup>+</sup> BAp:I<sub>A<sup>b</sup>-specific T cells correlate with antileukemia immunity upon Treg depletion (9). We attempted to recreate an inflammatory environment to generate many Ly6C<sup>+</sup> BAp:I<sub>A<sup>b</sup>-specific T cells. To do this, we infected mice with LM+BAp, which caused a 65-fold increase in BAp:I<sub>A<sup>b</sup>-specific T cell numbers. In parallel, we infected mice with either LCMV-Armstrong or VSV-Indiana and then delivered 200 μg BAp i.v. at 3 and 5 d postinfection. This allowed us to use the inflammation caused by acute viral infection to induce a strong BAp:I<sub>A<sup>b</sup>-specific CD4<sup>+</sup> T cell response (termed LCMV+BAp or VSV+BAp). At peak infection LCMV+BAp caused a 74-fold proliferation of BAp:I<sub>A<sup>b</sup>-specific T cells, whereas VSV+BAp caused a 114-fold proliferation of BAp:I<sub>A<sup>b</sup>-specific T cells (Fig. 3A). These results show that BAp:I<sub>A<sup>b</sup>-specific T cell proliferation can be initiated by immunization. Additionally, we found that LCMV+BAp induced a high frequency of Ly6C<sup>+</sup> memory BAp:I<sub>A<sup>b</sup>-specific T cells following leukemia rechallenge, whereas LM+BAp induced substantially fewer Ly6C<sup>+</sup> memory BAp:I<sub>A<sup>b</sup>-specific T cells following leukemia rechallenge (Fig. 3B). Because our previous work showed that Ly6C was expressed on most BAp:I<sub>A<sup>b</sup>-specific T cells upon Treg depletion (which also resulted in significantly less leukemic burden and significantly longer survival of leukemic mice), we reasoned that acute viral infections that result in increased Ly6C expression might induce protective BAp-specific immunity.

**BAp-specific adaptive immunity confers long-term survival for leukemic mice**

Our PCA suggested that MHC-II expression, and thus Ag presentation, was important in describing the immune response to leukemia. We have identified one peptide from BCR-ABL (BAp) that is processed and presented on MHC-II in vivo (9). Thus, we hypothesized that immunization with BAp plus strong adjuvants might mediate protection from BCR-ABL<sup>a</sup> B-ALL in mice. To test this, we infected mice with either LCMV-Armstrong (with and without BAp) or VSV-Indiana (with and without BAp) and rechallenged mice with BCR-ABL<sup>a</sup> leukemia >40 d later, a memory time point when no acute inflammation remained (Fig. 3C). Mice that were infected with an acute viral pathogen plus BAp survived long-term. In contrast, mice that were infected with an acute viral pathogen in the absence of BAp succumbed to leukemia rapidly. The hazard ratio comparing all “+BAp” vaccinations to all “−BAp” vaccinations in Fig. 3C was 0.24 with a 95% CI from 0.12 to 0.46 (p < 0.0001). Thus, BAp-specific adaptive immunity confers long-term survival in this model. Because BAp only binds to MHC-II and not MHC class I (data not shown), our results support the conclusion that BAp:I<sub>A<sup>b</sup>-specific T cells are critical for protecting against BCR-ABL<sup>a</sup> leukemia in our prophylactic vaccination studies.

**IFN-γ potentiates antileukemia immunity during prophylactic vaccination**

In CD4<sup>+</sup> T cells, IFN-γ is normally produced by Th1 cells, which can have a role in antitumor immunity (20). We have previously shown that in unvaccinated mice most BAp:I<sub>A<sup>b</sup>-specific T cells responding to BCR-ABL<sup>a</sup> leukemia are Tregs and thus are likely not making IFN-γ. Consistent with this idea, we found that the ability of host T cells to make IFN-γ in unvaccinated mice did not affect survival following leukemia inoculation, because ifng<sup>−/−</sup> hosts succumbed to leukemia similarly to C57BL/6 hosts (Fig. 3D). However, we hypothesized that IFN-γ might play a role in the adaptive immune response to leukemia following prophylactic vaccination. To

| Table I. SD, proportion of variance, and cumulative proportion accounted for by each PC for the data plotted in Fig. 1 |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | PC1  | PC2  | PC3  | PC4  | PC5  |
| SD             | 1.67 | 1.05 | 0.73 | 0.73 | 0.26 |
| Proportion of variance | 0.55 | 0.22 | 0.11 | 0.11 | 0.014 |
| Cumulative proportion | 0.55 | 0.77 | 0.88 | 0.99 | 1.00 |

PC1 and PC2 together described 77% of the variation in leukemic burden.

**FIGURE 2.** Modulation of individual Ag presentation and costimulation molecules improves survival of leukemic mice. (A) C57BL/6 mice were inoculated with 2500 leukemic cells and treated every other day with 100 μg anti-PDL1 until moribund. (B) Mice were treated as in (A), except with 100 μg anti-CTLA4. (C) Mice were treated as in (A), except with 100 μg anti-PDL1 plus 100 μg anti-CTLA4. (D) Mice were treated as in (A), except with 200 μg anti-CD40. Two or more independent experiments with four or more replicates are shown in each group; the log-rank (Mantel–Cox) test was used to establish significance in all panels.
test this, we vaccinated Ifng2/2 mice with LCMV-Armstrong+BAp and rechallenged with leukemia at >40 d postvaccination. Vaccination of IFN-γ-deficient mice did not increase survival following challenge with leukemia, when compared with unvaccinated control mice. In contrast, vaccination of Ifng-replete mice resulted in significant long-term survival when compared with unvaccinated controls (Fig. 3E). Thus, IFN-γ production was one mechanism required for effective antileukemia immunity following prophylactic vaccination.

**Specific pathogens mediate effective prophylactic vaccination for BCR-ABL+ B-ALL**

Immune memory is a critical component of prophylactic vaccination. To determine whether effective BAp:1-Aβ-specific memory T cells were formed by vaccination, we infected mice with LM+BAp or LCMV+BAp and waited 40 d to enumerate BAp:1-Aβ-specific memory T cells. We recovered significantly more memory BAp:1-Aβ-specific T cells from LCMV+BAp-infected mice than from
LM+BAp–infected mice (Fig. 4A). We then vaccinated mice with either LCMV+BAp or LM+BAp and rechallenged them by transferring 2500 leukemic cells into the mice 30 d later. Vaccination with LCMV+BAp, but not LM+BAp, led to a significant increase in the number of BAp:I-A<sup>b</sup>–specific T cells following leukemia challenge and decreased leukemic burden (4-fold, Fig. 4B, 4C). Thus, the increase in BAp:I-A<sup>b</sup> memory T cell numbers following LCMV+BAp but not LM+BAp vaccination correlated with disease outcome.

The quality and quantity of BAp:I-A<sup>b</sup>–specific memory T cells was different comparing LM+BAp vaccination to LCMV+BAp vaccination. We observed that the IQR of leukemic burdens in the mice vaccinated with LCMV+BAp was broad (IQR = 1.4 × 10<sup>6</sup>, Fig. 4C), showing that protection mediated by LCMV+BAp vaccination was more effective in some mice than in others. We previously observed that Ly6C expression was increased on BAp:I-A<sup>b</sup>–specific T cells when Tregs were depleted (9). Therefore, we examined whether Ly6C expression on BAp:I-A<sup>b</sup>–specific T cells correlated with leukemic burden. Mice with high leukemic burden despite prophylactic vaccination (and thus considered “failed vaccinated mice”) had a significantly lower percentage of Ly6C<sup>+</sup> BAp:I-A<sup>b</sup>–specific T cells than did the “successfully vaccinated mice” (Fig. 4D). Additionally, significantly more BAp:I-A<sup>b</sup>–specific T cells expressed Ly6C after LCMV+BAp vaccination (which lowered leukemic burden) than did LM+BAp (which had no effect on leukemic burden) (Fig. 3B). Importantly, the number of Ly6C<sup>+</sup> BAp:I-A<sup>b</sup>–specific T cells was inversely correlated with leukemic burden in LCMV+BAp–vaccinated mice (Fig. 4E). In contrast, leukemic burden did not correlate with total CD4<sup>+</sup>Ly6C<sup>+</sup> cells in these mice (Fig. 4F). We also observed that Tregs made up a smaller portion of the BAp:I-A<sup>b</sup>–specific T cell population in mice that were prophylactically vaccinated with LCMV+BAp than in unvaccinated mice (Fig. 4G). These results support a functional role for Ly6C<sup>+</sup>FOXP3<sup>+</sup> BAp:I-A<sup>b</sup>–specific T cells during the immune response to leukemia following prophylactic vaccination.

**Ag presentation and costimulation on leukemic cells are modulated by prophylactic vaccination**

Our PCA suggests that high ratios of CD40/PDL1 and MHC-II/PDL1 may be predictive of low leukemic burden. We examined the leukemic cells from LCMV+BAp–vaccinated mice and LM+BAp–vaccinated mice. First, we found that leukemias from mice that were successfully vaccinated with LCMV+BAp had higher expression of CD40 and MHC-II than did their “failed vaccination” counterparts (Fig. 5A, 5B). Second, we found that CD40/PDL1 and MHC-II/PDL1 increased on LCMV+BAp–vaccinated mice (which was an effective vaccination regimen, Fig. 5C, 5D) but not significantly on LM+BAp–vaccinated mice (an ineffective

**FIGURE 4.** Prophylactic vaccination induces protective immune responses against BCR-ABL<sup>+</sup> leukemia. (A) Mice were infected as in Fig. 3A and rested 30 d, when BAp:I-A<sup>b</sup>–specific T cells counts were compared with those in naive mice. Shown are BAp:I-A<sup>b</sup>–specific log(y + 1) T cell counts of BAp:I-A<sup>b</sup>–specific memory cells following vaccinations, gated on CD11a<sup>hi</sup>CD44<sup>hi</sup> cells. (B) Mice were unvaccinated or vaccinated with LCMV+BAp or LM+BAp, and 2500 BCR-ABL<sup>+</sup> cells were transferred 30 d postinfection. Shown are BAp:I-A<sup>b</sup>–specific log(y + 1) T cell counts; two or more independent experiments are shown for each infection. (C) Mice were treated as in (B), and leukemic burden was analyzed. Lines are median values; numbers represent fold changes in median. (D) Percentage Ly6C<sup>+</sup> on BAp:I-A<sup>b</sup>–specific T cells harvested from LCMV+BAp–vaccinated mice. (E) Ly6C<sup>+</sup> BAp:I-A<sup>b</sup>–specific T cell count negatively correlates with leukemic burden from secondary lymphoid organs. Spearman correlation r = −0.8201, p < 0.05. (F) Ly6C<sup>+</sup>CD4<sup>+</sup> T cell count does not correlate with leukemic burden from secondary lymphoid organs. Spearman correlation r = 0.3515, p > 0.05. (G) Percentage BAp:I-A<sup>b</sup>–specific Tregs recovered from leukemic Foxp3-GFP mice unvaccinated or vaccinated with LCMV+BAp. All comparisons were done by Kruskal–Wallis and Dunn tests (more than two groups) or a Mann–Whitney U test (two groups). Two or more independent experiments with four or more replicates are shown for each group.
vaccination regimen, Fig. 5E, 5F). Thus, prophylactic vaccination with acute viral pathogens plus BAp results in protection from leukemia and correlates with expression of the biomarkers that we previously demonstrated were linked to strong antileukemia immune responses (Fig. 1).

**Therapeutic heterologous vaccination drives long-term survival**

We hypothesized that a proinflammatory environment might counter leukemia-derived immune suppression while also inducing BAp-specific adaptive immunity, and thus inhibit leukemia progression. To test this hypothesis, we therapeutically vaccinated mice, which had established leukemia, using either homologous vaccinations with LCMV-Armstrong+BAp or heterologous vaccinations with LCMV-Armstrong+BAp, LM+BAp, and VSV-Indiana+BAp (Fig. 6A). Homologous vaccination with LCMV+BAp significantly prolonged survival, although all mice ultimately succumbed to leukemia. Heterologous vaccination should create a more robust proinflammatory response, because Abs created during the primary infection will not neutralize the secondary and tertiary infections. Indeed, heterologous vaccination was significantly more effective and led to long-term survival (more than twice the median untreated survival) in ∼10% of mice. Thus, repeated vaccination with heterologous agents was an effective treatment strategy in mice with BCR-ABL+ B-ALL.

The immune response to acute viral and bacterial infection is canonically proinflammatory. However, because mice with active leukemia have high doses of leukemia Ags during this proinflammatory state, this may cause chronic Ag stimulation, a situation where PDL1 signaling is highly expressed (21). Additionally, our initial findings showing that CD44 was not highly expressed on all BAp:I-A^b^-specific T cells responding to leukemia suggest that BAp-specific T cell priming is not optimal (9). CTLA4 blocks interaction of CD28 with B7-1 and B7-2 molecules, thereby reducing T cell functionality (22–24). Thus, we hypothesized that therapeutically vaccinated mice that were treated with dual PDL1/CTLA4 checkpoint blockade might show improved survival. This treatment strategy led to a significant increase in survival beyond that seen for either PDL1+CTLA4 blockade (Fig. 2C) or therapeutic vaccination (Fig. 6A), with 31% of mice surviving long-term. Because this long-term survival is far past the time point when inflammation would remain from the therapeutic vaccination, it suggests that an adaptive immune response is mediating long-term survival.

To understand some of the mechanisms allowing effective therapeutic vaccination, we compared homologous, heterologous, and heterologous plus checkpoint blockade treatments and assessed BAp:I-A^b^-specific T cell expansion and effector function. To do this, we inoculated mice with BCR-ABL+ leukemia and started therapeutic vaccination at the same time point. We then harvested the mice 21 d later and enumerated BAp:I-A^b^-specific T cells. We found that all regimens induced robust proliferation (∼1250-fold over naive precursor numbers); however, there was no difference in the number of BAp:I-A^b^-specific T cells recovered between any of the three treatment groups (Fig. 6B). Because total numbers of BAp:I-A^b^-specific T cells did not help give insight into the mechanisms that allowed effective therapeutic vaccination, we

![Figure 5](https://example.com/figure5.png)

**Figure 5.** Prophylactic vaccination induces Ag presentation and costimulation on leukemic cells. (A) Mice were vaccinated with LCMV+BAp and inoculated with leukemia 30 d later. CD40 mean fluorescence intensity from leukemic cells harvested from successfully vaccinated mice or failed vaccinated mice derived from Fig. 4C. A Mann–Whitney U test was used to establish significance. (B) MHC-II mean fluorescence intensity from leukemic cells harvested from successfully vaccinated mice or failed Vaccinated mice. A Mann–Whitney U test was used to establish significance. (C) Ratio of mean fluorescence intensity of CD40/PDL1 on leukemic cells was calculated from mice vaccinated with LCMV+BAp, and a correlation was calculated between this ratio (x-axis) and the log leukemic cell count (y-axis). (D) Ratio of mean fluorescence intensity of MHC-II/PDL1 on leukemic cells was calculated from mice vaccinated with LCMV+BAp, and a correlation was calculated between this ratio (x-axis) and the log leukemic cell count (y-axis). (E) Ratio of mean fluorescence intensity of CD40/PDL1 on leukemic cells was calculated from mice vaccinated with LCMV+BAp, and a correlation was calculated between this ratio (x-axis) and the log leukemic cell count (y-axis). (F) Ratio of mean fluorescence intensity of MHC-II/PDL1 on leukemic cells was calculated from mice vaccinated with LCMV+BAp, and a correlation was calculated between this ratio (x-axis) and the log leukemic cell count (y-axis). The values on graphs are from the Spearman correlation test. Two or more independent experiments with four or more replicates are shown for each group.
examined the phenotype of the BAp:I-A\*b–specific T cells. We reasoned that BAp:I-A\*b–specific T cells should take on a more Th1-like phenotype in response to therapeutic vaccination, and that this phenotype should correlate with improved disease outcome. However, we found no significant increase in IFN-\(\gamma\) or TNF-\(\alpha\) (two canonical Th1 cytokines) when comparing all three treatment groups. Interestingly, in mice that received heterologous vaccination plus checkpoint blockade, we found that a larger fraction of BAp:I-A\*b–specific T cells produced more IL-10, granzyme B, and both IL-17 and granzyme B together (Fig. 7A, 7C–F), all of which have previously been associated with proinflammatory tumor clearance (25–28). Additionally, we found that PD1 expression on BAp:I-A\*b–specific T cells positively correlated with leukemic burden in all therapeutically vaccinated mice (Fig. 7B), and that PD1 expression was lowest on these cells in heterologous vaccination plus checkpoint blockade–treated mice (Fig. 7A). Taken together, these results demonstrate that polyfunctional CD4\(^+\) leukemia-specific T cells produce a combination of IL-10, IL-17, and granzyme B, and this correlated with effective antileukemia adaptive immunity.

**Discussion**

BCR-ABL\(^+\) B-ALL is only transiently responsive to current therapies (29), with a 5-y survival of \(\sim\)35% (30, 31). Given this low survival rate, additional therapies are needed. One approach that has not been well explored in this disease is checkpoint...
blockade–based immunotherapy. Checkpoint blockade works well in malignancies with many nonsynonymous mutations and can lead to improved long-term survival in patients with such cancers (4, 32–36). Presumably, this is because the increased number of nonsynonymous mutations allows for a concurrent increase in the number of neoantigen-specific T cells. Comparatively, in cancers with low numbers of nonsynonymous mutations, such as B-ALL (36), checkpoint blockade–based immunotherapy targeting the endogenous immune response is relatively unstudied. In fact, current paradigms suggest that cancers with low numbers of nonsynonymous mutations may not be effectively treated using anti-CTLA4 and anti-PD1 checkpoint blockade (35). In the present study, we show that an endogenous T cell response can be effective in controlling BCR-ABL+ B-ALL, but this requires both checkpoint blockade and an intensive heterologous vaccination strategy.

In this study, we found that a strong immune response correlated with decreased leukemia burden. MHC-II expression on leukemic cells correlated with disease outcome, which hinted that CD4+ T cells were important for antileukemia immunity. We have previously shown that the leukemia Ag BAp is presented on MHC-II (9). In contrast, BAp does not bind to MHC class I in C57BL/6 mice (data not shown). These findings, in combination with our studies showing that the presence of BAp during prophylactic vaccination is required for protection from leukemia (Fig. 3C), provide evidence supporting a role for MHC-II–mediated presentation of BAp in antileukemia immunity. Moreover, an effective antileukemia immune response requires IFN-γ and correlates with increased induction of Ly6C on the BAp:I-Aβ–specific T cells (Fig. 3D, 3E).

Our PCA strongly suggested a role for targeting the immune checkpoint molecules PD1L and CTLA4 as part of a therapeutic strategy for BCR-ABL+ B-ALL. Costimulatory and coinhibitory molecules play a role in cancer progression (4, 37–42). In our model we observed statistically significant increases in survival upon monotherapy with either anti-PD1L or anti-CTLA4, or dual checkpoint blockade therapy with both anti-PD1L and anti-CTLA4. However, despite achieving statistical significance, the effects of checkpoint blockade alone were modest (increased survival of 2–4 d), and thus possibly of limited biological impact. The limited impact of checkpoint blockade treatment alone in BCR-ABL+ leukemia fits prevailing concepts regarding checkpoint blockade. Current models suggest that cancers with low numbers of nonsynonymous mutations will not be susceptible to checkpoint blockade (35, 36). Our leukemia model likely has few nonsynonymous mutations, and checkpoint blockade is not very effective in this leukemia model. Thus, our observations are consistent with the idea that small numbers of nonsynonymous mutations result in poor anticancer responses following checkpoint blockade therapy.

Immune checkpoint blockade therapy alone was only minimally effective in treating leukemic mice in our model. Thus, we explored therapeutic vaccination immunotherapy. Two lines of evidence precipitated this strategy. First, previous reports show that therapeutic heterologous vaccination can be effective in other cancers, albeit those with higher mutation rates (43). Second, it was clear that MHC-II–mediated Ag presentation was important for leukemia outcome, and the pathogens used in our therapeutic vaccination scheme all induce MHC-II expression on APCs (44–46). When mice were therapeutically vaccinated with these MHC-II–inducing proinflammatory pathogens, we saw increased survival (Fig. 6). We used heterologous vaccination because this approach has previously been shown to be effective at inducing a robust T cell response (47–49). In this approach, we used multiple infectious adjuvants to generate a proinflammatory environment that should promote robust adaptive immune activation. Similar approaches have been used prophylactically (47) and therapeutically for cancer (43, 49). However, our study examines therapeutic heterologous vaccination in combination with checkpoint blockade specifically to target CD4+ T cells in cancer, an underexplored field.

We found that therapeutic vaccination synergized with anti-PDL1 and anti-CTLA4 therapies to improve long-term survival in mice with BCR-ABL+ leukemia. Thirty-one percent of the mice that received therapeutic heterologous vaccination in combination with anti-PDL1 and anti-CTLA4 checkpoint blockade exhibited long-term survival. In contrast, only 10% of mice treated with therapeutic heterologous vaccination alone survived long-term. Furthermore, no leukemia-bearing mice treated therapeutically with checkpoint blockade alone exhibited long-term survival. These results suggest that even malignancies with few nonsynonymous mutations (such as B-ALL) can be responsive to immunotherapies that classically work well only in malignancies with high levels of nonsynonymous mutations (35). Importantly, such results are contingent upon intensive therapeutic vaccination approaches. One possible explanation for the synergistic effect of vaccination plus checkpoint blockade is that leukemia-derived Ag is available for the entire duration of the therapeutic vaccination regimen. This chronic Ag stimulation may lead to continual high expression of PD1L and CD80/86 on leukemic cells, which may explain the synergy between therapeutic vaccination (which is susceptible to inhibition by PD1L/PD1 and CD80/86/CTLA4 pathways) and dual checkpoint blockade (which inhibits those pathways). Finally and importantly, note that oncolytic viruses (which include VSV, used in our scheme) have been used for anticancer immunotherapy in the past (50) and are currently being used in clinical trials as a treatment option for cancer (51, 52). Thus, the approach taken to treat leukemia in our murine model is feasible to consider for human patients with BCR-ABL+ leukemia.

Our data provide initial mechanistic insights into how therapeutic vaccination therapy plus checkpoint blockade can lead to leukemia rejection by the C57BL/6 host. First, we saw a trend toward decreased PD1 expression on BAp:I-Aβ–specific T cells that correlated significantly with decreased leukemic burden (Fig. 7A, 7B). Checkpoint blockade could interfere with this potential mechanism of tolerance induction. Second, during the therapeutic vaccination response, we saw that many BAp:I-Aβ–specific T cells were polyfunctional (producing granzyme B and multiple cytokines such as IFN-γ, TNF-α, IL-10, and IL-17). Importantly, in the most effective vaccination regimen (therapeutic heterologous vaccination plus checkpoint blockade), we saw a significantly increased fraction of BAp:I-Aβ–specific T cells that produced granzyme B, IL-10, and a combination of granzyme B plus IL-17. This observation demonstrates that effective therapeutic vaccination induces formation of polyfunctional leukemia-specific CD4+ T cells. Future studies are needed to delineate the importance of these cytokines and granzyme B expression. However, it is intriguing that although IL-10 is more typically associated with immunosuppression, previous literature supports a role for T cell–derived IL-10 in antitumor immunotherapy (25, 53, 54). Additionally, IL-17 and granzyme B have both been implicated in T cell responses to cancer (26, 28, 55). Thus, we envision two possibilities for how and when BAp:I-Aβ–specific T cells might elicit antileukemia immunity after therapeutic vaccination. First, because the most effective therapeutic vaccination regimen we used (heterologous vaccination plus checkpoint blockade) yielded the greatest fraction of granzyme B–producing BAp:I-Aβ–specific T cells, it is possible that these cells directly kill MHC-II+ BCR-ABL+ leukemic cells. Second, it is possible these polyfunctional
BAp:I-Ab–specific T cells induce BAp:I-Ab–specific memory T cells, which may be required for long-term leukemia control. In support of this idea, Th17 cells responding to tumors in other models have a long lifespan, which may be associated with memory formation (28, 56). Therefore, our observations support the idea that polyfunctional BAp:I-Ab–specific T cells are induced...
by intensive therapeutic vaccination, and that these cells contribute to effective leukemia control.

The current paradigm suggests that neoantigen-specific T cells respond better to tumors because the repertoire of these cells have not been pruned by thymic central tolerance (36). This idea implies that cross-reactive T cells will respond poorly to tumors because the repertoires of these cells have been limited by thymic central tolerance. We have previously shown that BAp-I-A*-specific T cells are cross-reactive with self-Ag and that the BAp-I-A*-specific T cell repertoire is limited by thymic central tolerance (9). Nonetheless, we observed in this study that BAp-specific adaptive immunity is crucial for antileukemia immunity following prophylactic vaccination. Thus, our observations provide a counterpoint to the idea that neoantigen-specific T cells are a prerequisite for effective endogenous anticancer T cell responses (36).

Taken broadly, our observations suggest that fusion proteins created by chromosomal translocations may be viable immunotherapies targets even when the fusions do not create neoantigens. This is particularly relevant because chromosomal translocations often result in “driver” mutations, thus leaving minimal opportunity for cancer immuneediting to occur.

Checkpoint blockade is thought to work best in tumors with high numbers of nonsynonymous mutations (35). Our results support this concept, as checkpoint blockade was only minimally effective in B-ALL, a leukemia that generally has lower numbers of nonsynonymous mutations (5). However, we also demonstrate that intensive heterologous vaccination synergizes with checkpoint blockade to unmask a strong immune response that is capable of controlling this highly aggressive and uniformly fatal form of leukemia in mice. In conclusion, our work establishes that immunotherapy approaches can induce long-term survival with B-ALL, even though mice with B-ALL are refractory to checkpoint blockade–based immunotherapy (36).

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Disclosures

R.T.W. is currently an employee at Aegon, Inc. The work in this manuscript was completed prior to his accepting a position there. His previous employer, Puma Biotechnology, has no interests in leukemia, and thus we do not think there is an inherent conflict of interest. The interaction with R.T.W. dates back to the time when he was an Asst. Prof. at St. Jude Children’s Research Hospital (Memphis, TN). The other authors have no financial conflicts of interest.

References


Appendix J
Evidence for Strain-Specific Immunity to Pneumonia in Bighorn Sheep
ABSTRACT  Transmission of pathogens commonly carried by domestic sheep and goats poses a serious threat to bighorn sheep (Ovis canadensis) populations. All-age pneumonia die-offs usually ensue, followed by asymptomatic carriage of Mycoplasma ovipneumoniae by some of the survivors. Lambs born into these chronically infected populations often succumb to pneumonia, but adults are usually healthy. Surprisingly, we found that introduction of a new genotype (strain) of M. ovipneumoniae into a chronically infected bighorn sheep population in the Hells Canyon region of Washington and Oregon was accompanied by adult morbidity (100%) and pneumonia-induced mortality (33%) similar to that reported in epizootics following exposure of naïve bighorn sheep. This suggests an immune mismatch occurred that led to ineffective cross-strain protection. To understand the broader context surrounding this event, we conducted a retrospective analysis of M. ovipneumoniae strains detected in 14 interconnected populations in Hells Canyon over nearly 3 decades. We used multi-locus sequence typing of DNA extracts from 123 upper respiratory tract and fresh, frozen, and formalin-fixed lung samples to identify 5 distinct strains of M. ovipneumoniae associated with all-age disease outbreaks between 1986 and 2014, a pattern consistent with repeated transmission events (spillover) from reservoir hosts. Phylogenetic analysis showed that the strain associated with the outbreak observed in this study was likely of domestic goat origin, whereas strains from other recent disease outbreaks probably originated in domestic sheep. Some strains persisted and spread across populations, whereas others faded out or were replaced. Lack of cross-strain immunity in the face of recurrent spillovers from reservoir hosts may account for a significant proportion of the disease outbreaks in bighorn sheep that continue to happen regularly despite a century of exposure to domestic sheep and goats. Strain-specific immunity could also complicate efforts to develop vaccines. The results of our study support existing management direction to prevent contacts that could lead to pathogen transmission from domestic small ruminants to wild sheep, even if the wild sheep have previously been exposed. Our data also show that under current management, spillover is continuing to occur, suggesting that enhanced efforts are indicated to avoid introducing new strains of M. ovipneumoniae into wild sheep populations. We recommend looking for new management approaches, such as clearing M. ovipneumoniae infection from domestic animal reservoirs in bighorn sheep range, and placing greater emphasis on existing strategies to elicit more active cooperation by the public and to increase vigilance on the part of resource managers. © 2016 The Wildlife Society.

KEY WORDS bighorn sheep, disease ecology, Hells Canyon, livestock-wildlife interface, molecular epidemiology, multi-locus sequence typing, Mycoplasma ovipneumoniae, Ovis canadensis.
threats and potential solutions will help wildlife and land managers make appropriate risk management decisions that will succeed in resolving the problem of pneumonia in bighorn sheep.

Based on the hypothesis that \textit{Mannheimia haemolytica} expressing leukotoxin is the key causal pathogen, researchers have tested numerous vaccines to boost immunity to disease in bighorn sheep. However, so far no vaccine has protected wild sheep commingled with domestic sheep or goats in captive settings or shown potential for efficacy in free-ranging animals (Callan et al. 1991, Kraabel et al. 1998, Cassirer et al. 2001, Subramaniam et al. 2011, Sirochman et al. 2012). There may be several reasons for the elusiveness of an effective vaccine. First, there is a basic question as to the role of \textit{M. haemolytica} in the disease (Besser et al. 2013) and second there are significant technical difficulties associated with vaccine development and application. Experimental challenge with leukotoxin-positive \textit{M. haemolytica}, a well-described respiratory pathogen in domestic ruminants, is lethal to bighorn sheep in captivity (Foreyt et al. 1994, Dassanayake et al. 2009). However, \textit{M. haemolytica} is only weakly associated with pneumonia epizootics in free-ranging bighorn sheep populations (Besser et al. 2012b). The pathology, microbiology, and the course of disease experimentally induced with \textit{M. haemolytica} also do not match observations from the field (Besser et al. 2014). Recently, application of sensitive molecular diagnostic techniques on high quality samples led to identification of \textit{Mycoplasma ovipneumoniae}, a previously overlooked bacterium, as the pathogen most strongly supported as a primary causal agent of pneumonia in bighorn sheep (Besser et al. 2008, 2012a, b).

\textit{M. ovipneumoniae} is host-specific to Caprinae, and is frequently carried asymptptomatically by domestic sheep and goats (Martin and Aitken 2000). When introduced into naive bighorn sheep populations, outbreaks of polymicrobial pneumonia ensue, sometimes resulting in high mortality in all age classes (Besser et al. 2008, 2014). After all-age pneumonia outbreaks, surviving adults usually maintain good health and normal life spans, although some individuals chronically carry \textit{M. ovipneumoniae} in their upper respiratory tract (Besser et al. 2013). Both carriers and non-carriers are resistant to disease although this protection fails to prevent epizootics in lambs (Plowright et al. 2013, Manlove et al. 2016).

\textit{M. ovipneumoniae} is also associated with mild and transient respiratory disease, usually in juveniles, in its normal domestic sheep and goat hosts (DaMassa et al. 1992, Martin and Aitken 2000). However, several investigators have reported that \textit{M. ovipneumoniae} infections in domestic sheep and goats can cause severe pneumonia, particularly when multiple strains are present (Parham et al. 2006, Rifiatbegović et al. 2011). This could be linked to a strain-specific immune response that fails to provide universal protection. Many pathogens are able to evade host immune responses by expressing a diversity of surface-exposed targets for neutralizing antibodies. From influenza virus to \textit{Mycoplasma} spp., antigenic variation within and across strains enables immune escape by pathogens and also complicates development of vaccines (Citti et al. 2010, Vink et al. 2012, Quiñones-Parra et al. 2014).

We documented the effects of invasion of a novel strain of \textit{M. ovipneumoniae} into a group of free-ranging bighorn sheep that had harbored adult carriers for nearly 20 years following an all-age pneumonia outbreak (Cassirer et al. 1996, Plowright et al. 2013). Our expectation was that these adults were immune to the pathogen and that invasion of a new strain would not cause disease.

**STUDY AREA**

We conducted this study near Heller Bar at the mouth of the Grande Ronde River in Asotin County, Washington, USA (46.079° N, −116.986° W). The area was located in low elevation (250–1,250 m) canyon grasslands and cliffs along the breaks of the Grande Ronde and Snake Rivers on the northern edge of Hells Canyon. Summers were hot (x highs in Jul and Aug = 26–32°C) and winters were mild (x lows in Dec and Jan = −2 to 2°C). Average annual precipitation was 31 cm. July and August were the driest months and peak precipitation occurred in May. Plant associations were dominated by perennial bunchgrass (\textit{Pseudoroegneria spicata} and \textit{Festuca idahoensis}) communities, with deciduous riparian shrub strings and upland shrub-fields. Douglas-fir (\textit{Pseudotsuga menziesii}) and ponderosa pine (\textit{Pinus ponderosa}) stands occurred on northerly aspects. In addition to bighorn sheep, common ungulates in the study area included mule deer (\textit{Odocoileus hemionus}), white-tailed deer (\textit{O. virginianus}), and elk (\textit{Cervus elaphus}). Potential predators of bighorn sheep included cougars (\textit{Felis concolor}), bobcats (\textit{Lynx rufus}), coyotes (\textit{Canis latrans}), wolves (\textit{Canis lupus}), and black bears (\textit{Ursus americanus}). Over 50% of the area was publicly owned and managed by federal and state agencies chiefly for wildlife, recreation, and seasonal (spring) cattle grazing. A low density, unincorporated rural community was scattered on adjoining private rangelands at the mouth of the Grande Ronde River and along the adjacent Snake River.

Following extirpation in the early 1900s, the Washington Department of Fish and Wildlife reintroduced bighorn sheep to the Joseph Creek Wildlife Area near Heller Bar. Between 1977 and 1989, 39 sheep were translocated from Washington, Oregon, and Montana to establish the Black Butte population (Johnson 1995). This became one of 16 interconnected populations that comprise the Hells Canyon bighorn sheep metapopulation. The Black Butte population increased to approximately 215 animals before a pneumonia outbreak occurred in 1995; 70% of the sheep died or were transferred to captivity in an attempt to stop the epidemic (Cassirer et al. 1996). The source of the outbreak was thought to be domestic sheep or goats on private lands within the Black Butte bighorn sheep population range (Rudolph et al. 2003). The population never recovered because of chronically low recruitment due to pneumonia-induced mortality in lambs (Plowright et al. 2013). By 2013, only 36 bighorn sheep were observed in surveys, and the population was estimated at 45 (Cassirer et al. 2013, Washington Department of Fish and Wildlife, unpublished data). Three
spatially distinct female groups occur in the population: Heller Bar, Shumaker, and Joseph Canyon. The groups are connected by movements of males, but we observed no female interactions across groups during this study. The Heller Bar female group was most accessible from the road and was the subject of this investigation.

METHODS

Observations
We monitored the Heller Bar female group during 2013 and 2014 as part of a study of contact patterns and lamb survival. We could individually identify 4 (31%) and 11 (85%) of the 13 adult females in 2013 and 2014, respectively, by numbered ear tags and color-coded and numbered very high frequency (VHF) radio-collars. One unmarked female was missing a horn, so all 13 sheep were individually identifiable in 2014. We located marked animals from the ground by radio-telemetry and then observed them through binoculars and a spotting scope. We conducted frequent and intensive observations between 1 May and 16 July to document productivity and neonatal survival (2013 median observation interval = 4 days, median duration of each observation = 3 hr; 2014 median observation interval = 1.5 days, median duration = 2 hr). Frequency of observation from 17 July through 26 August was every 10 days in 2013 and every 5 days in 2014, and from 26 August through the first week in October we observed the sheep once a month in 2013 and every 10 days in 2014. Median duration of observations from 17 July through October was 1 hour in both years. At each observation we recorded female and lamb health and behavior. Animals observed with nasal discharge, droopy ears, head shaking, or lethargy received a clinical score of 1, and animals observed coughing received a clinical score of 2. Sheep with no evidence of disease received a clinical score of 0.

Radio-collars on adults were equipped with a switch that triggered a fast pulse mortality signal if no movement was detected for 4 hours. We conducted site investigations, and where possible, retrieved carcasses when whole when mortalities were detected. Where this was not possible, we conducted field necropsies and collected the head, the respiratory tract, and grossly abnormal tissues when available. We detected lamb mortalities through observation and retrieved whole dead lambs when autolysis was not too advanced for diagnostic testing (Cassirer and Sinclair 2007). We assigned lamb mortality dates as the midpoint between the last live observation and either the date when the carcass was found, or the date when the dam was first observed without a lamb if no carcass was located. We assumed a female had lost her lamb when it was found dead or when the number of lambs declined and she was never again observed with a lamb that year. All cadavers and tissues were submitted to the Washington Animal Disease and Diagnostic Laboratory (WADDL; Pullman, WA, USA) for analysis.

Health Sampling
We captured and sampled females in February (n = 11 of 13) and July (n = 2 of 11) 2014. In October 2014, we resampled all 8 remaining sheep when we transferred them to captivity. We conducted captures via helicopter netgun and by darting from the ground with chemical immobilizing agents. All capture and handling followed animal care protocols approved by the Washington Department of Fish and Wildlife. Sampling entailed collecting throat swabs and placing them in buffered glycerol or Port-a-cul transport media (Becton, Dickinson, and Company, Franklin Lakes, NJ, USA, #220144, #221606) for aerobic culture to detect Pasteurellaceae, swabbing nasal passages and placing swabs in mycoplasma broth (#102, Hardy Diagnostics, Santa Maria, CA, USA) for culture enrichment and polymerase chain reaction (PCR) detection of M. ovipneumoniae (Ziegler et al. 2014), and collection of serum for detection of antibodies to M. ovipneumoniae (competitive enzyme-linked immunosorbent assay [ELISA]), bovine respiratory syncytial virus (virus neutralization [VN]), bovine virus diarrhea (VN), infectious bovine rhinotracheitis (VN), and bovine parainfluenza-3 (PI-3, VN). Diagnostic testing on the above samples was conducted by WADDL.

We also collected upper respiratory washes by flushing nasal passages with 50 ml of phosphate-buffered saline and swabbed the oropharynx. We kept samples cool and processed them within 48 hours of collection, or stored them at −20° C. We extracted DNA (DNeasy, Qiagen, Redwood City, CA, USA) from swabs, from 10-ml aliquots of nasal wash, and from lung tissues of animals that died during the study as well as 2 pneumonic lambs that died in adjacent populations in 2013 to test for presence of pneumonia agents. We used a multiplex PCR to detect Pasteurellaceae including Bibersteinia trehalosi, Pasteurella multocida, and Mannheimia spp. (Besser et al. 2012b) and performed PCR for lktA, the gene encoding leukotoxin A, the major virulence factor of Mannheimia spp. and B. trehalosi (Walsh et al. 2016). If an agent was detected by either PCR or culture on any sample, we classified the animal as positive for that agent.

Strain Typing
Health of the Hells Canyon bighorn sheep metapopulation has been intensively monitored since the 1995 pneumonia outbreak in the Black Butte population, and intermittently prior to this. Therefore, we had access to fixed, frozen, and fresh lung tissue and swab samples collected from 1995–2015 in the Heller Bar female group and from 1986–2015 in adjacent female groups and populations. We extracted DNA from a subset of these sources to detect and strain type M. ovipneumoniae within the study population and the metapopulation through time. Detection was based on conventional (McAuliffe et al. 2003) and realtime (Ziegler et al. 2014) PCR.

We used multi-locus sequence typing (MLST) to characterize strains using partial DNA sequences of the 16S–23S intergenic spacer region (IGS), the small ribosomal subunit (16S), and the genes encoding RNA polymerase B (rpoB) and gyrase B (gyrB). We amplified these targets with PCR using a suite of existing and newly developed primers (Table 1). Because of the high degree of DNA sequence
variation in *M. ovipneumoniae*, the biggest challenge in developing this ensemble of loci was identification of targets with sufficiently conserved primer binding site sequences to enable consistent PCR amplification for subsequent sequencing. The loci selected for this study each exhibited large numbers and diversity of the alleles detected at each target locus. We aligned sequences using multi-locus sequence typing (MLST) in animals that died and those that survived. We used a Kaplan–Meier staggered entry estimator and a Cox proportional hazard model to analyze survival of females and lambs. We fit trend lines to 7-day moving averages of clinical scores using a lowess smoothing factor derived from locally weighted moving mean scores spanning 25% of the full dataset (2–3 weeks). We used a Fisher’s exact test to analyze prevalence of pneumonia agents before, during, and after the outbreak and to compare agents present in adult and lamb mortalities. We used a Kruskal–Wallis rank-sum test to determine whether neutralizing titers to PI-3 differed before, during, and after the outbreak. We used a 1-way analysis of variance (ANOVA) and Tukey contrasts to test for differences in serologic antibody titers to *M. ovipneumoniae* over the course of the outbreak and a 2-sided t-test to compare pre-outbreak antibody titers to *M. ovipneumoniae* in animals that died and those that survived. We used a Wilcoxon rank sum test with continuity correction to analyze duration of clinical signs. We conducted analyses with package survival (Therneau 2015), and stats and base packages in R (R Core Team 2015).

### RESULTS

#### Survival and Clinical Signs of Disease

In 2013, we observed all marked females with lambs, and median parturition date was 18 May (range = 5–20 May). In 2014, we observed 10 of 13 females with live lambs and median parturition date was 8 May (range = 27 Apr–17 May). One marked female and her lamb died, presumably of dystocia, on 16 May 2014 (e5 and L5, Fig. 1a), 1 marked female was observed with a dead 2-day old lamb (e10, Fig. 1a), and 1 unmarked female appeared to be pregnant but was never observed with a lamb (e22, Fig. 1a).

We observed clinical signs of pneumonia in lambs starting 1 June in 2013 and 26 May in 2014. Symptoms continued until the day the last lamb died, which was 30 June in 2013, and 2 July in 2014 (Fig. 1). Median lamb mortality date was 28 June in 2013 and 24 June in 2014, at a median age 218 days.

### Statistical Analysis

We used a Kaplan–Meier staggered entry estimator and a Cox proportional hazard model to analyze survival of females and lambs. We fit trend lines to 7-day moving averages of clinical scores using a lowess smoothing factor derived from locally weighted moving mean scores spanning 25% of the full dataset (2–3 weeks). We used a Fisher’s exact test to analyze prevalence of pneumonia agents before, during, and after the outbreak and to compare agents present in adult and lamb mortalities. We used a Kruskal–Wallis rank-sum test to determine whether neutralizing titers to PI-3 differed before, during, and after the outbreak. We used a 1-way analysis of variance (ANOVA) and Tukey contrasts to test for differences in serologic antibody titers to *M. ovipneumoniae* over the course of the outbreak and a 2-sided t-test to compare pre-outbreak antibody titers to *M. ovipneumoniae* in animals that died and those that survived. We used a Wilcoxon rank sum test with continuity correction to analyze duration of clinical signs. We conducted analyses with package survival (Therneau 2015), and stats and base packages in R (R Core Team 2015).

#### Table 1. Oligonucleotide primers for polymerase chain reactions (PCR) used to amplify *Mycoplasma ovipneumoniae* multi-locus sequence typing (MLST) targets. Nesting refers to external and internal primer sets used for nested PCR reactions for amplification of the MLST loci, IG, *rpoB*, and *gyrB*, when amplification from the default (internal) primers produced insufficient DNA template for sequencing.

<table>
<thead>
<tr>
<th>Target</th>
<th>Nesting</th>
<th>Oligonucleotide primer sequence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>LM-F</td>
<td></td>
<td>TGAACCGGAAATGTTAGCCTT</td>
<td>McAuliffe et al. (2003)</td>
</tr>
<tr>
<td>LM-R</td>
<td></td>
<td>GACTTCATCCTGCACCTCCTGT</td>
<td>McAuliffe et al. (2003)</td>
</tr>
<tr>
<td>Ex-IGS-F</td>
<td>External</td>
<td>GTTAACCTCGGAGACCATTG</td>
<td>This paper</td>
</tr>
<tr>
<td>Ex-IGS-R</td>
<td>External</td>
<td>GTTGCTAGTGTTGGGTTCC</td>
<td>This paper</td>
</tr>
<tr>
<td>IG-F</td>
<td>Internal</td>
<td>GGAACACTTCCTTCTCAGGG</td>
<td>Besser et al. (20126)</td>
</tr>
<tr>
<td>IG-R</td>
<td>Internal</td>
<td>CCAAGGCATCCACAAATAC</td>
<td>Besser et al. (20126)</td>
</tr>
<tr>
<td>Ex- <em>rpoB</em> – F</td>
<td>External</td>
<td>AGTTATACCAAAAAATTAGGATC</td>
<td>This paper</td>
</tr>
<tr>
<td>Ex- <em>rpoB</em> – R</td>
<td>Internal</td>
<td>GCTCAAAAGTTCACATTTCNCGA</td>
<td>This paper</td>
</tr>
<tr>
<td><em>rpoB</em> – F</td>
<td>Internal</td>
<td>TCGGTTCCAGAAATCTCTTCT</td>
<td>This paper</td>
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<tr>
<td><em>rpoB</em> – R</td>
<td>Internal</td>
<td>TCGGTTGTTGGGTTGCTCTTC</td>
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<tr>
<td>Ex- <em>gyrB</em> – F</td>
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<td>Internal</td>
<td>ACGGATTAATGTCACAAAGTTG</td>
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</tr>
</tbody>
</table>

of 40 and 44 days, respectively. All lambs died both years and there were no differences in lamb survival curves between years (log rank; $= 0.01$, $P = 0.92$) or between 2014 and the previous 10 years (Fig. 1c). We submitted 11 lambs for necropsy (6 in 2013 and 5 in 2014). With the exception of 1 lamb that died at approximately 2 days of age in 2014 (L10, Fig. 1a), all lambs presented characteristic lesions of bighorn lamb respiratory disease, including moderate to

![Figure 1. Clinical signs of pneumonia and survival of bighorn sheep in the Black Butte, Washington population during summers 2013 ($n = 4$ adult F and 4 lambs) and 2014 ($n = 13$ adult F and 10 lambs). (a) Time series of field observations of pneumonia symptoms in adult females (e) and their lambs (L). Observations ending prior to September indicate individual died. (b) Smoothed average daily clinical scores for adult females and lambs. (c) Adult female and lamb survival between May and October 2014 and survival between May and October during the 10 previous years, 2004–2013 (Kaplan–Meier curves and 90% CIs).]
Table 2. Prevalence of Mannheimia spp. (Mh), Bibersteinia trehalosi (Bt), Pasteurella multocida (Pm), Pasteurellaceae leukotoxin encoding gene (LktA), and Mycoplasma ovipneumoniae (Movi) in asymptomatic bighorn sheep females (F) sampled before and after a pneumonia outbreak, symptomatic females during the outbreak, and in the lungs of pneumatic females that died in the Black Butte population and pneumatic lambs that died in Black Butte, (10) and adjacent populations (2), Washington and Oregon, USA.

<table>
<thead>
<tr>
<th>Agent</th>
<th>F before (n = 11)</th>
<th>F during (symptomatic) (n = 2)</th>
<th>F pneumonia mortalities (n = 3)</th>
<th>Lamb pneumonia mortalities (n = 12)</th>
<th>F after (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mh</td>
<td>0.91</td>
<td>1.00</td>
<td>0.00</td>
<td>0.70</td>
<td>0.75</td>
</tr>
<tr>
<td>Bt</td>
<td>0.82</td>
<td>1.00</td>
<td>1.00</td>
<td>0.80</td>
<td>1.00</td>
</tr>
<tr>
<td>Pm</td>
<td>0.73</td>
<td>1.00</td>
<td>1.00</td>
<td>0.10</td>
<td>1.00</td>
</tr>
<tr>
<td>LktA</td>
<td>0.27</td>
<td>0.50</td>
<td>0.33</td>
<td>0.50</td>
<td>0.38</td>
</tr>
<tr>
<td>Movi</td>
<td>0.09</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>0.13</td>
</tr>
</tbody>
</table>

severe, subacute to chronic bronchopneumonia and otitis media.

Median age at first observation of clinical signs in lambs was 20 days (range: 7–35), median duration of clinical signs was 22 days (range: 1–59), and median age at death was 42 days (range: 22–66). Assuming that lambs were infected by 4 days of age and lung lesions were present by 10 days of age (Besser et al. 2008), we estimated a median latent period of 16 days (range: 3–31) between infection and first observation of disease symptoms in lambs and a median infection period of 38 days (range: 18–62) prior to death.

In adults, the only evidence of possible respiratory disease in 2013 was a single observation of coughing on 14 June, and all adults survived. In 2014, we observed clinical signs of pneumonia in adults starting in May. By June, all adults exhibited symptoms of pneumonia including severe prolonged coughing (Fig. 1a and b). Five adult females (38%) died between May and August 2014 (difference between summers, log rank1 = 5.98, P = 0.01) and the hazard of an adult female dying in the summer of 2014 was 6.83 times higher (SE = 0.83, P = 0.02) than in any of the previous 10 summers (2004–2013, Fig. 1c). We did not observe pneumonia symptoms prior to the first adult death on 16 May 2014. Subsequent mortalities followed observation of clinical signs of pneumonia and occurred on 26 June, 18 July, 27 July, and 3 August (Fig. 1a). Median duration of clinical signs was 36 days (range = 18–81 days), which was longer than observed in lambs (median = 22 days, range = 1–59, W = 136.5, P < 0.001). Duration of clinical signs did not differ between adults that died and those that survived (Fig. 1a, Wilcoxon rank sum W = 20, P = 1.0). We submitted samples to WADDL from all (4 of 5) mortalities where sufficient tissues were available for diagnosis. No gross or histological evidence of respiratory disease was found in the female that died on 16 May, although evaluation of tissues at WADDL was limited because of autolysis. The 3 adult females submitted for necropsy between late June and early August were diagnosed with chronic, moderate to severe bronchopneumonia. The survivors appeared to make a full recovery with no evidence of ongoing disease.

Microbiology and Immune Responses

*M. ovipneumoniae* was the only pneumonia agent detected more frequently in the lungs of adults that died of pneumonia than in the upper respiratory tract of healthy adults before and after the outbreak (χ² = 26.66, P < 0.001; Table 2). Prevalence of other suspected pneumonia agents in the upper respiratory tract of symptomatic adults sampled during the outbreak was similar to that detected in the lungs of adults that died of pneumonia except that *Mannheimia* spp. were detected in the upper respiratory tract of live adults but not in the lungs of adults that died. We found no differences in prevalence of pneumonia agents present in upper respiratory tract samples collected before and after the outbreak (χ² < 2.6, P > 0.2) or in the lungs of lambs that died in 2013 and 2014 (χ² < 0.39, P > 0.5). Detection of *Mannheimia* spp. was more common in lamb mortalities than in adult mortalities (χ² = 4.55, P = 0.07), and *P. multocida* was more frequently detected in the lungs of dead adults than lambs (χ² = 8.78, P = 0.01; Table 2).

We detected serologic evidence of exposure to PI-3 (VN titer > 4) and *M. ovipneumoniae* (ELISA inhibition > 50%) in adults before, during, and after the 2014 pneumonia outbreak. We did not detect evidence of exposure to other respiratory viruses during the study. Average *M. ovipneumoniae* ELISA percent inhibition (%I) values prior to the outbreak (69%) increased significantly to 89%I and 82%I during and after the disease event, respectively (F = 57.2, P = 0.01; Table 2). Individuals that died during the outbreak had higher *M. ovipneumoniae* ELISA %I values prior to the outbreak (x = 79%) than those that survived (x = 59%, t = 3.02, P = 0.02; Table 2). Median PI-3 titers of 256 (log2 7.7) did not change significantly over the course of the outbreak (χ² = 4.55, P = 0.23).

**Strain Typing**

We genotyped *M. ovipneumoniae* from all Heller Bar bighorn sheep where it was detected in 2013 (n = 7) and 2014 (n = 11). We found a single strain in 2013 based on identical DNA sequences of each of the 4 MLST loci, and that strain was also detected in the first lamb to die of pneumonia in 2014. All subsequent detections of *M. ovipneumoniae* differed from the 2013 strain at 3 MLST loci. The IGS sequences differed by 32 single nucleotide polymorphisms (SNP) and 3 base insertions or deletions (indels, 8.7% divergent), rpoB by 16 SNP (2.8% divergent) and gyrB by 16 SNP (4% divergent). The 165 sequences did not differ between strains.

We report strain differences by IGS sequences because we were unable to amplify rpoB and gyrB from DNA extracted 220
from formalin-fixed, paraffin-embedded lung tissues, the only specimens available prior to 2006. Because each of these strains differed by indels in IGS, the strains are conveniently designated by their differing IGS lengths. The 2013 Black Butte strain, IGS 404, has been detected in this population since the 1995 pneumonia outbreak (Fig. 3b). The 2014 strain, IGS 393, had never previously been detected in this population or any other bighorn sheep population in Hells Canyon or elsewhere in the western United States (among >700 other isolates that have been IGS typed).

In October 2014, we removed survivors from the Heller Bar female group in an attempt to prevent further spread of this strain. Nonetheless, 1 month after the removal, we detected the IGS 393 strain type in an adult male removed from the town of Asotin, Washington and we detected it again 4 months later in a 9-month-old lamb in the Shumaker female group, located between the Joseph and Heller Bar

**Figure 3.** Spatiotemporal distribution of the 16S-23S intergenic spacer (IGS) genotypes of *Mycoplasma ovipneumoniae* in the Hells Canyon bighorn sheep metapopulation in Washington, Oregon, and Idaho, USA in (a) 1986 and 1992 (b) 1995–2013; and (c) 2014–2015. Each colored marker represents one strain type, pie charts display strain types within populations and are scaled by sample size. Pie charts containing >1 color indicate that 2 strain types were present in a population during that time interval but not necessarily detected in the same year. Gray shaded polygons denote bighorn sheep populations: AS = Asotin, BB = Black Butte, BC = Big Canyon, BR = Bear Creek, IM = Imnaha, LH = Lower Hells Canyon, LM = Lookout Mountain, MU = Muir, MV = Mountain View, MY = Myers Creek, RB = Redbird, SM = Sheep Mountain, TU = Tucannon, UO = Upper Hells Canyon, Oregon, UI = Upper Hells Canyon Idaho.
groups in the Black Butte population. Retrospective analysis revealed that this strain was present in the lungs of a male that died of pneumonia in the Joseph Creek group in December 2013, representing the index case of disease associated with the IGS 393 strain type. The IGS 393 strain has not been detected in any surrounding populations, which remain carriers of the original IGS 404 strain (Fig. 3c).

Two other strains were detected in specimens obtained during or after disease outbreaks in other Hells Canyon populations (Coggins 1988, Foreyt et al. 1990), and have not been detected since. These samples were from the Lostine population in 1986–1987 (IGS 419) and the Mountain View population in 1992 (IGS 402; Fig. 3a). A third strain (IGS 415) associated with a pneumonia outbreak in the Sheep Mountain population in 2000 was apparently replaced by the IGS 404 strain by 2006 based on samples typed from 2006 and 2015 in that population. The IGS 415 strain was subsequently infrequently detected in 4 other populations between 2003 and 2009 and has not been found since then (Fig. 3b).

Sequence divergence among the 3 recently detected strains where all 4 genes could be analyzed (i.e., IGS 393, 404, and 415) was between 9% and 10%. These strains were well dispersed across 20 genotypes of \textit{M. ovipneumoniae} collected from 9 domestic sheep flocks in the western United States and Australia and 10 domestic goat flocks in the western United States and China. The IGS 404 and 415 types, first identified in the Black Butte and Sheep Mountain populations, respectively, were more closely related to the domestic sheep lineage, whereas the IGS 393 type detected in the Black Butte population in 2013 and 2014 clustered with the domestic goat clade (Fig. 4).

**DISCUSSION**

This is the first study to report on an all-age pneumonia outbreak in an intensively sampled population of free-ranging bighorn sheep with health, survival, and observational data collected before, during, and after the disease event. The collection and analysis of this information, employing recently developed molecular methods for pathogen detection and genotyping, allowed us to attribute severe disease in a bighorn sheep population with long-standing \textit{M. ovipneumoniae} carriage to introduction of a novel strain of \textit{M. ovipneumoniae}. This conclusion is supported by the detection of the never before recorded IGS 393 strain in the pneumonic lungs of adults that died, and in the upper respiratory tract of adults with clinical signs during the outbreak. We also observed a significant increase in antibody titers to \textit{M. ovipneumoniae} during the outbreak denoting an active immune challenge and we documented that previous exposure and ongoing carriage of the IGS 404 strain was not protective against disease. To the contrary, lower survival of adults with higher serologic titers prior to the disease outbreak could reflect a harmful autoimmune reaction associated with antibody response to \textit{M. ovipneumoniae} in bighorn sheep as has been suggested for domestic sheep (Niang et al. 1998a). Strain-specific immunity, as measured by serologic antibody inhibition of \textit{M. ovipneumoniae}, was similarly reported by Alley et al. (1999) for domestic sheep.

Adult mortality associated with this strain introduction was within the range previously observed during pneumonia outbreaks in naïve animals in this metapopulation (28–42%; Cassirer et al. 2013). Lamb mortality followed an identical time course regardless of strain, consistent with a lack of...
protective immunity in neonates. The timing of the onset of clinical signs following infection in lambs (latent period) was similar to that reported in experimental challenge of adults (Besser et al. 2014). However, disease progression in lambs was more rapid and severe than observed in free-ranging adults in this study or in experimental exposure of naïve adults in captivity (Besser et al. 2014).

Although pneumonia in bighorn sheep is a polymicrobial disease, pathogens other than \textit{M. ovipneumoniae}, including \textit{ltkA} positive \textit{Pasteurellaceae} and respiratory viruses, were either not detected or showed no association with disease. Whereas \textit{M. ovipneumoniae} was present in all pneumonic adults and lambs, prevalence of \textit{Pasteurellaceae} varied between age classes. This could be due to conditions associated with growth of an opportunistic pathogen or to other factors. Sample sizes were too small to draw broader inference. Anaerobic bacteria, not tested in this study, are a large component of the microbiome in pneumonic bighorn sheep lung tissue and may also play a larger role as secondary pathogens than previously suspected (Besser et al. 2008).

The novel strain of \textit{M. ovipneumoniae} detected in this study differed from the resident strain by 52 independent genetic mutations on 4 loci. This unique strain was not a variant of a resident strain and had never before been detected in over 700 samples strain-typed from Hells Canyon and other bighorn sheep populations. Therefore, the most likely source of this Caprinae-specific pathogen was a domestic sheep or goat. Phylogenetic analysis indicated that this strain was most likely of domestic goat origin.

This strain introduction likely occurred from a domestic goat on or from private lands within bighorn sheep range despite substantial efforts by wildlife managers and nongovernmental organizations to prevent contact. Management strategies included distributing educational material to flock owners and the general public, purchasing and removing a domestic sheep flock, and removing individual bighorn or domestic sheep and goats when they were at risk of contact. Our retrospective analysis showed that unique strains of \textit{M. ovipneumoniae} were associated with 4 other epidemiologically unrelated all-age pneumonia outbreaks in Hells Canyon, as would be expected from similar spillover events. Two of these historical strains (IGS 402 and 419) apparently remained localized, one spread and subsequently disappeared (IGS 415), whereas the fourth (IGS 404) has persisted and proliferated over a span of ≥20 years. It is not clear why some strains of \textit{M. ovipneumoniae} persist and others apparently do not. The IGS 404 strain may have driven fade-out of other extant strains when it spread, as introduction of the IGS 393 strain did in this study. Strain replacement might occur when the carrier host immune response is cross-reactive but protection is strain-specific. Under these conditions a new strain could have a competitive advantage and exclude the original strain.

Although evidence suggests that this epizootic was caused by introduction of a novel \textit{M. ovipneumoniae} strain, it is also possible that pneumonia outbreaks could be precipitated in carrier bighorn sheep populations if appropriate mutations occur in strains to which resistance has previously been acquired. Mutations in key virulence genes or in genes coding for the \textit{M. ovipneumoniae} capsule, which likely plays a role in adherence to host cells and in evading antibody recognition (Niang et al. 1998b, Razin et al. 1998), could cause disease and are unlikely to be detected by our strain-typing method. Another plausible mechanism of pneumonia resurgence would be reintroduction of the same strain of \textit{M. ovipneumoniae} into a population following either pathogen fade-out and waning immunity (Sydnestricker et al. 2005) or recruitment of unexposed susceptible individuals. Finally, other factors may play a role in triggering outbreaks such as pathogen dose, host contact patterns, immunocompetence, and invasion of secondary pathogens. Identifying the conditions most frequently associated with pneumonia outbreaks in previously exposed populations and a wider investigation of the genetic diversity and host-specificity of \textit{M. ovipneumoniae} strains would provide valuable insights into the adaptive immune response in bighorn sheep and the ecology of this disease.

**MANAGEMENT IMPLICATIONS**

Lack of cross-strain immunity to \textit{M. ovipneumoniae} could be one explanation for the regular occurrence of pneumonia epizootics in bighorn sheep populations over a century after initial contact with domestic sheep. Single strain infection in bighorn sheep populations contrasts with \textit{M. ovipneumoniae} carriage in domestic sheep where numerous strains typically coexist within a flock (Alley et al. 1999, Harvey et al. 2007). In the absence of cross-strain immunity, these flocks may serve as a constant source of novel strains capable of causing disease in bighorn sheep. Although vaccination could potentially reduce pathogen burden or prevalence within bighorn sheep populations, it is not clear that a vaccine would protect bighorn sheep from severe disease if exposed to new strains. Our results instead support preventing spillover as a primary strategy for managing disease in bighorn sheep. This could be accomplished by maintaining separation between bighorn sheep and domestic sheep and goats, by clearing \textit{M. ovipneumoniae} infection from domestic hosts, and by exercising caution to avoid mixing \textit{M. ovipneumoniae} strains among bighorn sheep populations during translocations. The management strategies implemented near the bighorn sheep in this study were apparently unsuccessful in preventing transmission, underscoring the difficulty of maintaining separation. New approaches, more active cooperation by the public, and greater vigilance on the part of resource managers may be key to preventing pneumonia outbreaks in bighorn sheep.

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LITERATURE CITED


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Peer-reviewed Publications

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Accounting for immunopathology in cross-scale models of infectious disease, RCN-IDEAS, K. Manlove and Pej Rohani $2,500
2013
Assessing the effectiveness of Montana’s occupant protection programs, Montana Department of Transportation, L. Stanley (Principal Investigator) and K. Manlove (Co-Investigator), $81,249
2011
Understanding the spread of pneumonia within bighorn sheep populations, Morris Animal Foundation, R. Plowright and P. Hudson (Principal Investigators), E. F. Cassirer, T. Besser, A. Dobson, P. Cross, B. Dickson, L. Bienen (Co-Investigators), K. Manlove (named graduate student). $195,245