Epidemiologic Approaches to Investigating Pathogen Spillover and Spread Across Diverse Systems

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DEDICATION

This dissertation is dedicated to my family, who have unfailingly and enthusiastically supported me in my quest to become a scientist and conservationist since I was a child. The kindness and curiosity that my mom, Kris, has for animals and nature is what seeded my passion for the natural world. My dad, Tom, always believed that I could be the best at anything I did and encouraged me to take on difficult endeavors. My sister, Kirsten, has been a loving supporter and encouraged me through all my adventures. My biggest cheerleader has been my brother, Shane, who always reminded me that I am accomplishing my dreams whenever I had moments of doubt. No one has been prouder of me than my grandma, Anne, who is always excited to hear stories of my adventures with animals. And lastly, Valentino, the heart of our family and the sweetest soul I have ever known – I will forever miss your calming energy as you napped by my side while I worked, giving me silent comfort and peace. Your joy in the simple things reminded me to slow down and live in the moment.

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Epidemiologic Approaches to Investigating Pathogen Spillover and Spread Across Diverse Systems

ABSTRACT

Rapid ecological changes have led to an increase in pathogen spillover risk between different host species, including between animals and humans. Spillover occurs when pathogens are transmitted to a new species which had previously not encountered the disease. The process of spillover and subsequent spread of a pathogen in a new host population can include three to four phases, which are often circular: 1) maintenance of disease in a reservoir host, 2) initial spillover into a novel host due to high-risk interactions, and 3) rapid pathogen spread causing an epidemic in the new, immunologically naïve host population, which can potentially be followed by 4) maintenance of disease in the new host. Disease dynamics in these phases of emergence are explored in three unique systems with relevance to both animal and human health.

In chapter 1, animal-human interfaces with risk of pathogen spillover were characterized along wildlife "supply chains" in Africa and Asia, to guide prevention efforts that will preempt spillover events. Observational surveys of sites along the wildlife supply chain were conducted by the PREDICT Consortium to characterize the settings in which wild animals are sourced, traded, and sold. Questionnaires were also administered to hunters and supply chain workers to assess their exposure to zoonotic disease and any spillover prevention measures implemented. Findings from this study inform community education efforts regarding zoonotic pathogen transmission, wildlife trade policies, and biosecurity and PPE guidelines.

In chapter 2, the efficacy of behavior changes to mitigate the expansion of an emerging epidemic are evaluated in the months immediately following a spillover event. The progenitor

virus of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was likely introduced into humans by a wildlife host, then adapted to spread human-to-human, causing a global pandemic in the span of a few months. The efficacy of social distancing was evaluated at the state level during the first two months of the pandemic in the United States by examining the relationship between daily SARS-COV-2 case incidence and human community mobility. Lag times between decreases in mobility and case counts were measured, and social distancing was found to be most effective when put into place early in an epidemic. These findings inform management of emerging infectious disease outbreaks by identifying areas where social distancing was the most effective in reducing disease transmission (e.g., indoor public spaces such as workplaces and transit station), and the expected time frame between behavioral changes and measurable changes in disease incidence.

In chapter 3, factors contributing to the maintenance of disease were investigated in the decades following spillover from reservoir host to novel host species. Endangered Peninsular bighorn sheep (*Ovis canadensis nelsoni*) have suffered population declines due to infectious diseases introduced from domestic sheep (the original reservoir host), which now circulate within bighorn sheep herds in the absence of continued spillover. Demographic and geographic risk factors for pathogen exposure in individual bighorn sheep were examined, and the impact that pathogen exposure has on adult survival and lamb recruitment was measured at the herd level. These results will inform targeted management and conservation of bighorn sheep as they face the compounding challenges of disease, habitat loss, and climate change.

The factors contributing to pathogen spillover and spread are highly complex, necessitating the study of these pathways in many diverse systems. The research presented here provides insights into the maintenance, spillover, and control of pathogens across select host

species and ecological systems. The animal-human interfaces identified at live animal markets will help us identify targets for surveillance of pathogens with pandemic potential in the pre-emergence setting, and guide local education and mitigation measures to prevent spillover. Implementing rapid behavior changes such as social distancing can slow the spread of a newly introduced pathogen in the absence of other control measures, such as vaccination. The impact of introduced pathogens on bighorn sheep survival and reproduction may be compounded by increasing temperature and decreasing precipitation, which can be expected to worsen due to climate change. These interwoven threats necessitate the longitudinal monitoring of bighorn sheep survival and systematic, range-wide surveillance of disease prevalence and food/water resources to guide conservation strategies. These findings can be extrapolated to other systems, so we are better prepared to identify, prevent, and respond to future emerging pathogens.

INTRODUCTION:

Surveillance strategies for detection of pathogen spillover and spread

Pathogen emergence, spillover, and spread are related and overlapping terms describing how a pathogen might expand the diversity or geographic extent of its host range. An "emerging" pathogen is one that has recently appeared in a new host species, or rapidly increased in either incidence or geographic area within an established host [1]. Pathogen "spillover" occurs when a pathogen is introduced into a new host species, then subsequently spreads within that population. This can occur when a pathogen originated in animals but adapted to infect humans ("zoonotic spillover"), wildlife contracts a disease from humans ("reverse zoonosis"), or when pathogens move from one animal host species to another animal host ("cross-species transmission") [2–4]. The risk of these emergence and spillover events involves a web of factors including host and pathogen biology, spatial distribution and contact rates among hosts, landscape ecology, and disease dynamics [5]. Host interactions which could potentially transmit pathogens increase with perturbations of ecosystems, including expansion of agriculture, habitat loss and encroachment of human infrastructure into natural habitats, movement of wild and domestic animals, and loss of biodiversity [1,6,7].

The introduction of a novel infectious disease can have devastating effects on naive host populations. Introduced disease in wildlife have resulted in subsequent epidemics that have caused the decline of native populations to the point of being listing as endangered by the International Union for Conservation of Nature (IUCN), with notable examples including chytridiomycosis (*Batrachochytrium* spp.) in amphibians, white-nose syndrome (*Pseudogymnoascus destructans*) in bats, plague (*Yersinia pestis*) in black footed ferrets

(*Mustela nigripes*), and avian malaria (*Plasmodium relictum*) in native Hawaiian forest birds [8–11]. Zoonotic spillover from wildlife into humans has resulted in the rapid development of large scale pandemics caused by influenza viruses, coronaviruses, and Ebola virus [12–15]. The spillover of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from its wildlife reservoir resulted in the declaration of a global pandemic within four months of the virus being identified, and has since caused widespread and devastating effects on human health and economies [16]. Cross-species transmission from domestic animals to wildlife has also been observed, such as domestic dogs (*Canis lupus familiaris*) transmitting rabies and canine distemper to African carnivores, and domestic cattle (*Bos taurus*) introducing *Brucella abortus* to North American wild elk (*Cervus elaphus*) and bison (*Bison bison*) [17–19]. The impacts of introduced disease can be critical in endemic and immunologically isolated species, especially those which are small in number due to previous population declines or limited geographic distribution, such as those living on islands [20,21].

Concurrent threats such as climate change, habitat loss, and anthropogenic disturbances can alter cross-species infectious disease transmission through changes in species abundance and distribution, host contact rates, toxin exposure, and stress [1]. The fragmentation of wildlife habitat directly increases the risk of cross-species pathogen transmission by increasing the access of humans and domestic animals to wildlife habitat and driving wildlife into human-occupied landscapes [22,23]. These external variables can combine with infectious disease to increase physiologic stress, which has been linked to changes in gene expression and immune function, increased susceptibility to and shedding of pathogens, and more severe clinical disease in multiple species [24–28]. Diligent surveillance for emerging pathogens, and monitoring for the changing impacts of known pathogens, due to ecological changes is important for preserving

wildlife health and preventing future epidemics of zoonotic pathogens, such as Ebola virus and SARS-CoV-2 [29,30].

The number of emerging infectious diseases has increased steadily over the past 80 decades, and the majority of emerging zoonotic diseases have originated in wildlife [31,32]. Preventing zoonotic spillover includes the early detection of pathogens in wildlife reservoirs and identification of interfaces where spillover to humans or domestic animals may occur. This often requires organized, proactive surveillance programs because wildlife health is much less frequently observed compared to that of domestic animals and humans, but the detection of pathogens before and immediately after spillover is critical for mitigation efforts to be effective.

The World Organization for Animal Health (OIE) considers wildlife pathogen surveillance to be the "single most important component of a national wildlife health programme" [33]. The epidemiologic role of surveillance in wildlife disease is to record and analyze the presence of disease over time, as an ongoing process, in order to make management interventions [34]. Ideally, surveillance systems provide early detection of disease before it becomes a population threat. The prevention of epidemics and associated host population declines are most effective when a disease is detected early, before a large proportion of animals have been infected. This means monitoring apparently healthy populations is imperative, although it can often be a challenge to fund and support these programs over the long term.

Disease surveillance systems are classically split into active and passive strategies [34]. Active systems often involve the capture of animals for sample collection and application of tracking technologies such as radio transmitters [35]. This allows researchers to target a representative sample of the population, allows for longitudinal monitoring of survival, and the timely detection of mortalities. Active surveillance is often utilized for high consequence

pathogens, such as those of economic or public health importance [36]. These active systems generally involve more time, money, and resource investment and are often more invasive, potentially putting animals at greater risk for negative consequences. However, active surveillance can also include non-invasive techniques such as scat collection, hair snares, and field cameras. These non-invasive tools provide less detailed information on the individual animals being sampled, and are often used for evaluating population rather than individual health [35]. Passive systems include syndromic surveillance using data from patients presenting to hospitals or rehabilitation centers, and opportunistic sampling, such as collection of animals killed on roads or the sampling of animals killed by hunters [34,37].

Regardless of the type of surveillance system utilized, there are inherent challenges to the detection of disease in wildlife. Free-ranging wildlife can be difficult to capture and handle, with some species being dangerous to humans or particularly prone to poor outcomes such as capture myopathy. Migratory, cryptic, or rare species can be difficult to locate and observe, let alone capture, and disease may go undetected in these populations for years [36]. Many animal mortalities go undetected due to difficult to navigate terrain or habitats, and the mortalities that are observed because the animal was marked and/or died in a human occupied area may be just the "tip of the iceberg." Pathogens which are rare or for which there is no effective diagnostic test are particularly difficult surveillance targets. These limitations can lead to biases in our measurements of disease metrics such as prevalence, incidence, and case fatality [34].

The importance of proactive surveillance for wildlife diseases is magnified by the risk of pathogen spillover between different host species, including between animals and humans, due to rapid ecological disturbances such and climate change and habitat fragmentation [38]. Spillover occurs when pathogens are transmitted to a new species which had previously not encountered

the disease. The process of spillover and subsequent spread of a pathogen in a new host population can include three to four phases, which are often circular: 1) maintenance of disease in a reservoir host, 2) initial spillover into a novel host due to high-risk interactions, and 3) rapid pathogen spread causing an epidemic in the new, immunologically naïve host population, which can potentially be followed by 4) maintenance of disease in the new host. These phases can be adapted to different wildlife-pathogen systems, such as the "infect-shed-spill-spread" cascade, whereby wildlife pathogens are amplified in reservoir hosts then spillover into humans or domestic animals (or vice versa) as a result of anthropogenic land-use changes [38]. Disease dynamics in these phases of pathogen emergence and spillover are explored in three unique systems with relevance to both animal and human health.

Surveillance in the pre-emergence, pre-spillover setting

Expansion of pathogens into new host species or geographic ranges can result in epidemics due to the large number of immunologically naive hosts available for infection. However, not every spillover or emergence event results in an epidemic. For example, animal pathogens may occasionally infect an individual or small group of humans, but the pathogen will not spread through the human population unless the pathogen mutates to readily transmit human-to-human [5,39]. Proactively testing a wide range of hosts to detect of these single spillover or early emergence events increases our knowledge of the classes of pathogens which most readily infect new host species and gives us a temporal advantage in responding to epidemics while case numbers are still small. The disadvantage of this strategy is that it is time, labor, and resources

intensive and will produce a large proportion of negative test results if healthy, non-clinical individuals are sampled.

Targeted surveillance for pathogens in the original reservoir host has been used as an early warning system for potential spillover events in several host-pathogen systems. Testing wild birds for West Nile Virus (WNV) has been used for decades to predict outbreaks of human cases. One study found that molecular detection of WNV in wild birds was found to precede the onset of human cases by >3 months, and detection of dead crows preceded molecular detection of WNV in birds by several months [40]. This information can help direct early prevention methods to minimize transmission, such as mosquito control measures, months in advance of human outbreaks. However, surveillance of the reservoir host is most useful when there are symptomatic or dead animals which can be easily identified for sampling, and is less efficient in asymptomatic hosts that necessitate widespread population sampling to detect a pathogen. Rabies is a global zoonotic pathogen for which several domestic and wild animal hosts serve as reservoirs. Human exposure usually happens through contact with wildlife or an unvaccinated domestic animal, therefore testing of these animal hosts has become a key component of rabies surveillance systems worldwide, providing information on the local burden of disease and risk of human exposure [41,42]. A recently developed "Wildlife Morbidity and Mortality Event Alert System" utilizes machine learning to monitor a network of wildlife rehabilitation organizations and detect unusual elevations in wildlife morbidity and mortality due to specific clinical syndromes [37]. This system generates alerts of possible pathogen emergence or local outbreaks,

which can identify at-risk taxa and direct targeted diagnostic testing to identify the pathogenic cause of the increase in morbidity/mortality.

The concurrent, proactive sampling of humans and animals for pathogens at sites with animal-human interfaces improves the likelihood that a pathogen will be detected prior to a spillover event, or during the early phases of emergence. There are three sampling strategies which may improve the efficiency of animal-human interface sampling. The first is to target high-risk species which have been shown to harbor pathogens of zoonotic or epidemic potential, such as bats, rodents, non-human primates, carnivores, and ungulates [43]. This method would not detect pathogens moving through intermediate host taxa that are not sampled because they are considered lower risk, such as has been seen with SARS-CoV-1 in civets [44]. The second strategy is to sample multiple host species, but test only for high-risk pathogen groups which have historically caused pandemics, such as coronaviruses and influenza viruses [45]. This strategy would miss other pathogen groups which may spillover less commonly or unexpectedly. The third is to have humans self-present for testing if they feel ill, especially with certain disease syndromes which are commonly associated with zoonotic disease, such as febrile illness [46,47]. This method would not detect a pathogen until after the initial spillover event into humans, limiting response options. All of these methods would increase the detection sensitivity of high-risk pathogen groups while decreasing the resources necessary for surveillance, but narrow the range of hosts or pathogens which are monitored.

The first chapter of this dissertation addresses the need for proactive surveillance prior to spillover occurring. Animal-human interfaces with risk of pathogen spillover were characterized along wildlife “supply chains” in Africa and Asia, to guide prevention efforts that will preempt spillover events. Site surveys and worker questionnaires were conducted at sites along the

wildlife supply chain where wild animals are sourced, traded, and sold. Study sites were selected according to a stratified sampling design across countries, with a second layer of judgement sampling determining the exact sites chosen within each country based on local knowledge of interfaces between animals and humans. This design maximized the chances for sampling an interface where circumstances for pathogen spillover may be present, while still sampling a variety of countries which may differ based on local ecology, host species present, and cultures driving the wildlife trade.

Post-spillover surveillance and implications for early epidemic control

Immediately following a successful spillover event, a pathogen can spread rapidly in the new, immunologically naïve host population to which it has adapted, causing an epidemic [4]. The magnitude of this initial spread can determine the morbidity and mortality impact the disease will have on the new host, as well as the probability that the disease will become established, or endemic, in the new host population. For example, diseases introduced into areas with a low host density or a high proportion of vaccinated hosts have a higher risk of "fadeout," or pathogen extinction due to inadequate transmission [48–50]. The control of this initial spread is therefore a key point in epidemic response [51]. Often, the control measures immediately available at the start of an epidemic of novel disease (for which vaccination is not yet available) are primarily non-pharmaceutical, behavioral changes that reduce the number of potentially disease-transmitting contacts among hosts.

Reductions in close contacts among hosts have been demonstrated to slow the transmission of directly transmitted, human pathogens such as influenza [52]. Quantifying social behaviors such as close contacts can be difficult, but recent advances in mobile phone global positioning system (GPS) technology has allowed us to examine contact rates among humans in

a remote, anonymous way. In wildlife, these contacts can be measured using proximity loggers. Proximity loggers utilize low power, ultra-high frequency (UHF) radio transmitters and receivers to record when two devices come within a preset distance of one another, creating reciprocal records of social encounters between animal dyads. These records can include the date, time, and duration of an encounter between two specific animals, or between an animal and a stationary receiver "base station." Proximity loggers are an advance on traditional telemetry technologies because they are more likely to detect all encounters between a pair of hosts, compared to traditional methods implemented during the analysis phase, such as designating an "encounter" as when two GPS collared animals came within a specified geographic distance of one another during a specified time window. However, encounters are not always recorded identically between proximity loggers due to differences in device sensitivity and/or external factors such as terrain, and decisions regarding how to resolve these discrepancies during data processing can lead to under or overestimates of the frequency and duration of encounters [53].

The ability to directly measure host contact rates is a huge benefit to understanding disease dynamics and informing models of disease transmission. Research measuring contact rates among female elk (*Cervus canadensis*) in Wyoming using proximity loggers found that interaction rates varied with herd size in a pattern between what would be predicted by traditional frequency or density-dependence models [54]. Combined proximity and VHF transmitters have been used to measure the relationships among animal density, home range overlap, and contact rates in Channel Island foxes (*Urocyon littoralis*), which then informed spatially explicit simulation models of early pathogen spread immediately after an introduction event, to evaluate epidemic prevention tools such as vaccination [48,49,55]. The simultaneous use of proximity loggers on cattle, white tailed deer (*Odocoileus virginianus*), raccoon (*Procyon*

lotor), Virginia opossum (*Didelphis virginiana*), and stationary base stations identified seasonal differences in both indirect contact rates around food/water sources and direct contacts between individuals that could impact the interspecific transmission of bovine tuberculosis (*Mycobacterium bovis*) [56].

The efficacy of reductions in social contacts to mitigate the expansion of an emerging epidemic immediately following spillover to a new host were evaluated in the second chapter of this dissertation, in the context of the COVID-19 pandemic. The progenitor virus of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; the viral cause of COVID-19) was likely introduced into humans by a wildlife host, then adapted to spread human-to-human, rapidly causing a global pandemic in the span of a few months. Due to limited testing capabilities during the first months of the pandemic, testing was initially restricted to symptomatic individuals who fit the Centers for Disease Control and Prevention COVID-19 case definition [57]. This syndromic surveillance increased the positive predictive value of testing despite the disease still being quite rare within the general population. The negative consequence of this strategy was that community transmission in the USA went largely undetected during the early pandemic period because asymptomatic or mildly symptomatic people, or those not self-presenting, were not sampled. The efficacy of social distancing to reduce SARS-CoV-2 transmission, despite these testing limitations, was evaluated during the first two months of the pandemic in the United States by examining the relationship between daily SARS-COV-2 case numbers and human community mobility, as measured by anonymized location data aggregated from individual mobile phones.

Surveillance for pathogens in reservoir hosts during the pre- or post-spillover phase

During the pre-spillover phase, “reservoir hosts” (or “maintenance hosts”) are those in which a pathogen circulates in the population due to sustained transmission among individuals [58]. Reservoir hosts have often evolved with their pathogens so that they suffer relatively minor disease in comparison to the effects a pathogen might have on a new host [4]. After spillover and the initial epidemic, a pathogen may subsequently become established (or “endemic”) in the new host and circulate without continued spillover from the original source host. In these cases, the new host may become a maintenance host, but often experiences variable morbidity and mortality due to its lack of co-evolution with the pathogen, potentially resulting in host population declines. This has been seen in the spillover of simian immunodeficiency virus (which is associated with little to no disease in the primate reservoirs) into humans, which mutated into human immunodeficiency virus and is now well established in human populations globally, despite causing severe and fatal disease (acquired immunodeficiency syndrome) without pharmaceutical interventions [4].

Endemic pathogens do not necessarily infect or clinically affect all subgroups in a population equally. Evaluating differences in risk of exposure and clinical disease is a key part of maintenance host surveillance to aide in management and monitoring for changes in disease distribution or prevalence [35]. In wildlife, the best way to elucidate these differences in risk is often to capture a representative sample of each stratum of interest, such as different demographic and geographic groups. Direct capture of animals for sampling is ideal for performing comprehensive health exams and detecting all stages of exposure and infection (i.e., pre-symptomatic, symptomatic, recovered but seropositive). Due to logistical and financial constraints, it is often not feasible to sample an entire wildlife population or sample consistently every year. These sampling limitations may underestimate the prevalence of diseases that

differentially impact under-sampled strata, are rare, cause short outbreaks in the intervening years between sampling, or would result in a lower chance of an animal being sampled (such as those with high mortality rates and short periods of shedding or seropositivity).

An alternative strategy would be the targeted surveillance of high-risk groups, which can be especially helpful in detecting rare diseases. Pathogen detection can be maximized by sampling animals that have a high likelihood of exposure, such as those which have contact with reservoir hosts or are exhibiting clinical signs of disease [35]. Certain species of waterfowl have been targeted for highly pathogenic avian influenza virus sampling based on data showing that they have higher rates of viral shedding or mortality [59]. Testing for chronic wasting disease may also prioritize sampling of animals showing clinical signs consistent with the disease, such as neurologic abnormalities or emaciation [60]. Capturing obviously sick or debilitated animals for disease sampling is more likely to result in pathogen detection, inform on which pathogens are likely to cause symptomatic disease, and enable swift interventions, where appropriate. However, this targeted sampling of symptomatic animals would not enable the calculation of accurate prevalence estimates for the population as a whole, resulting in biased estimates of disease burden.

Diseases that cause readily visible, physical changes could be monitored non-invasively via remote camera surveillance. Candidate diseases include those which cause alopecia, nasal/ocular discharge, muscle wasting, lameness, and large abscesses. Camera traps have been used to monitor mange in several different wildlife species from feral hogs (*Sus scrofa*) to Iberian wolves (*Canis lupus*) and to quantify the severity of giraffe skin disease in Tanzanian giraffes (*Giraffa camelopardalis*) [61–64]. Cameras facilitate range-wide syndromic surveillance at sites which are difficult for field personnel to access, and can provide prevalence estimates of

symptomatic disease in almost real-time compared to intermittent range-wide sampling. However, there are limitations to the sensitivity and specificity of cameras for disease detection since the absence of lesions in photographs does not necessarily indicate the absence of disease, and different pathogens may result in a similar clinical presentation. Cameras have been used effectively to monitor the advancing front of Tasmanian devil facial tumor disease, a highly transmissible tumor of the soft tissues of the face and oral cavity [65]. However, the absence of lesions in photographs does not necessarily indicate the absence of disease, as tumors can be located within the oral cavity and not visible on photographs. Similarly, scar tissue can look very similar to tumors and histopathology is sometimes the only way to definitively diagnose a lesion as devil facial tumor disease.

Factors contributing to the maintenance of disease were investigated in the decades following spillover from reservoir host to novel host species in the third chapter of this dissertation. Endangered Peninsular bighorn sheep (*Ovis canadensis nelsoni*) have suffered population declines due to pneumonia caused by pathogens which originated in domestic sheep (*Ovis aries*; the original reservoir host) but now circulate within bighorn sheep herds in the absence of continued spillover. This chapter explores demographic and geographic risk factors for bighorn sheep exposure to several pathogens, and measures the impact that pathogens had on adult survival and lamb recruitment at the herd level.

The Peninsular Mountains are divided into nine "recovery regions," defined for bighorn sheep population management [66]. Bighorn sheep from each recovery region are captured approximately every two years and adult females are preferentially captured [67]. This results in a cross-sectional, roughly two-stage stratified cluster design, whereby the population is stratified by recovery region, then adult females are randomly sampled within each region. This design is

appropriate for establishing range-wide and region-specific prevalence estimates of disease, but is subject to biases, such as over or underestimating the prevalence of pathogens in lambs or males due to smaller sample sizes in these groups.

A subset of bighorn sheep are also collared with radio-transmitters. The monitoring of these transmitters facilitates longitudinal monitoring for estimates of population survival and mortality rates, and the prompt detection and collection of mortalities. Necropsy of carcasses provide a wealth of information, including cause of death, but this information only allows reactive management responses, not proactive prevention. Necropsy would have a higher probability of detecting high consequence pathogens which contributed to the cause of death, whereas pathogens detected during live captures would be more likely to cause disease that an animal had/could survive.

Bighorn sheep may also play a role as the reservoir host for several pathogens they share with sympatric species, bringing us full circle to the first phase of pathogen spillover: maintenance of disease in a reservoir host. Bighorn sheep pathogens examined in this chapter which also circulate in other local hosts include: 1) *Orbivirus* spp. is transmitted by *Culicoides* spp. biting midges and can infect sympatric ruminant species such as mule deer (*Odocoileus hemionus*), domestic sheep, and domestic cattle [68]. 2) *Anaplasma* spp. is vectored by various tick species and has been detected in local mule deer (California Department of Fish and Wildlife, Wildlife Investigations Laboratory, unpublished data) [69]. 3) *Toxopasma gondii* and *Leptospira* spp. are both transmitted through environments contaminated with urine or feces and infect a range of hosts from felids to ungulates [68,70,71]. Although the first introduction of these pathogens into or from bighorn sheep populations has not been established, bighorn sheep

share several pathogens with other host species and may play a role in maintaining these pathogens in the ecosystem.

Summary

The accelerating pace of habitat fragmentation, biodiversity loss, and host/pathogen translocations has resulted in an increasing number of pathogen spillover events in recent decades. Many of these spillover events result in the subsequent establishment of pathogens in novel hosts, often causing population declines in those secondary hosts. Wildlife pathogen surveillance must be tailored to the host and pathogen species of interest, but also to the phase of spillover and spread. Detection probabilities will vary among the pre-spillover, initial epidemic, and post-spillover maintenance phases based on the prevalence and distribution of disease. For example, disease prevalence may be high in the original reservoir host, but extremely low in the novel host immediate after spillover and before it has been widely transmitted. This variation necessitates different surveillance strategies for each phase of disease spillover and spread. The surveillance strategies reviewed here can be adapted to a range of species and systems, allowing researchers to collect the data needed to identify, prevent, and respond to future emerging pathogens.

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CHAPTER 1:

Characterization of animal-human interfaces with risk of pathogen spillover along wildlife supply chains in Africa and Asia

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Abstract

The global trade of wildlife, including the removal and transportation of animals from their natural habitat into large trade networks, enables close contact of diverse host species in crowded conditions that can facilitate pathogen spillover. To characterize the settings in which wild animals are sourced, traded, and sold, we conducted observational surveys of sites along the wildlife "supply chain" from source to sale, and administered questionnaires to hunters and supply chain workers in 23 countries in Africa and Asia. We collected data regarding animal taxa and husbandry, biosecurity, demographics, education, concerns about disease, and the use of personal protective equipment (PPE). The most frequently observed conditions that could potentiate pathogen spillover included the presence of animal waste (blood, tissue, excreta), multiple taxa housed together, not disinfecting animal crates/equipment or removing sick/dead animals, and a lack of veterinary care, handwashing facilities, and PPE use. Among hunters and supply chain workers, self-reporting of PPE use was higher if individuals were concerned about disease, had higher levels of education (hunters only), worked in a small market, hunted carnivores and pangolins, or hunted for personal use at home. Gloves, masks, and gowns/aprons were the least frequently used PPE, while shoes/boots were the most common. This paper utilizes a variety of local environmental and behavioral data to characterize high-risk interfaces for potential pathogen emergence and spillover along the wildlife supply chain. Our findings can

inform community education efforts regarding zoonotic pathogen transmission, wildlife trade policies, and biosecurity and PPE guidelines.

Significance Statement

The risk of zoonotic disease spillover from animals is increasingly recognized as a major threat to human health, with several recent human epidemics/pandemics traced back to pathogens originating in wildlife hosts. The global wildlife supply chain is associated with animal movements that increase the risk of pathogen transmission within and among species. We characterized interfaces with the potential for pathogen emergence and spillover along wildlife supply chains in Africa and Asia. We identified behaviors around animal husbandry, biosecurity, and PPE use that may potentiate pathogen spillover, and show that education about wildlife diseases may improve PPE use among hunters and supply chain workers. These findings can be used to guide mitigation efforts for the prevention of pathogen transmission and spillover.

Introduction

The risk of pathogen transmission among species is increasingly recognized as a major threat to human and animal population health at a global level. The number of emerging infectious disease (EID) events has increased steadily since 1940 (1), with zoonotic pathogens causing 56% of human infectious disease outbreaks globally between 1980 and 2013 (3) and an estimated >70% of all zoonotic EIDs found in humans having wildlife origins (1, 2). The World Bank estimates that from 1997 to 2009, ≥$80 billion was spent globally to respond to outbreaks of just six emerging zoonotic diseases (4). Recognition of the increasing threat that zoonotic diseases pose to human and animal health, food security, and economic stability has contributed to the growing One Health perspective, which addresses the interdependence of the health of humans, animals, and their shared environments. Perhaps no disease has demonstrated the interconnectedness of global health so explicitly as COVID-19, a viral respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The progenitor of SARS-CoV-2 is suspected to have originated in bats (5–8), with its introduction to humans possibly linked to habitat loss and wildlife movement (9). After adapting to humans, SARS-CoV-2 spread rapidly person-to-person and was declared a global pandemic by the World Health Organization in March 2020 (10). SARS-CoV-2 has had widespread and devastating effects on human health, national economies, and the way current human societies are structured, bringing the basic principles of zoonotic disease and epidemiology to worldwide consciousness.

Recent large-scale epidemics in humans have been traced back to pathogens that originated in animals and infected humans through a combination of ecological opportunity and cross-species transmission, a process referred to as “zoonotic spillover.” These epidemics have been caused by a variety of viruses, including influenza viruses, coronaviruses (severe acute

respiratory syndrome [SARS-CoV-1], Middle East respiratory syndrome [MERS-CoV]), Ebola virus, and Nipah virus (11–15). Wildlife can also contract diseases from humans through "reverse zoonosis or "anthropozoonoses;" for example, humans have transmitted respiratory viruses to wild mountain gorillas (*Gorilla beringei beringei*) (16, 17). In addition, cross-species transmission events (excluding humans) occur relatively frequently when infectious agents move from one animal host species to another, via shared ecological niches or mediated via anthropogenic disturbance, with the potential to continue circulating within the new host population. For example, domestic dogs (*Canis lupus familiaris*) have spread rabies and canine distemper to African carnivores (18, 19). These zoonotic and interspecies transmission events are more likely to occur at "high-risk interfaces" where the frequency and duration of contact between the pathogen reservoir and naive species is increased (20–22). These risky contacts increase with perturbations of ecosystems, including expansion of agriculture, habitat loss and encroachment of human infrastructure into natural habitats, movement of wild and domestic animals, and loss of biodiversity (9, 23, 24). Additionally, the number of zoonotic viruses in mammalian species is positively associated with declines in wildlife population size due to exploitation by humans and decreases in habitat area or quality (25, 26).

Overexploitation and overconsumption of wildlife are major threats to biodiversity, and the global wildlife trade (legal and illegal) is increasingly recognized for its negative impacts on wildlife populations, ecosystem integrity, and public health (27–30). Free-ranging wildlife is harvested for subsistence needs such as food and traditional medicines, and luxury uses such as clothing, jewelry, art, and the live animal trade for exotic foods and pets (27, 31, 32). Research shows that 23 – 36% of all bird, mammal, and amphibian species used for food or medicine are threatened with extinction (33), and the global wildlife trade has caused a 61.6% decline in

species abundance worldwide, with local species extirpations observed in 16.4% of populations examined (29). While the traditional use of wildlife for subsistence involves limited hunting and transport of animals within a local community, the consumption of wild animal products have become increasingly commercialized through developments in transportation infrastructure, the migration of people and their cultural preferences (for food, pets, clothing, medicine, and ornaments), and the globalization of trade (31, 32). The national and international trade networks that transport animals and their byproducts (31, 34) have reduced species abundance by 66 - 76%, whereas localized trade often has had more limited impacts (29). These reductions in biodiversity do not just threaten species survival, but also have far reaching impacts on planetary health through the loss of critical ecosystem services such as pollination, the availability of food and pharmaceutical compounds, and disease regulation (35, 36).

The entire wildlife "supply chain" from source to sale mixes potential hosts and pathogens in high-risk interfaces which may facilitate disease transmission (Figure 1). The supply chain can be classified into three primary "nodes," which include "source," "transit," and "sale." A "source" node is the origin of an animal and can include natural habitat where they are hunted or farms where they are raised. Natural areas tend to be wildlands or rural, while farms are typically peri-urban and located along trade routes to supply urban markets. Animals are then moved through "transit" nodes where they are transported by distributors and wholesalers, often at high densities and with multiple species together. "Sale" sites are markets and other locations (including on the worldwide web) where animals and their byproducts are sold to the end consumer. This often includes the mixing of several different taxa of live and dead animals from a wide geographic area. The commercial wildlife trade routes can be extensive, with animals often being moved internationally from point of origin to point of sale. The scale and reach of the

wildlife trade allows for animal species that would not normally co-occur in nature to interact and potentially exchange pathogens. For example, wildlife markets in Lao People's Democratic Republic (Lao PDR) are sites where wildlife are both sold alive and slaughtered on site, animals of many different taxa are housed together or in close proximity, and wildlife is often displayed in close contact with other foods such as fresh produce (37). In Cameroon, wildlife are usually killed by hunters at the site where they were captured before the wild meat is brought to open air markets (38). Many governments discourage but do not prohibit the sale of wild meat, and vendors might accommodate local policies by selling wildlife on the edges of markets (or "underground"), in substandard hygiene and biosecurity conditions (37, 38). Generally, source sites are associated with varying degrees of deforestation, agricultural development, loss of biodiversity, and other ecological disturbances that have been associated with the amplification of pathogens, while transit and sale sites are areas of increased cross-species pathogen transmission and zoonotic spillover risk due to high levels of contact among host species and deficient sanitation (39–42).

The extraction of wild species from their natural habitats and movement while still alive through commercial trade networks is of special concern with respect to the transmission and movement of zoonotic viruses. The live wild animal trade has the potential to facilitate pathogen transmission via dense and unhygienic animal housing conditions, and the close contact of multiple host species and people during the capture, transport, and slaughter of animals. (25, 43). Live animal markets and other components of the wildlife trade, such as the exotic pet industry, have been directly linked to zoonotic spillover and outbreaks in several species. Chytridiomycosis was introduced into native amphibian species globally through contact with non-native species imported via the trade in amphibians for research and exotic pets, resulting in

drastic population declines of endemic species (44, 45). Severe acute respiratory syndrome (SARS-CoV-1) was transmitted from masked palm civets (*Paguma larvata*) to humans at live animal markets, ultimately resulting in a pandemic following widespread human-to-human transmission (46, 47). Interestingly, one study found very low seroprevalence (mean = 10%) of SARS-CoV-1 in civets at the farms where they were bred, but a seroprevalence of up to 78% at live animal markets where they were sold (46). This serologic evidence, combined with genetic data suggesting bats as the natural reservoir of the SARS-CoV-1 progenitor (48), suggests that civets were an intermediate host and their infection resulted from the mixing of multiple competent host species at animal markets and/or the amplification of SARS-CoV-1 within the civets as they moved through the supply chain. Similarly, SARS-CoV-2 is most closely related to coronaviruses found in bats in China, but ecological, epidemiological, and genetic evidence do not support these viruses being the direct progenitor (49). A recent investigation into of the origins of SARS-CoV-2 suggests there may have been an intermediate host between bats and humans, although this potential intermediate host species has not yet been identified (49).

People working in the wildlife supply chain have potentially high occupational exposure to zoonotic pathogens, and cases of disease could be substantially under-reported in communities with limited access to healthcare. Hunters who trap free-ranging wildlife and butchers who prepare animal carcasses for consumption have very close contact with animal tissues, blood, and excrement that are common routes for pathogen transmission (42, 50). The close and frequent contact between numerous host species and people along this supply chain (many of which would not interact in a natural setting) in areas contaminated with the bodily fluids of live animals is an ideal setting for the spillover of pathogens (37). Of particular concern, viruses with

the greatest host plasticity (capable of infecting the most diverse range of host species) have been transmitted through contact with wild animals kept as pets or sold at these types of markets (51).

The wildlife supply chain poses a direct threat to human and animal health, both locally and globally, through the potential for disease spillover events that can propagate into human epidemics and wildlife epizootics. Yet, direct studies to quantify this risk across diverse wildlife trade systems and value chains around the world are lacking. Here we assemble the largest dataset to date of human behavioral and ecological risk factors for pathogen spillover within the global wildlife trade network. We gathered data from sites along wildlife supply chains in Africa and Asia, with a special focus on sites where wildlife was sold for food, to identify differences in the presence of factors that may facilitate zoonotic amplification and spillover across continents, seasons, urbanization gradient, and the node of the supply chain. We also investigated factors associated with the use of personal protective equipment (PPE) use in hunters and supply chain workers to inform risk mitigation in these activities.

Results

Characterization of sites in the wildlife supply chain

The level of urbanization varied significantly across the nodes of the wildlife supply chain ($p < 0.001$; Figure 3). Source nodes (n = 34) were more commonly rural (58.8%, n = 20) and less commonly peri-urban (20.6%, n = 7) or urban (20.6%, n = 7; $p = 0.007$). Sale nodes (n = 73) were more commonly urban (57.5%, n = 42) and less commonly peri-urban (23.3%, n = 17) or rural (19.2%, n =14; $p < 0.001$). Transit nodes (n = 25) were most often at rural sites (72.0%, n = 18) and rarely at peri-urban (12.0%, n = 3) or urban (16.0%, n = 4 sites; $p < 0.001$).

Animal blood and dead tissues were present at 54.8% (n = 69/126) of sites (Figure 2) and were more common at urban sites (OR = 30.68, 95% CI = 1.29 – 868.56) and points of sale nodes (OR = 44.20, 95% CI = 2.17 – 1,220.64; Table S1). Animal urine and feces were commonly observed (78.6%, n = 99/126; Figure 2), and observed more frequently in Asia compared to Africa/Middle East (OR = 24.78, 95% CI = 2.92 – 344.49) but less commonly at transit nodes compared to source nodes (OR = 0.02, 95% CI = 0.00 – 0.28; Table S1). Continent, season, urbanization, and node of the supply chain were not significant predictors of drinking water being unprotected (31.0%, n = 39/126) or shared with animals (24.6%, n = 31/126), or bathing water being shared with animals (48.4%, n = 61/126; Figure 2, Table S1).

The removal of sick or dead animals was not observed at most sites (73.8%, n = 93/126; Figure 2), but was missing less often from sites in Asia compared to Africa/Middle East (OR = 0.02, 95% CI = 0.00 – 0.13) and missing from sale nodes less often than source nodes (OR = 0.02, 95% CI = 0.00 – 0.21; Table S1). There were no significant predictors of the disinfection of animal crates and equipment (Table S1), which was an uncommon practice and absent from 85.7% (n = 108/126) of study sites overall (Figure 2). Handwashing facilities were absent from 54.0% (n = 68/126; Figure 2), and were missing less often from sites in Asia compared to Africa/Middle East (OR = 0.01, 95% CI = 0.00 – 0.09) (Table S1).

Multiple taxa were housed together at 69.0% (n = 87/126) of sites sampled, and wild and domestic taxa were housed together in cages or pens at 7.1% (n = 9/126) of sites. Veterinary care was absent at 73.8% (n = 93/126) of sites (Figure 2, Table S1). Continent, season, urbanization, and node along the supply chain did not predict any of these factors (Table S1).

All site characterization model results (percentage of sites, sample sizes, odds ratios, and 95% confidence intervals) can be found in Table S1.

Characterization of animal taxa in the wildlife supply chain

Although sites were chosen because they were previously identified as part of the wildlife supply chain, wildlife species were not observed at every site. Wildlife taxa were observed alive or freshly slaughtered at 45.2% (n = 57/126) of sites, and dead at 46.8% (n = 59/126) of sites. Results from all logistic regression models below (odds ratios and credible intervals) predicting the presence of animal taxa can be found in Table S1.

For wildlife observed alive or slaughtered on site, non-human primates (10.3%, n = 13/126) were less common in Asia than Africa/Middle East, and more common at urban sites compared to rural sites. Wild birds (29.4%, n = 37/126) were more common in Asia and at urban sites. Ungulates (8.7%, n = 11/126) were less common at Asian study sites. Continent, season, urbanization, and node along the supply chain did not predict the presence of rodents/shrews (19.0%, n = 24/126), bats (9.5%, n = 12/126), or pangolins (7.9%, n = 10/126) observed alive or slaughtered on site in this study. Carnivores were not observed at enough study sites (4.0%, n = 5/126) to perform regression analysis.

Among wildlife carcasses identified, rodents/shrews (36.5%, n = 46/126) were more common at transit nodes, and bats (16.7%, n = 21/126) were less common at sale nodes compared to source nodes. Non-human primates (27.0%, n = 34/126) and pangolins (14.3%, n = 18/126) were both less common at study sites in Asia and more common at transit nodes compared to source nodes across Asia and Africa/Middle East. Carnivores (11.1%, n = 14/126) were more common at peri-urban sites compared to rural sites. Ungulates (23.8%, n = 30/126) were less common in Asia and more common at sale nodes. The presence of wild birds (13.5%, n = 17/126) was not associated with continent, season, urbanization, or node along the supply chain.

For domestic animals observed alive or slaughtered on site, poultry/fowl (65.1%, n = 82/126) were more commonly observed at study sites in Asia compared to sites in Africa/Middle East. Goats/sheep (50.0%, n = 63/126) and camels (13.5%, n = 17/126) were observed less frequently at transit nodes and dogs/cats (22.2%, n = 28/126) were observed less frequently at sale nodes compared to source nodes. Continent, season, urbanization, and node along the supply chain were not significant predictors of the presence of swine (21.4%, n = 27/126) or cattle/buffalo (45.2%, n = 57/126). Horses were not observed at enough study sites (4.0%, n = 5/126) to perform regression analysis.

Among domestic animal carcasses identified, poultry/fowl (35.7%, n = 45/126) were more commonly observed at study sites in Asia and sale nodes compared to source nodes. Goats/sheep (38.9%, n = 49/126) were more common at urban sites compared to rural sites, and sale nodes compared to source nodes. Camels (7.1%, n = 9/126) were less common at Asian sites and during the wet season, but more common at urban sites and sale nodes. Cattle/buffalo (47.6%, n = 60/126) were more common in Asia, at urban sites, and at both transit and sale nodes, compared to source nodes. Dogs and cats (7.9%, n = 10/126) were more common at sites in Asia. Continent, season, urbanization, and node along the supply chain were not significant predictors of the presence of swine (31.7%, n = 40/126). Horses were not observed at enough study sites (1.6%, n = 2/126) to perform regression analysis.

Use of protective equipment among supply chain workers

Among people working in the wildlife supply chain, 30% (n = 159/530) of individuals reported wearing any type of PPE at work, and this was more common if they reported being worried about disease (OR = 8.04, 95% CI = 4.36 – 15.11; Table S2). People were less likely to report use of PPE (of any type) if they were in Asia compared to Africa/Middle East (OR = 0.06,

95% CI = 0.00 – 0.74) or worked at large markets compared to small markets (OR = 0.23, 95% CI = 0.05 – 0.94; Table S2). Gender, education level, selling live animals, housing multiple species together in one enclosure overnight, and the taxa sold were not significant predictors of PPE use in this study. Because animal taxonomic orders were not significant predictors of PPE use in preliminary models, animal taxa were collapsed into "wildlife" and "domestic" groups in the final model, which were also not significant. The duration of time a person had worked at a site was also not a significant predictor of PPE use.

Of the 159 individuals (28 females, 131 males) in the supply chain who reported using PPE, shoes or boots were used by 71.1% (n = 113), protective clothing was used by 40.9% (n = 65), gowns/aprons were used by 9.4% (n = 15), gloves were used by 17.0% (n = 27), and masks were used by 12.6% (n = 20). One quarter of people reported using any type of PPE while handling animals (25.2%, n = 40) and half reported always using their PPE while they were working (51.6%, n = 82). Of the 331 (35 females, 296 males) total supply chain workers who reported slaughtering an animal in the past year, 28.7% (n = 95/331, 15 females, 80 males) reported using PPE and 30.5% of those (n = 29/95, all males) reported using PPE specifically while slaughtering or butchering animals.

Use of protective equipment among hunters

Among hunters, 28.7% (n = 286/995) of individuals reported using PPE, which was more likely if they were worried about disease (OR = 2.24 95% CI = 1.45 – 3.49) or had had a primary (OR = 2.09, 95% CI = 1.17 – 3.76), secondary (OR = 2.36, 95% CI = 1.28 – 4.44), or tertiary (OR = 2.74, 95% CI = 1.16 – 6.41) school education (Table S3). Hunters were less likely to report PPE use in Asia compared to Africa/Middle East (OR = 0.33, 95% CI = 0.12 – 0.81; Table S3). Hunters who hunted for home use were more likely to use PPE (OR = 2.13, 95% CI =

1.02 – 4.58), while hunting for the purposes of sale or nuisance wildlife control was not associated with PPE use (Table S3). Hunters were also more likely to use PPE if they reported hunting carnivores (OR = 1.85, 95% CI = 1.05 – 3.33) or pangolins (OR = 2.65, 95% CI = 1.36 – 5.23) in the past year, but less likely to use PPE if they hunted wild birds (OR = 0.60, 95% CI = 0.38 – 0.94; Table S3). There was no association between PPE use and the hunting of rodents/shrews, bats, non-human primates, birds, or ungulates (Table S3). Gender and experience with an outbreak of dead wild animals in the previous year were also not significant predictors of PPE use (Table S3).

Of the 286 hunters (11 females, 275 males) who reported using PPE, shoes or boots were used by 95.8% (n = 274), protective clothing was used by 36.4% (n = 104), gowns/aprons were used by 2.8% (n = 8), gloves were used by 9.4% (n = 27), and masks were used by 3.8% (n = 11).

Approximately one third of hunters reported using PPE while handling animals (27.0%, n = 77/285) and 64.9% (n = 185/285) always used PPE while they were working (groups overlapped). Of the 803 hunters (71 females, 732 males) who reported slaughtering an animal in the past year, 31.8% (n = 255/803, 8 females, 247 males) reported using PPE and 15.4% (n = 39/254, all males) of those reported using PPE while slaughtering or butchering animals. Sample sizes differ among groups because one respondent did not answer all follow-up questions regarding the specific types and circumstances of their PPE use.

Discussion

This study describes the distribution of risk factors for zoonotic pathogen spillover along the wildlife supply chain in Africa/Middle East and Asia, especially at sites where wildlife was

sold for food. Overall, we found limited biosafety practices or biosecurity measures implemented, which raises concerns around the potential for disease transmission in these settings. Coinciding with this, we identified several common conditions that may facilitate zoonotic disease emergence, including housing multiple taxa together, the presence of live or freshly slaughtered animals, and the presence of animal blood, tissues, and excreta. Certain conditions were more common at urban sites and point-of-sale nodes, raising additional concerns for the risk of pathogen transmission among a wide variety of species and spillover to humans at locations with high population densities.

Housing multiple taxonomic orders of wildlife together was very commonly observed at sites sampled in this study, perhaps due to logistical and space constraints in resource-poor settings and a possible lack of knowledge regarding the increased risk this behavior holds for cross-species disease transmission. This practice increases the risk of cross-species pathogen transmission through close contact among different species and contamination with bodily fluids such as respiratory excretions. Even when animals of different species or orders were separated into different cages, these cages were often placed extremely close together or stacked on top of one another, potentially allowing animals to be in effective contact. While wild and domestic species were not frequently observed housed together (only 7% of sites), inter-species contact between caged and free-roaming animals was likely possible. At many markets, domesticated species roamed freely and local free-ranging wildlife, such as rodents and birds, could interact with caged animals for sale (52). In addition, wildlife found together within the supply chain were often species that would not come into close contact naturally based on non-overlapping ecological niches (37, 53). Previous work quantifying wildlife at markets in Lao PDR found a wide range of wildlife taxonomic groups present in the same market, many of which were

capable of harboring zoonoses of significance to humans (36 zoonotic pathogens previously documented in the mammalian species alone) (37). For both wild and domestic animals, the transit node of the supply chain is a bottleneck where animals housed at high densities have close contact, enabling opportunity for transmission of pathogens that have been pre-adapted or are evolving adaptations to zoonotic transmission. Separating animals by species and only housing animals together if they were captured in the same location would mitigate cross-species transmission risk but would be hard to implement in resource poor settings and without significant investment in education campaigns.

Both urban sites and sale nodes were areas with relatively high human densities, and sale nodes tended to be sites where multiple species were housed at markets after being transported from various source locations, increasing the possibility of a species encountering a new pathogen to which that species has not been previously exposed. Observed patterns, such as animal blood/tissues being more common at sale nodes relative to source nodes, might have been impacted by the inclusion of both natural areas and farms as source nodes, even though these areas can differ greatly in their level of urbanization, species present, and animal slaughtering activities performed. Future work could explore finer scale differences among source nodes which may impact disease amplification and zoonotic spillover at these sites.

Although there were no specific predictors of unprotected drinking water or drinking/bathing water being shared among humans and animals, both practices were observed at least once at one-quarter to one-half of sites. Our analysis did not measure changes in the proportion of time that water sources are shared, but these behaviors were consistently observed across seasons, continents, and human densities, which is of concern given the potential for water-borne pathogen transmission (54).

The proper use of PPE can prevent disease transmission by limiting contact with infectious materials during close contact with animals and while cleaning animal tissues and excreta (55). The use of PPE can act as a harm reduction practice in occupations with high zoonotic disease exposure while the primary goal of reducing high-risk, unstainable wildlife trade is concurrently addressed. The greater PPE use reported among people with higher levels of education and those concerned about disease suggests that education regarding the health risks of working with animals may increase its use among hunters and supply chain workers. However, we cannot rule out the possibility of response bias in interviewees' responses to questions regarding their personal use of PPE. Although we did not detect gender differences in PPE use, we may not have had the power to detect gender differences due to the interviewee pool was heavily skewed towards males.

Among supply chain workers, working in a large market reduced the likelihood of PPE use. This could be related to a culture of apathy towards PPE, e.g., in larger markets with more workers, a person is more likely to become accustomed to co-workers not using PPE and not suffering any apparent ill effects. Studies of human healthcare workers have found that a strong "safety culture" (the use of PPE by supervisors and peers) and enforcement of regulations were important in maintaining positive reinforcement to encourage personnel to consistently use PPE (56–58). Markets may be able to create a "safety culture" by employing strong public education campaigns to encourage voluntary PPE use (e.g., radio commercials, posters in markets, direct outreach), setting PPE requirements for vendors, and enlisting the support of local public health groups to provide PPE to workers.

Gloves, masks, and gowns/aprons were the least frequently utilized types of PPE across all groups. Shoes/boots were the most common PPE used, but these are also the least likely to

protect a user from exposure to a pathogen and may contaminate homes and other locations if not adequately disinfected. Determining the type of PPE that people have access to and are willing to consistently use is an important part of establishing recommendations for various professions. For example, the long-term durability of shoe/boots may be one reason they were more commonly utilized than single-use, disposable PPE such as masks and gloves which must be continuously repurchased. While increasing the use of PPE and biosafety practices may be beneficial from a health perspective, these are also products/procedures that may be costly or difficult for people to obtain, such as clean water and soap for handwashing. We recommend incorporating discussions about practical and sustainable forms of PPE for a given occupation, including those which can be safely reused and which low-cost alternatives may be effective (e.g., reusable cloth masks vs. disposable surgical masks), as part of educating communities about zoonotic disease, occupational disease exposures, and forms of high-risk contacts with animals.

PPE use was also consistently lower at study sites sampled in Asia. This may reflect a true difference in PPE use, either due to cultural norms or monetary/logistical access to supplies, or may reflect inconsistences in data collection, definitions, or biased sampling across sites. Fine-scale patterns of PPE use may have been masked by the types of sites sampled in this study or the granularity of the data. We may observe a general increase in PPE use now that the public has become more aware of the role wildlife play in disease transmission in light of the SARS-CoV-2 pandemic, the global adoption of masking, and the awareness of zoonotic disease risk from live animal markets.

Many species observed in the wildlife supply chain were hunted and/or traded illegally, according to local and international wildlife protection laws and trade regulations, including the

Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (37, 53, 59–61). Some species observed in the wildlife supply chain are also undergoing significant population declines and are listed as near threatened, vulnerable, or endangered by the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (62), contributing to local and global reductions in biodiversity and ecosystem resilience (21, 22, 63). A recent study found that among mammals listed as threatened by the IUCN (n = 1,125 examined), 54.8% were experiencing population declines due to exploitation or reductions in habitat area or quality, and these species were also predicted to host a greater number of zoonotic viruses compared to species listed as threatened for other reasons (25). If wildlife populations continue to rapidly decline due to habitat loss and exploitation by humans, two of the primary causes of species decline worldwide, their role in the spillover of zoonotic pathogens may increase as well.

Despite the impacts that unregulated hunting and commercial sale of wildlife have on biodiversity and conservation, wildlife are still a critical, locally available, and culturally important food source for many communities across the globe (64–67). Until access to sustainable food and income is secure and the demand for exotic pets and wildlife-derived products declines, our findings could inform community education efforts regarding zoonotic pathogen transmission risk and the development of PPE adoption campaigns informed by the behavioral evidence. High risk interfaces along the wildlife supply chain identified in this study could be modified to reduce the risk of cross-species transmission and zoonotic spillover to humans by 1) eliminating the sale of live animals and high-risk taxa at markets, 2) housing animals together only if they are of the same species and from the same location, 3) cleaning and disinfecting equipment and slaughter facilities, 4) increasing the use of highly effective PPE such

as gloves and masks, and 5) securing year-round sources of clean water for drinking and handwashing. These guidelines apply to domestic as well as wildlife species. As the global community calls for changes to the way wildlife is traded and consumed in light of the SARS-CoV-2 pandemic, nuanced and targeted policy changes that can be adapted based on local community needs, traditions, and conditions, will be important to conserve wildlife while also avoiding unintended consequences such as impacts to food security and sustainability of livelihoods (66).

Materials and Methods

Characterization of sites in the wildlife supply chain

We identified sites along wildlife supply chains in Africa and the Middle East ("Africa/Middle East") and Asia and Southeast Asia ("Asia") where there were animal-human interfaces with potential for pathogen spillover, with a special focus on sites where wildlife was sold for consumption. These were sites where epidemiologic conditions, such as a high level of close contact between humans and wild animals at markets where live wildlife were for sale, could facilitate cross-species disease transmission or zoonotic spillover.

Sites along the wildlife supply chain (n = 126) were visited between July 2015 and November 2018 by trained observers, with the goal of visiting each site at least once during the wet and dry season of each year (Figure 4). During each visit, observers used standardized survey forms to record the season (wet or dry), node along the supply chain (source, transit, sale), and local urbanization gradient (rural, peri-urban, urban). They also recorded the presence of risky behaviors and circumstances related to zoonotic spillover: wild and domestic animal taxa present, if multiple taxa were housed together, if wild and domestic taxa were housed

together, if a veterinarian cared for animals at the site, biosecurity measures (handwashing facilities, removal of sick or dead animals from live animal settings, disinfection of animal crates and equipment), presence of animal waste (urine and/or feces, tissue and/or blood), and if human drinking and bathing water sources were unprotected or shared with animals. Wild taxa observed were categorized as rodents/shrews, bats, non-human primates, birds, carnivores, ungulates, and pangolins. Domestic taxa observed were categorized as poultry/other fowl, goats, sheep, cattle/buffalo, camels, swine, dogs/cats. The condition of wildlife taxa (live, dead) was also recorded and aggregated based on zoonotic risk; animals present alive and/or slaughtered on site were grouped together, and animals present dead and intact and/or sold in parts ("dead") were grouped together.

Site visits resulted in 562 observation events across 61 sites in 13 African/Middle Eastern countries (Cameroon, Democratic Republic of the Congo, Egypt, Ethiopia, Ghana, Guinea, Republic of Côte d'Ivoire, Jordan, Kenya, Republic of the Congo, Sierra Leone, Tanzania, Uganda) and 65 sites in eight Asian countries (Bangladesh, Cambodia, Indonesia, Lao People's Democratic Republic [Lao PDR], Myanmar, Nepal, Thailand, Vietnam).

Of the 126 sites sampled, six of them included multiple nodes (source, transit, sale) of the wildlife supply chain. To evaluate the relationship between urbanization and node of the supply chain without resampling by season, we aggregated the study sites by urbanization and node only, resulting in a total of 132 unique site events. Comparisons between node and urbanization classifications were made using chi-square or Fisher's exact test (to accommodate small cell sizes) with significance at $p \leq 0.05$ in the R programming language (69).

Observation event data for the 126 sites were aggregated separately based on season, node of the wildlife supply chain, and urbanization into 169 "site events" used for regression

modeling. When sites were visited and characterized multiple times (i.e., more than one observation per site), a risk characteristic was considered "present" if the characteristic was observed at least once on any visit. These site event data informed Bayesian, multilevel, logistic regression models testing the association of continent, season, node of the supply chain, and urbanization (predictors) with the presence of the zoonotic risk characteristics listed above describing animal husbandry, animal waste, biosecurity, and taxa present (outcomes). Regression models were only performed for outcome variables with ≥9 events observed across the 169 site events to avoid inaccurate estimates due to lack of outcome variability.

Use of protective equipment in the wildlife supply chain

People within the wildlife supply chain were interviewed regarding their contact with animals and PPE use if they hunted, trapped, or fished wild animals (hunters), or if they traded wild/domestic animals or owned a business in a market (wildlife supply chain workers, including markets). Interviewees were selected if they reported hunting or market/supply chain involvement associated with a given site but were not always recruited within markets themselves. Interview questions for both groups came from standardized behavioral risk questionnaires that were administered by local researchers in the interviewee's native language.

Data were collected from a total of 530 supply chain workers (83 females, 447 males) from 29 sites in nine countries in Africa and the Middle East (317 workers; Republic of Côte d'Ivoire, Ghana, Cameroon, Republic of the Congo, Democratic Republic of the Congo, Kenya, Tanzania, Egypt, and Jordan) and 27 sites in six countries in Asia and Southeast Asia (213 workers; Nepal, Bangladesh, Cambodia, Indonesia, Myanmar, and Vietnam) between June 2016 and September 2018 (Figure 4).

Data were collected from 995 hunters (127 females, 868 males) from 64 sites in 10 countries in Africa (477 hunters; Cameroon, Democratic Republic of the Congo, Egypt, Ghana, Republic of Côte d'Ivoire, Kenya, Senegal, Sierra Leone, Tanzania, Uganda) and 77 sites in nine countries in Asia and Southeast Asia (518 hunters; Bangladesh, Cambodia, India, Indonesia, Lao PDR, Myanmar, Nepal, Thailand, Vietnam) between September 2016 and December 2018 (Figure 4).

Responses from human surveys informed Bayesian, multilevel, logistic regression models testing for factors associated with the use of PPE. The outcome variable was whether or not an individual reported owning any PPE used for work (including gloves, masks, gowns/aprons, clothing, and shoes/boots), and predictive covariates of interest included continent, gender (female, male; as observed by the interviewer), highest level of education completed (none, primary school, secondary school, college/university/professional school ["tertiary"]), whether or not the person was worried about outbreaks of animal disease at the local market, if the person had experienced an outbreak of dead wild animals in the previous year, the size of the market as measured by the number of people working at the site ("small" = ≤1,000 people, "large" = >1,000 people), the duration of time the person had worked at the market (≤5 years or >5 years), the purpose of hunting wild animals (consumption or use of animal products at home, sale of live animals or animal products, culling or live trapping and translocation of nuisance animals), and the animal taxa hunted or sold within the past year. Wild taxa included rodents/shrews, bats, non-human primates, birds, carnivores, ungulates, and pangolins. Domestic taxa included poultry/other fowl, goats, sheep, cattle/buffalo, camels, swine, dogs/cats.

Follow-up questions included which specific types of PPE were used (gloves, masks, gowns/aprons, clothing, shoes/boots), in what circumstances this PPE was used (always while at work, while handling animals, while slaughtering or butchering animals), and if the individual had slaughtered an animal in the past year.

Model building

All regression models were performed using the "brms" package (70, 71) in the R programming language (69). Continent, country, and site name were controlled for as a nested random intercept to account for resampling by geographic location. Weakly informative priors [Normal(0, 2.5)] were selected for the intercept and beta parameters in order to account for complete or quasi-separation of data (72), and each model contained four chains with 10,000 iterations each. The 95% highest density interval was used as the credible interval (CI). Model parameters were included if they were considered biologically important (such as gender and season) or were associated with both the model outcome and other model parameters (confounders) according to bivariate comparisons. Bivariate comparisons were made using chi-square or Fisher's exact test (to accommodate small cell sizes) with significance at $p \leq 0.05$. Adjusted p-values for multiple comparisons were calculated using the Holm's correction (73). Animal taxa were modeled individually, then collapsed into broader "wildlife" and "domestic" groups if individual taxa were not significant predictors of PPE use.

Ethics statement

The University of California at Davis Institutional Review Board (#804522) approved all research activities, and research protocols were approved in all participating countries through local ethics committees and Institutional Review Boards.

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Figures and Tables

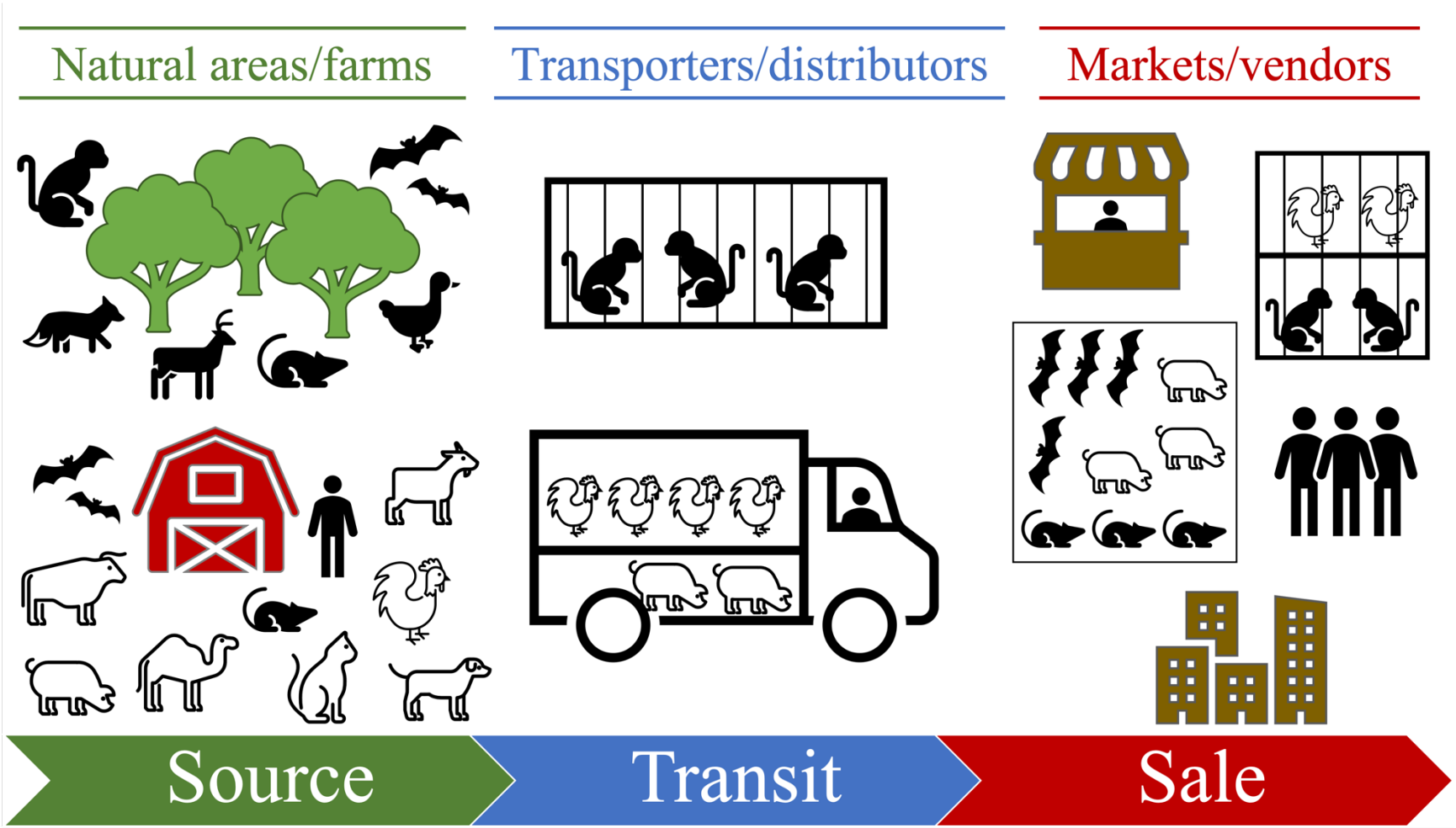

Figure 1. Schematic illustrating the wildlife supply chain through which wildlife and their byproducts mix as they move from source to sale. Animals shown in black are wildlife taxa, animals show in white are domestic taxa.

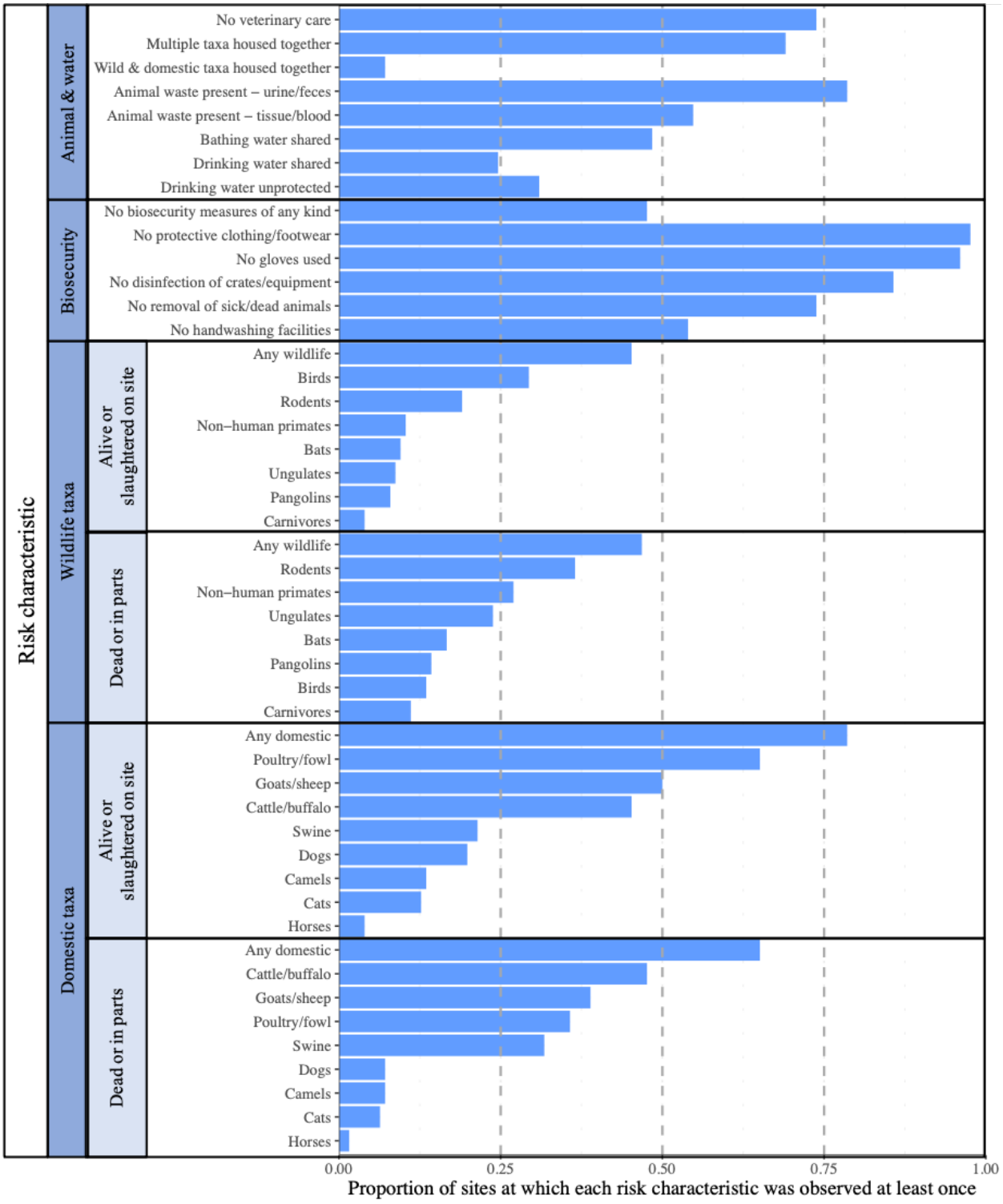

Figure 2. Barplot showing the proportion of study sites (n = 126) along the wildlife supply chain at which a given risk characteristic was observed at least once. Data were collected from sites across 21 countries in Africa/Middle East and Asia between July 2015 and November 2018.

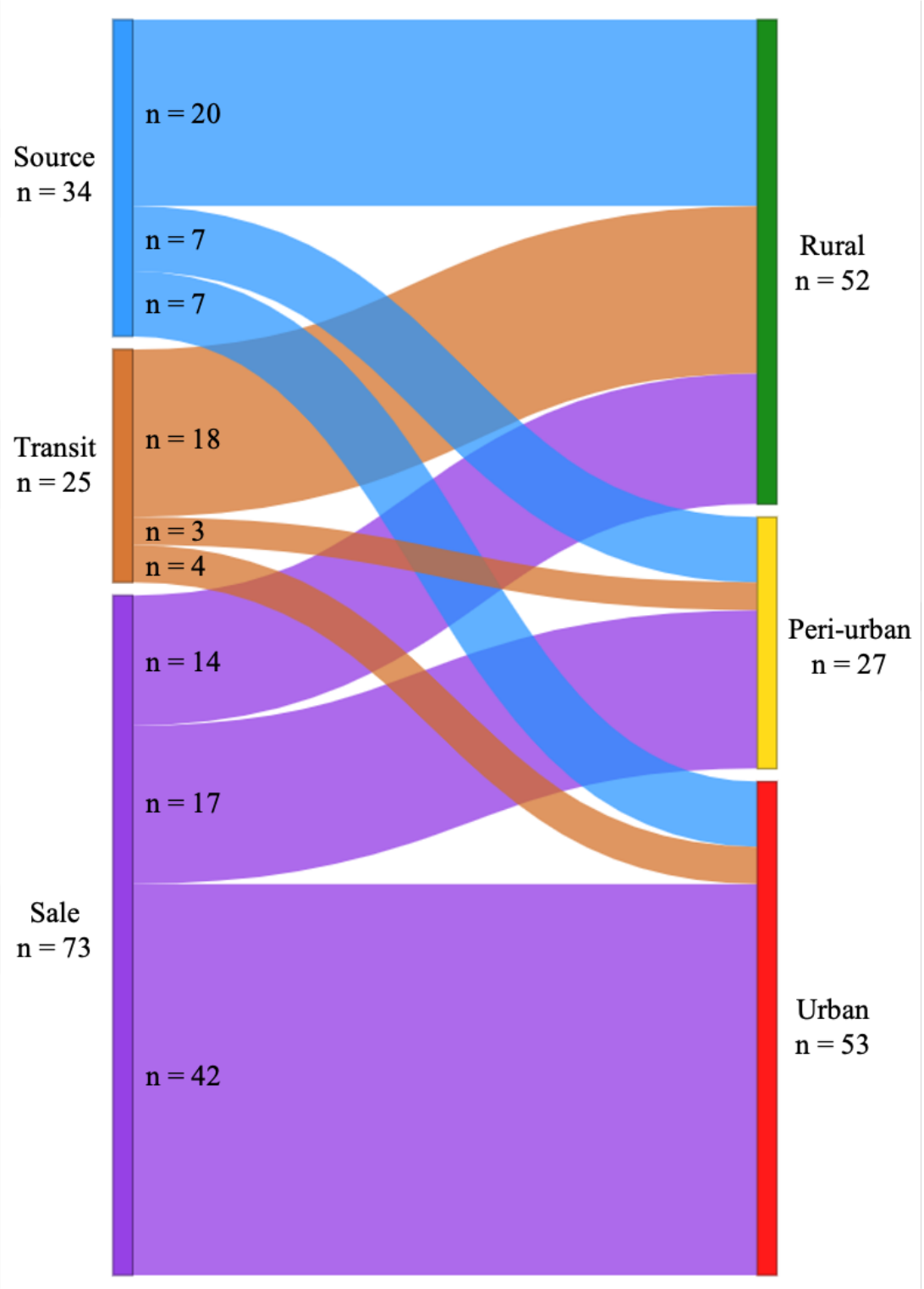

Figure 3. Sankey network plot illustrating the distribution of study sites (n = 126) among nodes along the wildlife supply chain (source, transit, sale) and local urbanization (rural, peri-urban, urban). Some study sites (n = 6) were comprised of multiple nodes of the supply chain, resulting in a total of 132 elements being included in the figure. The width of the nodes (vertical bars) and links (horizontal lines) are proportional to the number of observations in each category.

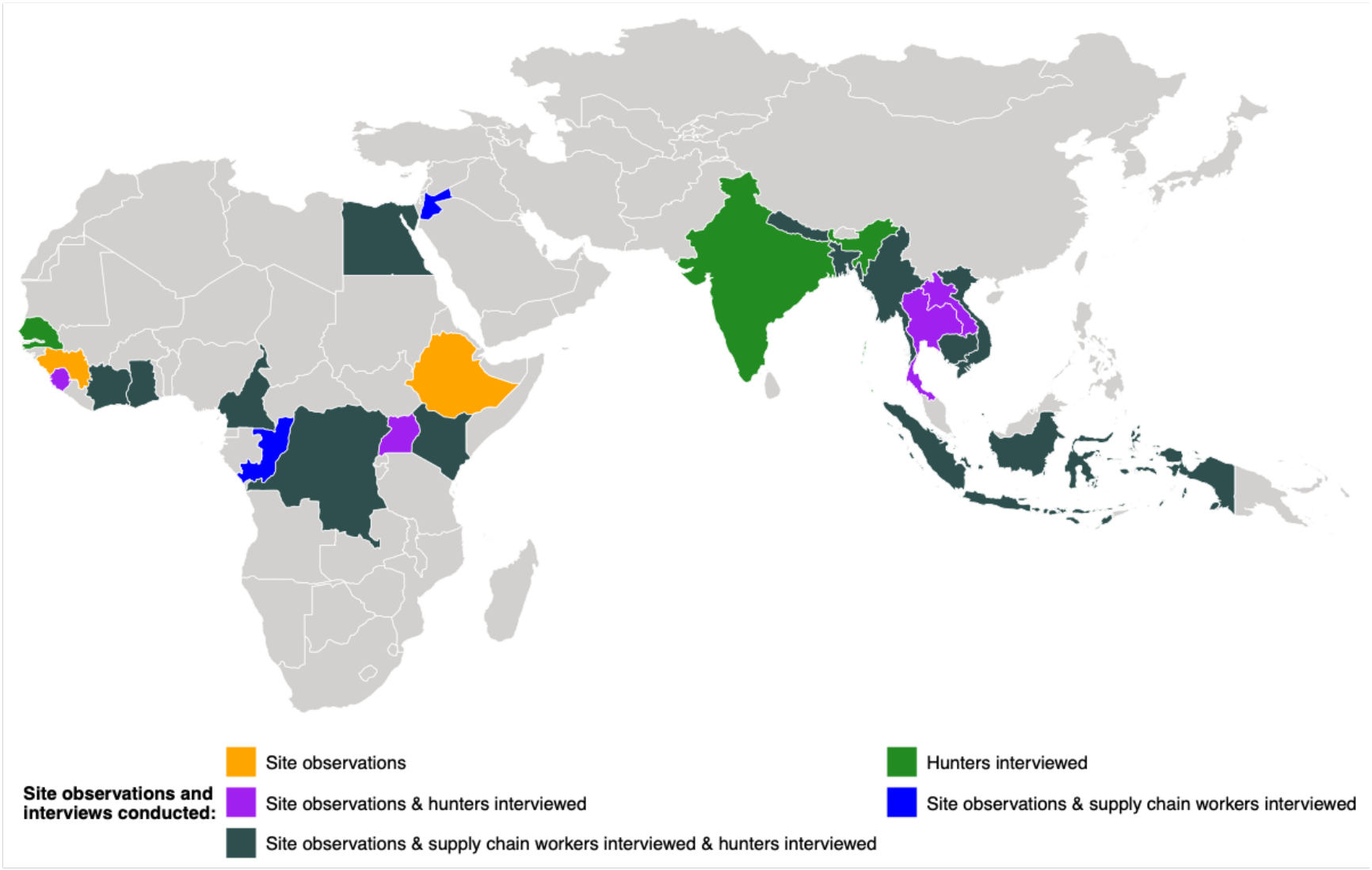
Site observations and interviews conducted:
Site observations
Site observations & hunters interviewed
Site observations & supply chain workers interviewed & hunters interviewed
Hunters interviewed
Site observations & supply chain workers interviewed

Figure 4. Map depicting the distribution of site observations and human interviews along the wildlife supply chain in Africa and Asia. Sites along the wildlife supply chain (source, transit, sale) were visited by trained observers who used standardized survey forms to record the presence of risky behaviors and circumstances related to zoonotic spillover, including animal husbandry and taxa present, animal waste, and biosecurity. Site visits resulted in 562 observation events across 61 sites in 13 African/Middle Eastern countries and 65 sites in eight Asian countries between July 2015 and November 2018. People within the wildlife supply chain were interviewed regarding their contact with animals and personal protective equipment use if they hunted, trapped, or fished wild animals (hunters), or if they worked within the wildlife supply chain. Data were collected from a total of 530 supply chain workers from 29 sites in nine countries in Africa and the Middle East and 27 sites in six countries in Asia and Southeast Asia between June 2016 and September 2018. Data were collected from 995 hunters from 64 sites in 10 countries in Africa and 77 sites in nine countries in Asia and Southeast Asia between September 2016 and December 2018.

CHAPTER 2:

The impact of social distancing on early SARS-CoV-2 transmission in the United States

Style formatted for *Zoonoses and Public Health*

SUMMARY

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a viral pathogen that quickly became a global pandemic in the winter of 2020. In response, governments issued social distancing orders to minimize transmission by reducing community contacts. We tested the efficacy of this social distancing at the state level during the first two months of the pandemic in the United States. We utilized data on daily SARS-COV-2 case numbers and human community mobility (anonymized, aggregated cellphone location data stratified into six categories used as an index of social distancing), the date of government-issued social distancing orders, demographics, urbanization, and public transportation. We implemented cross-correlation to identify lag times between declines in mobility and SARS-CoV-2 cases. Incorporating state-specific lag times, we tested for associations between case counts and mobility metrics using Bayesian multilevel models. Decreased mobility around grocery stores/pharmacies, retail/recreation locations, transit stations, and workplaces were correlated with decreases in SARS-CoV-2 cases with significant lag times of ≥21 days. Social distancing orders were associated with fewer cumulative SARS-CoV-2 cases when they were put in place earlier. Community mobility had already started declining prior to most social distancing orders, especially the more restrictive orders implemented later in the pandemic. Social distancing is an important tool that has been implemented throughout the pandemic to decrease SARS-CoV-2 transmission, although with significant social and economic impacts. Our results suggest that declines in cases were observed several weeks subsequent to implementation of social distancing measures, and that implementing social distancing earlier could potentially minimize the duration of time these policies need to be in effect. Our findings can inform ongoing management of this pandemic and other emerging infectious disease outbreaks by identifying

areas where reductions in mobility are associated with reduced disease transmission, and the expected time frame between behavioral changes and measurable population outcomes.

KEYWORDS

severe acute respiratory syndrome; COVID-19; coronavirus; social distancing; epidemic; pandemic

IMPACTS

- Reductions in community mobility around workplaces, transit stations, retail/recreation locations, and grocery store/pharmacies was associated with subsequent declines in SARS-CoV-2 cases, but these declines in cases occurred after lag times of three weeks or more. Parks visitation was not associated with SARS-CoV-2 case numbers.
- Mobility metrics declined prior to government-issued social distancing orders, but early implementation of these orders was correlated with lower cumulated SARS-CoV-2 cases.
- Social distancing can be an effective epidemic response tool to slow transmission while developing other control measures, such as vaccinations.

INTRODUCTION

A novel betacoronavirus emerged in December 2019 in Wuhan, Hubei province, China, where it caused a cluster of severe respiratory disease and pneumonia cases. It spread rapidly across the globe and was declared a pandemic by the World Health Organization in March 2020 (World Health Organization, 2020). The virus, named "severe acute respiratory syndrome coronavirus 2" (SARS-CoV-2) caused the disease "COVID-19" (Coronaviridae Study Group of the International Committee on Taxonomy of Viruses, 2020) and now been confirmed in nearly every country in the world and infected nearly 210 million people (Johns Hopkins Coronavirus Resource Center, 2021).

The SARS-CoV-2 virus is highly contagious, with a basic reproductive number of 3.14 (95% CI = 2.69 – 3.59; calculated as a pooled estimate) (Hussein et al., 2021). Person-to-person transmission is the driver of the current pandemic (Chan et al., 2020). Airborne transmission of respiratory secretions during close contact is currently believed to be the primary method of spread, although the role of other routes are still being investigated (Karia et al., 2020). The most common symptoms in people are fever, cough, and fatigue but a range of other symptoms are being identified that affect other organ systems (Grant et al., 2020). Due to high rates of pre-symptomatic and asymptomatic viral shedding (Li et al., 2020; Moghadas et al., 2020) transmission often occurs before infection is confirmed through diagnostic testing, posing challenges for contact tracing and quarantine.

The first positive case of SARS-CoV-2 in the United States of America (USA) was confirmed by the Centers for Disease Control and Prevention (CDC) on January 20, 2020 (Holshue et al., 2020) and all 50 states reported cases by mid-March 2020. Due to limited testing capabilities during the first months of the pandemic, testing was initially limited to individuals

with fever and lower respiratory tract symptoms who also had a history of travel from China or close contact with a laboratory-confirmed SARS-CoV-2 patient within 14 days of symptom onset (Patel, 2020). Due to these limitations, community transmission in the USA went largely undetected during the early pandemic period.

To slow SARS-CoV-2 spread while testing capabilities increased, every state government in the USA instituted some kind of social distancing order (SDO) during March and April 2020. These SDOs varied in their scope, level of intensity, and timing. Not all states enforced every type of SDO either; for example, only 38 states had instituted stay-at-home orders by April 30. This variation in the timing and location of SDOs provided an opportunity to investigate the impact of social distancing measures on the spread of SARS-CoV-2 at the state level.

Minimizing close contacts through social distancing is a key tool to rapidly decrease the propagation of directly transmitted pathogens, especially emerging infectious diseases for which vaccines are not yet available. Social distancing has limited the transmission of other infectious diseases, such as influenza (Fong et al., 2020), especially when distancing is instituted early in an epidemic and across a large proportion of the population (Kelso et al., 2009). However, widespread social distancing has significant impacts on livelihoods, social interactions, mental wellbeing, and economies worldwide (Bonaccorsi et al., 2020; Brodeur et al., 2020; Fitzpatrick et al., 2020; Nicola et al., 2020; Rajkumar, 2020). Due to these far-reaching consequences, there is a need for critical scientific examination of the effectiveness of social distancing, especially during the early phases of response when strict social distancing would be most effective, but the epidemic potential of an emerging disease is not yet well characterized.

We hypothesized that social distancing, as measured by reductions in movement, resulted in subsequent declines in daily SARS-CoV-2 case incidence at the state level during the first two

months of the pandemic in the USA. We expected that these case declines would occur after a temporal delay that varied by state, related to the timing of SDOs and the degree of mobility reduction achieved. We expected that the effect of social distancing on transmission was mediated by other factors that also increase contact among people, such as population size, number of people living in dense urban areas, and public transportation use.

MATERIALS AND METHODS

Data Collection

The daily count of new SARS-COV-2 cases were reported by state public health websites and compiled by the COVID Tracking Project (The Atlantic Monthly Group, 2020d). Most states reported only laboratory confirmed cases but nine states combined these with "probable" cases, defined as symptoms consistent with SARS-COV-2 and a history of exposure to a SARS-CoV-2 patient or travel to an area with high SARS-COV-2 prevalence, per the CDC case definition (Council of State and Territorial Epidemiologists, 2020). Both probable and confirmed cases, as reported by each state, were included in case incidence counts for modeling. Polymerase chain reaction (PCR) was the only diagnostic test widely available at the beginning of the pandemic (The Atlantic Monthly Group, 2020a), which reflects current or very recent infection. Some states began combining antibody test results (indicating previous infection) together with PCR test results in early May 2020 (The Atlantic Monthly Group, 2020c). We ended the study period ended on April 30, 2020 in order to better represent case incidence and not include previous infections. Each state entered the study on the first date they reported cumulative results from ≥10 tests (minimum date: February 29, 2020).

Inadequate testing during the early weeks of the epidemic resulted in apparent under-detection of cases. However, the relative trends of the epidemic curves are still useful for examining transmission patterns among states and correspond to similar trends in hospitalizations and deaths. Although hospitalization and mortality due to SARS-CoV-2 may be less likely to go undetected than infections, these measures are affected by additional confounders, such as comorbidities, disease severity, and discrepancies in access to health care. Case counts are therefore a more appropriate index of new infections because they are more tightly associated temporally with transmission events.

State-issued SDOs (Institute for Health Metrics and Evaluation, University of Washington., 2020) included the closure of educational facilities, large gathering restrictions, initial closures of any businesses, closure of all nonessential businesses, and stay-at-home orders. Cumulative SARS-COV-2 cases in each state on the date a SDO went into effect was used as an index of how early social distancing was implemented relative to when the pandemic arrived in each state, with fewer cases indicating that an SDO was enacted earlier.

Human movement ("mobility"), in the form of anonymized location data aggregated from individual mobile phones (Google LLC, 2020), was used as an index of dynamic changes in social distancing over time. "Baseline" was the median amount of movement for a particular day of the week summarized over January 3 – February 6, 2020 and represented "normal" mobility immediately prior to the pandemic. Mobility after February 6 was calculated as the proportionate change in daily mobility relative to baseline for each day of the week. Mobility data were stratified by six different location categories: parks, residential areas (e.g. houses, apartments), grocery stores and pharmacies, retail and recreation, transit stations, and workplaces. Residential

measurements were quantified as the average number of hours that a person spent at their residence, while all other categories were the number of visitors to a given type of location.

We accounted for non-time varying, state-level variables that may influence disease transmission. These include population size (U.S. Census Bureau, 2019) and density (people/km^2) (U.S. Census Bureau, 2020); annual number of airplanes passengers (Bureau of Transportation Statistics, 2018c) and trips on public transportation ("transit ridership") (Bureau of Transportation Statistics, 2018b); number of airports (Bureau of Transportation Statistics, 2020); proportion of the population: in poverty (Semega et al., 2019), without health insurance (Edward R. Berchick et al., 2019), classified as an essential worker or healthcare worker (United Way of the National Capital Area, 2020) and using public transportation for commuting (Bureau of Transportation Statistics, 2018a); and proportion of the population living in areas classified as urban (densely developed areas with ≥2,500 people), an urban cluster (2,500 – 50,000 people), or an urban area (≥50,000 people) (U.S. Census Bureau, 2018a). Race/ethnicity was measured as the proportion of the population self-identifying as Black, Indigenous American, Hispanic/Latino, or any "non-White" race (groups were not mutually exclusive) (U.S. Census Bureau, 2018b, 2018c). Covariates that were not proportions were scaled to range from 0 – 1 but retain the relative differences between values.

Data Analyses

Data evaluation and statistics were performed in R version 4.0.4 (R Core Team, 2021). Seven-day centered means (moving windows) were generated for the daily count of new SARS-CoV-2 cases and mobility values to smooth out variation related to the day of the week (e.g. case reporting was lower and parks mobility was higher on weekends) (The Atlantic Monthly Group, 2020b). This resulted in in 53 ± 4 days (mean ± SD) of data per state. We used case numbers

rather than another disease metric, such as proportion of positive tests, because these metrics would have shown a false decrease in disease burden during the first weeks of the pandemic due to the number of daily tests performed increasing exponentially at a much faster rate relative to the number of cases.

At the start of the pandemic when case numbers were increasing and mobility was starting to decline, these variables were negatively correlated. This correlation switched to positive as case numbers started to decline in some states. Lag times between reductions in mobility and initial declines in new cases were calculated using cross-correlation between case counts and mobility. Two independent measures were considered: 1) the number of days until the inflection point from negative to positive correlation ("minimal correlation"), which is hypothesized to be the amount of time it took for social distancing to contribute to initial declines in case numbers, and 2) the number of days until the maximum positive correlation, which could reflect the time it took for social distancing efforts to have maximal effects on case incidence.

The extent of social distancing that occurred prior to government-issued restrictions was calculated as the proportionate change in mobility that had already occurred on the day an SDO was put into place, relative to the maximum change in mobility each state achieved over the entire study period.

To test for associations between non-time varying, state-level predictors and pandemic severity, we implemented a Bayesian, negative binomial regression with a log link and no random effects. For these "cumulative case models," the outcome was the cumulative count of cases in each state on April 30. We tested for associations between predictors using Spearman's rank correlation, and correlated variables (significant at $p < 0.05$) were not included in the same model. Models were compared using leave-one-out cross-validation information criterion (LOO

IC) using the "loo" package (Vehtari et al., 2017, 2020). The predictor(s) in the top model was use in the next phase of modeling.

To test for associations between mobility and case counts, we implemented a Bayesian, multilevel, negative binomial regression with a log link. For these "mobility models," the outcome was the daily count of SARS-CoV-2 cases and the predictor was daily mobility. Mobility was lagged by the number of days until maximum positive correlation as identified by cross-correlation analysis; this lag was unique for each state. We included random intercepts for each state to account for resampling, and random slopes based on the number of days since a state entered the study to account for temporal autocorrelation between case numbers. Model building included running models with just mobility predictors, then adding the non-time varying predictor(s) from the top cumulative case model(s). Multiple mobility categories were not included together in the same models since they represented interrelated behaviors (for example, as workplace mobility declined due to more people working from home, residential mobility inherently increased concurrently). Models containing the same mobility predictors were compared using LOO IC (Vehtari et al., 2017, 2020).

Regression models were performed using the "brms" package (Bürkner, 2017, 2018). Weak priors were selected for the intercept [Normal(0, 10)], beta [Normal(0, 1)], and shape [Gamma(0.01, 0.01)] parameters and each model contained four chains with 10,000 iterations each. The 95% highest density interval was used as the credible interval (CI).

RESULTS

We demonstrate a strong positive association between social distancing in the form of reduced community mobility and decreases in SARS-CoV-2 case counts at the state level.

Specifically, decreased movement around grocery stores/pharmacies, retail/recreation locations, transit stations, and workplaces were associated with decreases in SARS-CoV-2 cases with significant lag times of 38 – 41 days. When put into place earlier, SDOs were associated with fewer cumulative cases, but community mobility had already began declining before SDOs were issued.

Trends in community mobility

Mobility for grocery stores/pharmacies showed a small spike of 18% (median) over baseline during the first three weeks of March in all states except Hawaii, corresponding to people shopping in preparation for stay-at-home orders and anticipated shortages. Mobility for grocery stores/pharmacies then declined to a maximum of 19% below baseline (median) and plateaued by April. Mobility in residential areas increased during the middle two weeks of March then plateaued to a maximum 18% above baseline (median), reflecting people staying at home more often. Mobility around parks was highly variable for all states and over time, with no consistent pattern, likely because many states had less restrictive policies regarding the use of outdoor spaces. The minimum use of parks ranged from 68% below to 20% above baseline (Figure 1, Supplemental Table 1, Supplemental Figure 1).

Mobility around retail/recreation locations, transit stations, and workplaces were extremely correlated ($R^2 = 0.88 – 0.90$, $p \leq 0.001$). Mobility in these categories declined in near parallel to a maximum of 46%, 48%, and 46% below baseline, respectively (median; Figure 1, Supplemental Table 1, Supplemental Figure 1). These categories represent areas that were avoided as the public became aware of the pandemic and were commonly closed when SDOs took effect.

Mobility started changing across all states and mobility categories at the beginning of the study, very often before SDOs were implemented (Supplemental Figure 1). Social distancing orders that tended to occur earlier (e.g., large gathering restrictions, closure of educational facilities and initial businesses) had more variability in the degree of mobility reduction that had already occurred at the time they were instituted (Supplemental Figure 2A-C). Nonessential services tended to be closed later (March 17 – April 3), by which time 33 of 34 states had already reached ≥70% of the maximal change in residential mobility, ≥60% of the maximum change in retail/recreational mobility, ≥60% of the maximal change in transit station mobility, and ≥69% of the maximal changes in workplace mobility (Supplemental Figure 2D). By the time of stay-at-home orders (March 19 – April 7), 100% of 38 states had already reached ≥70% of the maximal change in residential mobility, ≥60% of the maximum change in retail/recreational mobility, ≥68% of the maximal change in transit station mobility, and ≥69% of the maximal changes in workplace mobility (Supplemental Figure 2E). Parks and grocery stores/pharmacies were not included in this analysis due to these categories having more variable patterns of use during the time period in which SDOs were enacted. Four SDOs were not enacted by every state: gathering restrictions (enacted by 49 states), stay-at home orders (enacted by 38 states), any business closure (enacted by 47 states), and closure of non-essential services (enacted by 34 states).

Temporal correlation between mobility and case count

Grocery stores/pharmacies, transit stations, retail/recreation locations, and workplaces generally had a negative correlation with cases at the start of the study when mobility was declining and cases were increasing. Minimal correlation occurred after a 21 – 23-day lag and maximal positive correlation peaked after a 38 – 41-day lag (median; Supplemental table 2, Supplemental Figure 3A-D). Residential mobility initially had a positive correlation with cases,

peaking after a two-day lag and minimal correlation occurring after a 23-day lag when cases were declining but residential mobility had plateaued and remained high (Supplemental table 2; Supplemental Figure 3E). Parks mobility had variable correlations with cases numbers, both between states and over time, with most states having undulating cross correlation curves as park use fluctuated (Supplemental table 2; Supplemental Figure 3F).

The associations of mobility with SARS-CoV-2 cases

Significant positive associations with cumulative SARS-CoV-2 case counts were detected for population size and density, population in poverty, population living in any type of urban center, population commuting on public transportation, transit ridership, number of airports, airline passengers, and the number of SARS-CoV-2 cases on the day the following SDOs were enacted: educational facility closure, stay-at-home orders, initial business closure, and nonessential services closure (Supplemental table 3). The top cumulative case model contained the population living in an urban area (β = 36.47, 95% CI = 10.83 – 107.26) and the number of airports (β = 1.68, 95% CI = 1.30 – 2.22, R^2 Bayes = 0.3, LOO IC = 741.1), and these variables were included in mobility models (Supplemental Table 3).

Once urban area and airports were controlled for, lagged mobility around grocery stores/pharmacies (β = 2.25, 95% CI = 2.02 – 2.51), retail/recreation locations (β = 1.46, 95% CI = 1.32 – 1.62), transit stations (β = 1.46, 95% CI = 1.32 – 1.66), and workplaces (β = 1.72, 95% CI = 1.51 – 1.97) had positive associations with daily SARS-CoV-2 case count (Figure 2; Supplemental table 4). Parks were not associated with case count (β = 1.04, 95% CI = 0.90 – 1.20). Models had very similar point estimates and LOO IC values regardless of whether urban area and the number of airports were included (Supplemental table 4).

Mobility around residential areas was also positively associated with case count but the point estimates, 95% CI, and LOO IC were extremely large (β = 80,515,75.63, 95% CI = 5,488,097.66 – 11,874,990.68, LOO IC = 24,601.4; Figure 2; Supplemental table 4). These unrealistic estimates were due to case numbers continuing to increase exponentially as the residential mobility curve plateaued once people were spending a peak number of hours at home. We expect that increased time spent at home would not be a predictor of cases but rather a public reaction to increasing case numbers, although more time spent at home could in turn contribute to eventual reductions in overall community transmission.

DISCUSSION

This study is unique in incorporating data at daily timesteps to demonstrate that reductions in mobility specifically around grocery store/pharmacies, retail/recreation locations, transit stations, and workplaces are associated with subsequent declines in SARS-CoV-2 cases. These are areas with a high degree of mixing among people from different households and minimizing these interactions can be expected to slow the transmission of SARS-CoV-2. Our results support other studies using complimentary techniques to show that decreases in SARS-CoV-2 cases are associated with social distancing and/or the implementation of SDOs (Courtemanche et al., 2020; Dave et al., 2020; Friedson et al., 2020; Matrajt & Leung, 2020). Social distancing is a tool that can be implemented rapidly (compared to vaccine development) but is economically and socially costly, and unlikely to end an epidemic when used as a solitary strategy. This is especially true in the face of highly contagious and rapidly mutating pathogens, as demonstrated by the spread of highly transmissible SARS-CoV-2 variants (Washington et al., 2021).

The inflection point of correlation between SARS-CoV-2 cases and mobility occurred three weeks after initial reductions in mobility, and it took five to six weeks to see maximum correlation. These lag times are supported by previous work using difference-in-difference and synthetic control approaches to estimate that it took >3 weeks for cumulative cases counts to decline substantially after stay-at-home orders were put in place (Dave et al., 2020; Friedson et al., 2020). Lags between mobility and case detection may be due to several biological, logistical, and social factors. The virus incubation period, delays and limits to testing, and pre-symptomatic or asymptomatic transmission events would all contribute to delays in case detection after infection. Decreasing mobility will decrease new transmission events, but daily case counts may not decline immediately due to recently acquired infections which will continue to be detected as people start to feel ill and get tested. Although reductions in mobility can reduce new infections between households, the increased amount of time that people spend at home may increase within-household transmission secondary to household members being in close contact more often (Leclerc et al., 2020). Anticipated lag times, adjusted for evolving changes in testing availability, should be communicated to the public to manage expectations about the expected duration of social distancing interventions.

Lag times could be reduced through multiple mechanisms. Widely available testing early during an epidemic will provide individuals with the information they need to make informed decisions about personal behaviors such as isolation and quarantine. There is likely a feedback loop between the public perception of growing case numbers leading to declines in mobility, with these reductions in mobility subsequently leading to declines in case numbers. The directionality of this two-way interaction between behavior and disease transmission likely changed over the course of our study period. If the goal is to motivate timely changes in public

behaviors to reduce disease transmission, increased transparency and public communication regarding current epidemic conditions might also increase the speed and degree of mobility reduction prior to government guided SDOs.

Indeed, we found that the public had already started reducing their mobility by the time state-level SDOs were put into place, especially the more restrictive orders that occurred later in the pandemic. This is supported by event study regression analysis that found no effect of state-level stay-at-home orders on social mixing indices, and emergency declarations accounted for only 12% of social mixing reductions after 5 days, though this increased to 45% after 20 days (Gupta et al., 2020). This lack of state-level SDO impact may reflect unmeasured local ordinances and/or individual behavior changes in response to news coverage of the pandemic. Cancellation of popular events may have encouraged the public to alter their behavior ahead of SDOs, such as the cancellation of the remainder of the National Basketball Association season on March 11 in response to a player testing positive for SARS-CoV-2. However, our results demonstrate that SDOs were relatively more effective when put into place earlier, when there were fewer people infected, similar to work that found early adoption of stay-at-home orders resulted in the largest declines in SARS-CoV-2 cases (Dave et al., 2020). Despite this mixed support for direct effects, SDOs likely encouraged the public to continue social distancing by reinforcing the severity of the pandemic threat to public health and reducing opportunities for activities outside the home.

Important non-pharmaceutical interventions to control SARS-CoV-2 transmission were implemented concurrently or subsequently to SDOs, such as the use of facial coverings, staying >6 feet apart, and frequent hand washing. These actions have reduced transmission of SARS-CoV-2 and other pathogens (Chu et al., 2020; Fong et al., 2020; Lyu & Wehby, 2020) and public

awareness of these recommended interventions increased over time (Lin et al., 2020), although there was geographic and demographic variation in compliance (Fisher, 2020). Use of non-pharmaceutical interventions and declines in mobility are likely not independent, and there could have been additive or multiplicative interactions among these behaviors.

The non-time varying, state-level variables represented different but overlapping aspects of population density and clustering, all of which had positive correlations with case numbers, although likely for different reasons. For example, larger airports, especially those with a high number of international routes, could lead to higher risk of initial seeding of disease into a state, whereas numerous urban centers may contribute to sustained disease transmission once introduced. There can also be complicated relationships between urbanization, poverty, race, and health care access, as demonstrated by urbanization mediating the association between human immunodeficiency virus (HIV) prevalence and poverty levels, and racial/ethnic disparities in HIV prevalence being reduced once poverty was controlled for (Mackey et al., 2021; Vaughan et al., 2014; Walton & Willyard, 2020). Although we controlled for these factors in our modeling, they did not greatly improve model fit, demonstrating that most of the variability in the data was explained by the mobility parameters and state-level groupings. Although this association between higher human densities and more SARS-CoV-2 cases is intuitive, some areas with high population densities, such as Taiwan, have been largely successful in controlling their outbreaks through proactive testing and contact tracing, strict social distancing, widely available face masks, and limited international travel (Wang et al., 2020).

Park mobility was not significantly associated with SARS-CoV-2 cases, likely because people could utilize these areas while still physically distancing. Recent evidence demonstrates that outdoor spaces are low risk for SARS-CoV-2 transmission and support the use of outdoor

spaces to support physical and mental wellbeing during the pandemic (Bulfone et al., 2021; Leclerc et al., 2020).

Mobile phone data provides a wealth of information but also introduces potential biases if there are different mobility behaviors between people who have mobile phones compared to those who do not. Mobile phone use in the USA is high, with 81% of people owning a smartphone, but smartphone use was lower among older age groups and lower education and income levels (Taylor & Silver, 2019), groups which were disproportionally impacted by SARS-CoV-2 (Killerby et al., 2020; Leclerc et al., 2020).

This study demonstrates that social distancing, in the form of mobility reductions, is associated with subsequent declines in SARS-CoV-2 cases, albeit with significant lag times of three weeks or more. This decline, in combination with the fact that our study showed that the public made significant mobility reductions prior to state-level SDOs, highlights the importance of early and accurate public health communication to inform individuals of preventative measures they can personally implement, especially in the absence of government-mandated guidelines. Social distancing is likely to be most effective when done proactively at the start of an epidemic, which should be communicated to the public to encourage support and compliance with SDOs. Social distancing can be an effective epidemic response tool to slow transmission while developing testing and hospital capacity, quarantine protocols, and pharmaceutical interventions, such as vaccinations.

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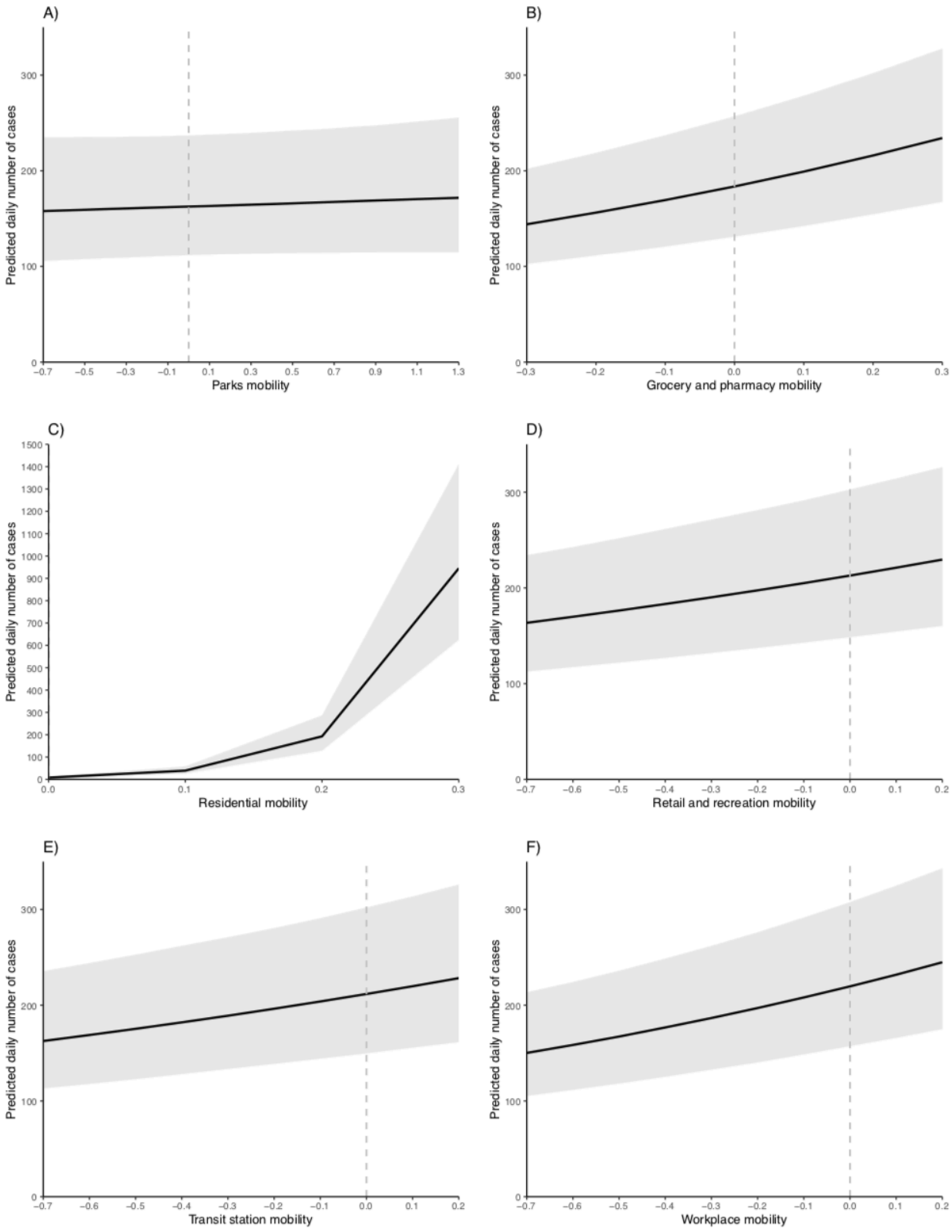
FIGURES

Figure 1: Maximum proportional change in mobility compared to baseline (baseline = 0) for each state, over the course of the study period (February 29 – April 30, 2020). Mobility was measured as one of five categories based on the type of locations used by the public: grocery and pharmacy locations, retail and recreation locations, transit stations, workplaces, and residential areas (e.g. houses, apartments). Residential mobility increased over the course of the study period (positive values relative to baseline), while all other mobility categories decreased (negative values relative to baseline). Note: Parks were not included in this figure because mobility around parks was highly variable and fluctuated erratically over time for all states.

Maximum proportionate change in mobility relative to baseline
0.0
-0.2
-0.4
-0.6
-0.8
Grocery and Pharmacy
Retail and Recreation
Transit Stations
Workplace
Residential
0.3
0.2
0.1
Alabama
Alaska
Arizona
Arkansas
California
Colorado
Connecticut
Delaware
Florida
Georgia
Hawaii
Idaho
Illinois
Indiana
Iowa
Kansas
Kentucky
Louisiana
Maine
Maryland
Massachusetts
Michigan
Minnesota
Mississippi
Missouri
Montana
Nebraska
Nevada
New Hampshire
New Jersey
New Mexico
New York
North Carolina
North Dakota
Ohio
Oklahoma
Oregon
Pennsylvania
Rhode Island
South Carolina
South Dakota
Temnessee
Texas
Utah
Vermont
Virginia
Washington
West Virginia
Wisconsin
Wyoming

Figure 2: Plot of predictions from the final six "mobility models," each testing the association between reductions in community mobility (as measured by anonymized location data aggregated from mobile phones) and the daily number of new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) cases. Each model measured mobility in one of six categories based on the type of locations used by the public: parks, grocery stores and pharmacies, residential areas (e.g. houses, apartments), retail and recreation locations, transit stations, and workplaces. The proportion of the population living in an urban area and the number of airports were also controlled for as covariates. The state was used as a random intercept and the number of days since a state entered the study was used as the random slope.


A)
Predicted daily number of cases
Parks mobility
B)
Predicted daily number of cases
Grocery and pharmacy mobility
C)
Predicted daily number of cases
Residential mobility
D)
Predicted daily number of cases
Retail and recreation mobility
E)
Predicted daily number of cases
Transit station mobility
F)
Predicted daily number of cases
Workplace mobility

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CHAPTER 3:

Epidemiology of infectious pathogens in endangered Peninsular bighorn sheep (*Ovis canadensis nelsoni*)

Style formatted for *Biological Conservation*

Abstract

Peninsular bighorn sheep (*Ovis canadensis nelsoni*) are found exclusively in Southern California and Baja Mexico. They are federally endangered due to multiple threats, including introduced infectious disease. From 1981 – 2017, we conducted surveillance for 16 infectious pathogens and estimated population sizes, adult survival, and lamb recruitment. We used mixed effects regression models to assess the impact of disease at the individual and population levels. Pathogen infection/exposure prevalence varied both spatially and temporally. Our findings indicate that the primary driver of exposure to a pathogen is the region in which an animal was captured, implying that transmission is driven by local ecological or behavioral factors. Higher *Mycoplasma ovipneumoniae* seropositivity was associated with lower lamb recruitment, consistent with lambs having high rates of pneumonia-associated mortality. This may be slowing population recovery, although there was no association between *M. ovipneumoniae* and adult survival. Orf virus seroprevalence was positively associated with adult survival in the previous year, suggesting that transmission is density dependent. Population size within a region was positively associated with adult survival. Network analyses identified three groups of pathogens that bighorn sheep tended to be co-infected with/co-exposed to, and these groups differed by mode of transmission. Peninsular bighorn sheep are recovering from small population sizes in a habitat of environmental extremes, compounded by introduced disease. Our research can help inform future targeted management and conservation of this population.

Key Words

bighorn sheep; *Ovis canadensis nelsoni*; Peninsular Range; endangered species; epizootic pneumonia; *Mycoplasma ovipneumoniae*

Highlights

- Peninsular bighorn sheep are federally endangered
- Pathogens associated with epidemic pneumonia are of concern
- *Mycoplasma ovipneumoniae* exposure was associated with lower lamb recruitment
- Orf virus exposure prevalence was higher following years of high adult survival
- Population size was associated with higher adult bighorn sheep survival

1. Introduction

Pneumonia epidemics are a source of mortality and decreased lamb recruitment in bighorn sheep (*Ovis canadensis*) throughout their range (Besser et al., 2013; DeForge et al., 1982; Nolen, 2010). Pneumonia was originally introduced to bighorn sheep through contact with domestic sheep (Foreyt and Jessup, 1982) but can be maintained by carrier bighorn sheep for years without continued spillover from domestic animals (Raghavan et al., 2016), causing intermittent epidemics and suppressing recruitment (Cassirer et al., 2018). Bighorn sheep pneumonia is a disease complex involving co-infection with infectious pathogens, environmental and immune factors, and host behavior and movement (Besser et al., 2013; Wobeser, 2007). Recent research indicates that *Mycoplasma ovipneumoniae* infection can cause pneumonia by decreasing respiratory immune function and allowing colonization by other pathogens (Besser et al., 2014, 2012; Dassanayake et al., 2010). Numerous management tools including vaccination, population reduction, and supplemental feeding have failed to prevent or control pneumonia outbreaks in bighorn sheep (Cassirer et al., 2018, 2001; Ward et al., 1999) but recent efforts to test and remove chronic *M. ovipneumoniae* carriers demonstrate promising results, including improved lamb survival (Garwood et al., 2020).

Peninsular bighorn sheep (*Ovis canadensis nelsoni*) reside in the Peninsular Ranges of southern California and Baja Mexico, and are currently considered a genetically distinct metapopulation of desert bighorn sheep (Buchalski et al., 2016). Peninsular bighorn sheep were listed as federally endangered in 1998 due to a multitude of population threats, including habitat loss and fragmentation, infectious disease, predation, and drought (US Fish and Wildlife Service, 2000). The Peninsular metapopulation has been steadily increasing in size from ~300 at the time

of listing to ~900 in 2016; however, infectious disease continues to threaten survival and recruitment (Colby and Botta, 2019).

Bighorn sheep behavior plays a role in the transmission and maintenance of disease. The Peninsular bighorn sheep metapopulation consists of at least 19 herds that inhabit the desert slopes, alluvial fans, and washes of the Peninsular Ranges (Colby and Botta, 2019). While most individuals within each herd are philopatric, a subset of ewes and rams will disperse to neighboring herds on a seasonal basis (Bighorn Institute, 2018; Buchalski et al., 2015; Colby and Botta, 2019). The Peninsular mountains are divided into nine "recovery regions" defined for bighorn sheep population management (US Fish and Wildlife Service, 2000) (Figure 1). Historically, these recovery regions roughly corresponded to different herds (Rubin et al., 1998) but some regions now contain multiple overlapping herds and inter-regional movements are regularly observed (Bighorn Institute, 2018; Colby and Botta, 2019).

Bighorn sheep movements are driven by food and water availability, which are especially scarce in drought years. California has had chronically low rainfall for several decades, including a severe drought from 2012 – 2016 that significantly reduced the surface water available for wildlife in desert ecosystems where bighorn sheep are found (U. S. Geological Survey, 2017). Bighorn sheep congregate in high densities at natural and artificial watering holes and urban areas where irrigation and landscaping provide resources (Bighorn Institute, 2018; Colby and Botta, 2019) (Figure 1). This co-mingling of animals from different herds, age classes, and disease statuses increases the risk of pathogen transmission, and higher density herds are associated with an increased risk of respiratory disease outbreaks (Esther S Rubin et al., 2002).

Bighorn sheep are an important model for risk factors that promote disease transmission within and among host species in a desert ecosystem that is evolving with climate change. The

multifactorial nature of pneumonia in bighorn sheep also makes them a model for identifying risk factors for disease exposure that are relevant to other species.

The goal of our research is to identify key epidemiologic factors driving the prevalence of disease in bighorn sheep, with special attention paid to pathogens associated with epidemic pneumonia. We aim to: 1) Estimate pathogen prevalence by age, sex, and recovery region; 2) Identify demographic and geographic predictors of pathogen infection/exposure in individual bighorn sheep; and 3) Identify associations between pathogen infection/exposure prevalence and adult survival and lamb recruitment. 4) Identify groups of pathogens that co-occur together within individual bighorn sheep.

2. Methods

2.1 Pathogen infection and exposure prevalence

We collected blood from wild-caught Peninsular bighorn sheep (735 individuals; 844 sampling events) from 1981 – 2017 and tested them for infection or exposure to up to 15 pathogens, including: *Anaplasma* spp., bluetongue virus (BTV), bovine herpesvirus-1, bovine respiratory syncytial virus (BRSV), bovine viral diarrhea virus types 1 and 2, *Brucella ovis, Chlamydia* spp., epizootic hemorrhagic disease virus (EHDV), *Leptospira* spp., *Mycoplasma ovipneumoniae*, ovine progressive pneumonia virus, orf virus, parainfluenza-3 virus (PI-3), and *Toxoplasma gondii* (Table 1).

Nasal/pharyngeal swabs were also collected from a subset of sheep (316 individuals; 349 sampling events) and tested for combinations of *M. ovipneumoniae* via polymerase chain reaction (PCR), *Pasteurellaceae* spp. via culture, and PI-3 via virus isolation (VI) to detect active infection (or very recent exposure). Virus isolation was also performed on blood to test for BT

and EHDV, but all other blood tests measured antibodies and more likely indicated previous exposure (Table 1). “Prevalence” hereafter refers to the proportion of positive tests, indicating infection or exposure depending on the test used.

BTV and EHDV are both orbiviruses and cross-react on agar gel precipitin (AGP) and agar gel immunodiffusion (AGID), so we created an “*Orbivirus* spp.” group which included animals positive for BTV and/or EHDV via AGP/AGID. We classified animals as exposed to BTV only if they tested positive on the more specific competitive enzyme-linked immunosorbent assay (cELISA). We classified animals as exposed to EHDV only if they were positive on AGP/AGID and negative on BTV cELISA, although this calculation excluded animals exposed to both viruses so was not included in modeling.

We did not include *Leptospira* spp. serovars in analyses due to cross-reaction on the modified agglutination test, and an animal was considered positive if any serovar was detected.

Age, sex, and recovery region were recorded at the time of capture. Age was usually recorded categorically based on dentition and horn growth rings, with lambs and yearlings grouped together and all other ages categorized as adults. The dataset was heavily skewed towards adult females (73.2%, n = 618/844) since they were the target population for radio-collaring and are the reproductive base of the population (Colby and Botta, 2019). Most individuals were only captured once (n = 641).

We calculated the overall prevalence of each pathogen across the entire study for each diagnostic test type, then stratified by age, sex, and recovery region. We tested for differences among two groups using a Fisher’s exact test and among ≥3 groups using a one-way analysis of variance (significance at $p \leq 0.05$). These calculations only included samples from first capture

events to eliminate re-testing errors. All statistics were performed in R version 4.0.4 (R Core Team, 2021).

Annual adult survival rates for each recovery region were previously calculated by California Department of Fish and Wildlife and Bighorn Institute using Kaplan Meier estimates from radio-collared bighorn sheep, modified to allow for staggered entry of new animals (Bighorn Institute, 2018; Colby and Botta, 2019; Ostermann et al., 2001). The ratio of lambs to ewes (lamb:ewe; an index of lamb survival to ~3 – 9 months) for each recovery region was estimated from field observations made during range-wide helicopter surveys, waterhole counts, or ground observations (Bighorn Institute, 2018; Colby and Botta, 2019).

2.2 Pathogen impacts on survival and reproduction

We created "population-level models" using Bayesian, multilevel, ordered beta regression to test for associations between adult survival rates or lamb:ewe ratios (outcomes), and pathogen prevalence, population size, and meteorologic covariates. The unit of analysis was the year-recovery region unit, and the random intercept was recovery region. We selected weakly informative priors for the intercept and beta parameters [Normal(0, 5)], and the phi parameter [exp(0.1)] (Kubinec, 2020). Lamb:ewe ratios, population size, and meteorologic covariates were scaled so values ranged from 0 – 1 but the relative differences between values were maintained.

The prevalence of each pathogen was calculated for each year-recovery region unit (i.e., "2010 – San Jacinto Mtns") for which ≥5 samples were tested (all capture events included). Models included individual pathogen prevalence and combinations of respiratory pathogens (*M. ovipneumoniae*, BRSV, and PI-3) as covariates, and we tested prevalence lag times of -1-year to +2-years.

We included annual population size estimates for each recovery region as a model covariate. We interpolated missing data by averaging values for years *t-1* and *t+1*, but only where estimates were missing for a single year.

Temperature and precipitation for each recovery region were included to control for meteorologic factors that may influence survival and reproduction. Rasters of daily meteorologic data (4x4 km resolution) were extracted from the "gridMET" dataset (Abatzoglou, 2013) using R package "climateR," then cropped by the geographic extent of each region and aggregated temporally as described below, resulting in a single summary value for each year-recovery region unit.

Temperature was calculated as the average of daily maximum temperatures (Celsius) from June – September for year *t*, which have historically been the hottest months in the Peninsular Range (Rubin et al., 2000; Turner et al., 2004).

Precipitation in the Peninsular Range is bimodal, with the largest volume and most consistent rains occurring November – February, and more variable monsoons occurring July – September (Rubin et al., 2000). We calculated annual precipitation as the sum of daily precipitation (centimeters) from November of year *t-1* through October of year *t*. We also aggregated precipitation annually for winter (November – April) and summer (May – October). Winter corresponded to the winter rains and bighorn sheep gestational and peak birthing period, while summer corresponded to the summer monsoons, post-lambing, and the rut (Colby and Botta, 2017; Rubin et al., 2000; Esther S Rubin et al., 2002).

2.3 Individual risk factors associated with pathogen exposure or infection

We created "individual-level models" using Bayesian, multilevel, logistic regression to evaluate the impact of age, sex, and recovery region on an animal's risk of pathogen

infection/exposure. Reference groups were adults, males, and the San Jacinto Mountains. The unit of analysis was the individual animal, and the random intercept was animal ID (all capture events included). We selected weakly informative priors [Normal(0, 2.5)] for the intercept and beta parameters to account for complete or quasi-separation of data (Ghosh et al., 2018).

For all regression models, we evaluated bivariate relationships between covariates with Spearman's rank correlation (significance at $p \leq 0.05$) and built models in R package "brms" (Bürkner, 2018, 2017). Models contained four chains with 10,000 iterations each. We calculated point estimates as the median value of the posterior and used the 95% highest density interval as the credible interval (CI). We only included models with ≥5 observations per covariate in results. We compared models with the same number of observations using leave-one-out cross-validation information criterion (LOO IC) in the "loo" package (Vehtari et al., 2020). Appendix A contains heatmaps illustrating model variables by year and recovery region.

2.4 Pathogen co-occurrence network

We identified groups of pathogens to which an individual was co-infected/co-exposed using social network methods. Since the diagnostic tests used can indicate infection or previous exposure, we use "co-occurrence" to mean that an animal was infected with a pair of pathogens during its life, but perhaps not concurrently. We generated a weighted, undirected pathogen network from the proportion of samples that were positive for two pathogens, given that they were tested for both pathogens (antibody tests or direct measures of infection; first capture events only). Centralization, density, and degree centrality were used to describe how tightly pathogens clustered (package "sna") (Butts, 2008). We calculated network modularity using the fast greedy modularity optimization algorithm (package "igraph") (Clauset et al., 2004). We converted the hierarchical community structure to a dendrogram to illustrate relative rates of co-occurrence

among pathogens (package "ape") (Paradis and Schliep, 2019) and visualized the network in Gephi (Bastian et al., 2009).

3. Results

3.1 Pathogen infection and exposure prevalence

A total of 735 first-capture samples were collected from 1981 – 2017. Pathogen and antibody prevalence estimates can be found in Table 1, including stratifications by age, sex, and recovery region. Not all diagnostic tests were performed in every year or recovery region.

The most common pathogen exposures detected were orf virus (71.8%), *M. ovipneumoniae* (cELISA, 60.2%), *Anaplasma* spp. (49.7%), *Chlamydia* spp. (42.8%), BRSV (39.3%), EHDV (serum virus neutralization [SVN], 24.4%), *Orbivirus* spp. (21.6%), PI-3 (hemagglutination inhibition [HI], 21.2%), and *T. gondii* (18.0%). Prevalence of active infections were lower than antibody tests for *M. ovipneumoniae* (PCR, 12.0%), PI-3 (VI, 11.4%), and EHDV (VI, 0.0%). All other pathogens were relatively uncommon or absent (Table 1).

Pasteurellaceae spp. are common commensal organisms of bighorn sheep (Ward et al., 1997; Wild and Miller, 1991), and we found that all samples cultured at least one species. *Pasteurella multocida* was not detected from first capture events, although the beta-hemolytic form was detected in one sample from a recaptured animal.

We detected multiple *Leptospira* serovars, including: *Leptospira interrogans* serovars bratislava *(19.2%, n = 10/52), pomona (1.6%, n = 5/316), icterohaemorrhagiae (0.3%, n = 1/316), and canicola (1.3 %, n = 4/316); Leptospira kirschneri* serovar *grippotyphosa (5.7%, n = 18/316); Leptospira borgpetersenii* serovar *hardjo (1.6%, n = 5/310).*

Males had higher rates of exposure to BRSV ($p = 0.01$), while females had higher exposure to *T. gondii* ($p < 0.001$) and *Orbivirus* spp. ($p = 0.03$). Lambs/yearlings tested positive for exposure to orf virus more often than adults ($p = 0.03$). There were differences among recovery regions in the prevalence/exposure of *Anaplasma* spp. ($p < 0.001$), BRSV ($p < 0.001$), *B. ovis* ($p < 0.001$), *Chlamydia* spp. ($p < 0.001$), *Leptospira* spp. ($p = 0.001$), *M. ovipneumoniae* (PCR, $p = 0.04$), orf virus ($p < 0.001$), PI-3 (HI; $p < 0.001$), *T. gondii* ($p = 0.02$), BTV (cELISA; $p = 0.01$), EHDV (SVN, $p = 0.01$; AGP/AGID, $p = 0.04$), *Orbivirus* spp. ($p = 0.001$), and *B. trehalosi* beta-hemolytic ($p < 0.001$).

All pathogens showed temporal changes in prevalence over the course of the 36-year study period (Appendix A). Common pathogens, such as *M. ovipneumoniae* (cELISA) and *Chlamydia* spp., were consistently present across the entire study period and all regions. BRSV, PI-3, and orf virus were more variable, sometimes ranging from 0% to 100% in the span of one year. *B. ovis* was only detected in the 1990s. *M. ovipneumoniae* (PCR) and BRSV showed increasing prevalence over time.

3.2 Pathogen impacts on reproduction

To evaluate which pathogens may affect bighorn sheep survival and reproduction, we performed regression modeling with the following pathogens as covariates (which had enough datapoints for modeling): *Anaplasma* spp., BTV (cELISA), BRSV, *Chlamydia* spp., orf virus, *Leptospira* spp., *M. ovipneumoniae* (PCR, cELISA), *Orbivirus* spp., and PI-3 (HI).

The bivariate relationship between adult survival and lamb:ewe ratios with population size, temperature, and precipitation were tested using correlations and univariable regression models (recovery region as a random effect) as the first step in model building. Population size was positively correlated ($R^2 = 0.34$, $p < 0.001$) and significantly associated (ß = 2.9, 95% CI =

1.7 – 4.1) with adult survival, but the directionality of this relationship could not be determined by this study. There was no relationship between population size and lamb:ewe ratios using correlation ($R^2 = 0.01$, $p = 0.90$) or regression modeling (ß = 0.5, 95% CI = -0.4 – 1.4). Temperature and precipitation were not correlated with either adult survival ($R^2 = -0.04 – 0.1$, $p \geq 0.15$) or lamb:ewe ratios ($R^2 = 0.04 – 0.08$, $p \geq 0.33$), and were also not associated with either outcome using univariate regression models (Appendix C). Despite this lack association, meteorologic covariates were included in population-level regression model building to test if they improved model fit because they have been previously established as important factors in bighorn sheep survival and reproduction.

M. ovipneumoniae exposure (cELISA) was negatively associated with lamb:ewe ratios in the current year (no lag) in models including population size (ß = -1.2, 95% CI = -2.2 – -0.3; Appendix E), population size and temperature (ß = -1.1, 95% CI = -2.1 – -0.1; Appendix F), population size and annual precipitation (ß = -1.2 , 95% CI = -2.3 – -0.2; Appendix G), population size and summer precipitation (ß = -1.2, 95% CI = -2.2 – -0.2; Appendix H), and population size and winter precipitation (ß = -1.3 , 95% CI = -2.3 – -0.2; Appendix I). In these models, population size and meteorologic covariates were not associated with lamb:ewe ratios.

Leptospira spp. had a positive association with lamb:ewe ratios after a 2-year lag both as a single covariate (ß = 2.7, 95% CI = 0.08 – 5.1; Appendix D) and in models with population size (ß = 2.7, 95% CI = 0.07 – 5.2; Appendix E), but there were not enough observations to include meteorologic variables.

Lamb:ewe ratios were not associated with other pathogens (alone or in combination), population size, temperature, or precipitation (Appendix D-I). The inclusion of population size

and summer temperature improved model fit in 20.6% (n = 7/34) of models. LOO IC standard errors overlapped for all other models.

3.3 Pathogen impacts on survival

The prevalence of orf virus was positively associated with adult survival in the previous year (-1 year lag; ß = 1.6, 95% CI = 0.4 – 2.9; Appendix D). This relationship persisted with the addition of population size (ß = 1.2, 95% CI = 0.0 – 2.5; Appendix E), and both population size and annual precipitation (ß = 1.2, 95% CI = 0.0 – 2.4; Appendix G) as covariates.

In the current year (no lag), PI-3 was associated with increases in survival once other covariates were accounted for: population size (ß = 1.8, 95% CI = 0.2 – 4.1; Appendix E), population size and temperature (ß = 1.8, 95% CI = 0.3 – 3.9; Appendix F), population size and annual precipitation (ß = 1.8 , 95% CI = 0.2 – 4.0; Appendix G), population size and summer precipitation (ß = 1.5, 95% CI = 0.1 – 3.7; Appendix H), and population size and winter precipitation (ß = 1.8 , 95% CI = 1.2 – 4.1; Appendix I). In these models, population size was also positively associated with survival rates, but meteorologic covariates were not.

No other pathogens were significant predictors of adult survival (alone or in combination) regardless of lag time (Appendix D). Population size was associated with higher survival rates in 22.4% (n = 51/228) of models across all pathogens (Appendix E-I). Higher summer temperatures were associated with lower survival rates in only 5.4% (n = 2/37) models, and higher summer precipitation was associated with higher survival rates in 18.9% (n = 7/37) models (Appendix H). Annual and winter precipitation were not significantly associated with survival in any models (Appendices G, I). The inconsistency in the significance of population size and meteorologic variables across models was likely because each model contained a different dataset; samples were not tested for every pathogen and survival rates and lamb:ewe ratios were not available for

every year-recovery region unit. The inclusion of population size and summer temperature improved model fit in 21.6% (n = 8/37) of models. LOO IC standard errors overlapped for all other models.

Several covariates showed temporal trends over the 36-year study period. Population size was positively correlated with year across all recovery regions ($R^2 = 0.67$, $p < 0.001$), reflecting overall sustained growth of the population over time. Temperature was positively correlated with year ($R^2 = 0.18$, $p < 0.001$), while annual ($R^2 = -0.14$, $p = 0.008$), summer ($R^2 = -0.17$, $p = 0.002$), and winter ($R^2 = -0.13$, $p = 0.02$) precipitation were negatively correlated with year. Increasing temperature and decreasing precipitation are consistent with the effects of global climate change.

3.4 Individual risk factors associated with pathogen exposure or infection

To evaluate pathogen infection/exposure risk factors, we performed regression modeling for the following pathogens (which had enough positive test results for modeling): *Anaplasma* spp., BTV (cELISA), BRSV, *B. ovis, Chlamydia* spp., *Leptospira* spp., *M. ovipneumoniae* (PCR and cELISA), *Orbivirus* spp., orf virus, PI-3 (HI), and *T. gondii* (Figure 2, Appendix B).

Odds of exposure to *M. ovipneumoniae* (by cELISA) were higher in the northern Santa Rosa Mountains and Vallecito Mountains (OR = 3.6 and 2.9, respectively), compared to the San Jacinto Mountains. Age, sex, and recovery region were not significant predictors of *M. ovipneumoniae* active infection (PCR).

BRSV and PI-3 had similar distributions, with most recovery regions having higher odds of exposure compared to the San Jacinto Mountains (OR = 4.4 – 45.4; Figure 2). Females were less likely to be exposed to BRSV than males (OR = 0.5).

Orbivirus spp. had higher odds of exposure in females (OR = 3.2) and lower odds of exposure in the northern half of the range (Figure 2). The northern San Ysidro Mountains, in the

middle of the range, had higher odds BTV exposure (OR = 8.2). The discrepancies in risk between BTV and *Orbivirus* spp. are likely due to the *Orbivirus* spp. models including animals exposed to EHDV and/or BTV and being slightly skewed towards more EHDV positives because cELISA replaced AGP/AGID as the test for BTV in 1993.

Bighorn sheep were more likely to be exposed to *B. ovis* in the southern half of the range, including the northern (OR = 21.1) and southern San Ysidro Mountains (OR = 10.1), and Carrizo Canyon (OR = 9.5). These positive samples were limited to 1990 – 1997, with 17 of 24 positive results occurring in 1992 in the northern San Ysidro Mountains and Carrizo Canyon. Carrizo Canyon, the southernmost recovery region, also had higher odds of exposure to orf virus (OR = 3.0).

Anaplasma spp. and *Chlamydia* spp. had patchy geographic distributions. The risk of exposure to *Anaplasma* spp. was lower in the northern Santa Rosa Mountains (OR = 0.14) and higher in the northern San Ysidro Mountains (OR 8.7). Odds of exposure to *Chlamydia* spp. were higher in the central Santa Rosa Mountains (OR = 6.4) but lower in the bordering northern and southern Santa Rosa Mountains (OR = 0.1 in both).

Exposure to *Leptospira* spp. was also scattered, with higher exposure odds in the southern Santa Rosa Mountains (OR = 5.5) and southern San Ysidro Mountains (OR = 7.2).

Females (OR = 11.1) and animals in the northern Santa Rosa Mountains (OR = 15.1) had higher odds of being exposed to *T. gondii*.

3.5 Pathogen co-occurrence network

Network analyses revealed three groups of pathogens which co-occurred together in individual sheep more often within a group than between groups (Figures 3, 4) and roughly clustered by mode of transmission. All pathogens co-occurred with many other pathogens (mean

= 13, range = 7 – 15) and group clustering was weak, as evidenced by low modularity (0.07), low centralization (0.01), and high density (0.86).

Pathogen Group A included directly transmitted pathogens, including the three respiratory pathogens. Group B included pathogens transmitted by *Culicoides* spp. biting midges (BTV, *Orbivirus* spp.) (Ruder et al., 2015) or indirectly through the environment (*Leptospira* spp., *T. gondii*) (Adler and de la Peña Moctezuma, 2010; Dubey, 2009; Ruder et al., 2015). Pathogen Group C included the directly transmitted *Pasteurellaceae* spp. and tick-vectored *Anaplasma* spp. Interestingly, *Anaplasma* spp. is vectored by the ixodid tick *Dermacentor hunteri*; adult ticks are found almost exclusively on bighorn sheep or in their preferred habitats (Crosbie et al., 1997; Crosbie and Boyce, 1998), although less host-specific ticks may also play a role in transmission.

4. Discussion

4.1 Pathogen impacts on survival and reproduction

Higher *M. ovipneumoniae* exposure (cELISA) was associated with lower lamb recruitment in the same year, although the same relationship did not hold true for active *M. ovipneumoniae* infections (PCR). Bighorn sheep infected with *M. ovipneumoniae* can die, clear the infection, or become carriers that persistently or intermittently shed the bacteria (Cassirer et al., 2013). After the initial epidemic, adult *M. ovipneumoniae* PCR prevalence tends to be low because most animals stop shedding after <1 year, although some animals can test positive for >3 years (Plowright et al., 2017). *M. ovipneumoniae* seropositivity (cELISA) is likely a better indicator of past disease exposure, although it represents a less specific time period than PCR. Increased lamb mortality is associated with the presence of even a few ewes shedding *M. ovipneumoniae*

and epidemics of pneumonia affect lamb survival and recruitment to a greater degree than adult survival (Cassirer et al., 2013; Manlove et al., 2014; Monello et al., 2001; Plowright et al., 2013). Although *M. ovipneumoniae* may be slowing population recovery through declines in reproduction/recruitment, the extinction risk of Peninsular bighorn sheep is inversely related to adult female survival (Esther S. Rubin et al., 2002), which was not found to be associated with *M. ovipneumoniae* infection/exposure in this study. However, the negative association between lamb:ewe ratios and *M. ovipneumoniae* may impact longer term herd health and growth.

Exposure to orf virus was positively associated with adult survival in the previous year, suggesting that this directly-transmitted pathogen is more common in years with higher bighorn sheep density resulting from high survival the previous year. This is consistent with the southern recovery region having both the highest orf virus exposure risk and the largest population, with ~256 individuals among four herds (Colby and Botta, 2019). Although contagious ecthyma (the disease caused by orf virus) is generally self-limiting and resolves within a few months, it can lead to secondary infections and mortality in animals that are young or have co-morbidities (Colby and Botta, 2018; Jones et al., 2018; Michelsen and Smith, 2009). Immunity is variable, with infectious carrier states and rapid reinfections observed in domestic sheep (Lewis, 1996; Nandi et al., 2011). This means the virus may not fadeout due to herd immunity, especially in larger herds. The absence of a negative association between orf virus exposure and survival or lamb:ewe ratios in subsequent years suggests this pathogen is not currently affecting population growth. However, as herds continue to grow and co-mingle among regions, the cumulative effects of co-morbidities may begin to play a role in population recovery.

Antibody prevalence of PI-3 and population size were associated with higher adult survival, consistent with PI-3 causing low mortality and circulating widely in large herds. PI-3

generally causes subclinical to mild respiratory signs as a sole agent in domestic sheep, but predisposes the respiratory tract to fatal secondary bacterial pneumonia, especially from *Pasteurellaceae* spp. (Woolums et al., 2009). This was demonstrated experimentally in bighorn sheep, where inoculation with PI-3 and respiratory syncytial virus resulted in mild pneumonia, but subsequent inoculation with *M. haemolytica* resulted in fatal pneumonia in four of four animals (Dassanayake et al., 2013). We did not have enough *Pasteurellaceae* spp. data to evaluate this association directly, but the pathogen network showed relatively low co-occurrence of PI-3 and *Pasteurellaceae* spp. (0.3% – 1.5% of samples).

Leptospira spp. was associated with higher lamb:ewe ratios, but only after a 2-year lag. *Leptospira* spp. is shed in urine, with transmission primarily occurring through contact with contaminated water, food, and soil. (Adler and de la Peña Moctezuma, 2010). Leptospirosis has been increasing globally (Vijayachari et al., 2008) and seroprevalence in Peninsular bighorn sheep has historically ranged from 0 – 20% (DeForge et al., 1997, 1982). The most biologically plausible explanation for this association is that *Leptospira* spp. exposure and reproduction are both associated with water availability (Wehausen et al., 1987) but through different ecological mechanisms and temporal time scales.

The lack of associations in this study between exposure to other pathogens and adult survival and lamb recruitment may have been due to data limitations resulting from the shifting priorities and capabilities of this multi-decade recovery project. Most of the diagnostic tests in this study measured previous exposure and we do not know the duration of seropositivity for many of these diseases. A seropositive animal may have been infected with a pathogen years prior to sampling and subsequently recovered, and our results might differ if we measured clinical disease, active infections, or directly observed lamb survival in the first few months of

life. Using recovery region as the unit of analysis may have masked herd-level differences, such as in the central Santa Rosa Mountains where there are "wild" and "urban" herds which utilize different habitats and have different pathogen exposure risks. Carrizo Canyon is a large region with four herds that have access to different topography, water sources, and forage quality. More importantly, disease-induced mortality is a multifactorial process that includes variables not included in our models. These include immune suppression due to comorbidities or physical stressors, variability in pathogen virulence, and dynamic behaviors such as contact during the lambing season or at water sources.

We found a positive relationship between population size and adult survival but could not establish the directionality of this relationship. Larger population sizes may be the result of improving survival rates as the population recovered, or there may be a survival benefit to larger groups, such as vigilance against predators.

There were trends towards higher survival rates with lower peak summer temperatures and higher summer rainfall, once population size and pathogen prevalence were controlled for, but these findings were inconsistent due to differences in datasets among models. Previous work found positive associations between precipitation and desert bighorn sheep reproduction and lamb survival (Bender and Weisenberger, 2005; Wehausen et al., 1987). It is possible that increasing temperatures and decreasing precipitation could start playing a larger role in bighorn sheep population sustainability as climate change alters weather patterns in the region, especially given the changes we expect these climactic variables to have on vegetation quality (Epps et al., 2004; Hess et al., 2008). Although modeling suggests that desert bighorn sheep living in drier mountain ranges at lower elevation are more likely to go extinct in the face of climate change (Epps et al., 2004), low elevation habitat in the Peninsular ranges appears to be better quality and

preferred by bighorn sheep, especially in drier years and during the lamb-rearing season. Unfortunately, work evaluating a subset of the Peninsular Mountains (overlapping with recovery regions 3 – 9) over the same time period as this study (1984 – 2017) found similar increases in mean annual summer temperature and decreases annual precipitation (October$_{t-1}$ to September$_{t}$), and determined that these changes were associated with widespread declines in perennial vegetation cover, with a stronger magnitude of effect at the lower elevations (<500 m) often preferred by bighorn sheep (Hantson et al., 2021).

4.2 Geography is a greater risk factor for pathogen infection/exposure than demographics

The primary risk factor for individual bighorn sheep pathogen infection/exposure was recovery region, which is likely a proxy for local ecological and behavioral factors. The northern Santa Rosa and Vallecito Mountains had higher odds of exposure to *M. ovipneumoniae*, BRSV, and PI-3, and both areas have relatively scarce natural water sources but high numbers of artificial ponds and guzzlers which could increase contact rates (Figure 1). The northern Santa Rosa mountains also have higher odds of *T. gondii* exposure, which could be related to fecal contamination of limited water sources where animals gather (Dubey, 2009), including irrigated golf courses. The need to utilize the same limited resources could spread disease through increased direct contacts and environmental contamination. In contrast, Coyote Canyon had some of the lowest disease risk and has six major riparian areas with year-round surface water that are utilized by two herds in this region, with little golf course use. There is also topographical and habitat variation within recovery regions, with the eastern areas of the southern Santa Rosa Mountains, Vallecito Mountains, and Carrizo Canyon being extremely xeric compared to the western areas. Previous work found that Peninsular bighorn sheep had higher exposure to multiple pathogens compared to other California populations, and the frequency of

exposure to ≥2 pathogens was higher at the northern latitudes within the range (Elliott et al., 1994).

Behavioral observations and genetic data has demonstrated a strong matrilineal structure between bighorn sheep herds, but within a herd animals generally associated freely (Boyce et al., 1999). This social structuring, with more contact among animals within a herd compared to between herds, could explain geographic differences in pathogen exposure through lower contact rates between herds/regions limiting transmission. There may also be differences in immune response, as bighorn sheep with lower heterozygosity in a locus within the Major Histocompatibility I gene complex are more likely to be persistent carriers of *M. ovipneumoniae* (Plowright et al., 2017), although there is little evidence of reduced genetic diversity in Peninsular bighorn sheep, despite previous population declines (Buchalski et al., 2015).

Geographic variation in these biotic and abiotic factors could affect the health of bighorn sheep differentially between regions, with additive or multiplicative effects on an individual's fitness. Further research into the fine scale variation of these factors across the Peninsular Range can help direct future bighorn sheep management.

Age was not a significant predictor of an animal's pathogen status, perhaps because lambs and yearlings were categorized together. Also, sampling generally happened in the fall, while most lambs are born in the spring. Many lambs that contract pathogens on high-density lambing grounds will die by ~4 months of age (Cassirer et al., 2018). This timing may have introduced a bias towards sampling lambs that were not exposed to disease. Similarly, the relatively low numbers of both lambs/yearlings (11.0%, n = 81/735) and males (19.6%, n = 144/735) may have decreased our power to detect differences among groups.

4.3 Bighorn sheep harbor numerous pathogens and co-infections/exposures are common

Bighorn sheep epidemic pneumonia is a complex disease process involving multiple infectious pathogens, environmental and immune factors, and host behaviors (Besser et al., 2013). We found that respiratory pathogens were relatively common in Peninsular bighorn sheep, and the pathogen co-occurrence network showed that pathogens examined in this study are widespread, with most sheep being exposed to/infected with multiple pathogens throughout their lives. Overall, very few pathogen pairs were never detected together. These pathogens may have never infected the same animal, or temporal changes in diagnostic testing may have precluded the detection of certain pathogens pairs. There are also potential biases towards detecting less virulent pathogens with higher survival rates and those which induce long-lasting antibodies.

M. ovipneumoniae exposure was detected across almost all years and recovery regions for which it was tested, demonstrating that the first spillover event from domestic sheep occurred prior to 1990 when testing began (and before the population was listed as endangered in 1998). Several pathogens, including BRSV, PI-3, and *Pasteurellaceae* spp., were also present starting in the first year of testing (1983, 1981, and 2001, respectively). Although we found limited evidence for population-level effects of pathogens other than *M. ovipneumoniae*, the long-term circulation of multiple pneumonia-associated pathogens may have a subclinical effect on population performance or exacerbate concurrent, non-disease stressors.

Bighorn sheep do not appear to gain protective cross-immunity against different strains of *M. ovipneumoniae* after infection (Cassirer et al., 2017), and different *M. ovipneumoniae* strains have been associated with varying levels of bighorn sheep morbidity/mortality (Besser et al., 2017). To date, 23 *M. ovipneumoniae* samples from Peninsular bighorn sheep have been genotyped using multi-locus sequence typing, and two ovine strains have been identified. One

strain, most closely related to bighorn sheep samples from the nearby Orocopia Mountains, is found throughout all recovery regions (Cassirer et al., 2018). A second strain, most similar to samples from Joshua Tree National Park, was identified in 2020 from sheep in two northern recovery regions (San Jacinto Mountains, central Santa Rosa Mountains; California Department of Fish and Wildlife, unpublished data). Continued monitoring and strain typing of PCR positive samples will be important to determine if this second strain will spread to the southern portions of the recovery zone. Infection with a novel strain may lead to new outbreaks of pneumonia and all age class mortality.

5. Conclusions

Peninsular bighorn sheep are recovering from critically small population sizes in an ecosystem which includes natural and urban habitats at the environmental extreme of what most species can survive. This paper demonstrates that *M. ovipneumoniae* is associated with lower lamb recruitment and identified recovery regions with elevated risk of pathogen infection/exposure to guide future management. Changes in bighorn sheep behavior and distribution in response to environmental changes, such as drought and anthropogenic development, may play a role in the maintenance or amplification of disease, especially in areas where bighorn sheep congregate in high-density groups, such as around limited water sources. Long-term, consistent, range-wide pathogen testing and population surveys will be critical to advance our understanding of pathogen transmission and the role of disease in Peninsular bighorn sheep population recovery. Consideration of environmental factors, such as the number and location of water sources and the impact the distribution of these resources has on bighorn

sheep contact rates and disease transmission, will also be important to adjust management strategies in the face of climate change and disease risks.

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7. Tables

Table 1. Summary of pathogen infection/exposure prevalence in Peninsular bighorn sheep across the entire study period (1981 – 2017), stratified by sex, age, and recovery region. Estimates only include samples from the first capture event for each individual. Fractions represent the number of positive tests over the number of tests performed. Sample sizes within each stratification level may not sum to the same totals as in the "overall" column because age and sex were not available for all samples. Bolded text indicates significant differences ($p \leq 0.05$) among groups within a stratification level (i.e., females vs. males). "S" = southern, "C" = central, "N" = northern, AGP = agar gel precipitin, AGID = agar gel immunodiffusion, CA = card agglutination, CF = complement fixation, ELISA = enzyme-linked immunosorbent assay, cELISA = competitive ELISA, IFA = immunofluorescence assay, HI = hemagglutination inhibition, LA = latex agglutination, MAT = modified agglutination test, PCR = polymerase chain reaction, SVN = serum virus neutralization, VI = virus isolation, NT = not tested.

		Overall	Sex		Age		Recovery Region								
Pathogen	Test type		Female	Male	Lamb or yearling	Adult	San Jacinto Mtns	N. Santa Rosa Mtns	C. Santa Rosa Mtns	S. Santa Rosa Mtns	Coyote Canyon	N. San Ysidro Mtns	S. San Ysidro Mtns	Vallecito Mtns	Carrizo Canyon
Anaplasma spp.	CA	49.7% (158/318)	55.4% (41/74)	48.1% (117/243)	51.0% (148/290)	34.6% (9/26)	**42.9% (12/28)**	**29.8% (14/47)**	**48.3% (14/29)**	**29.6% (8/27)**	**57.1% (20/35)**	**77.1% (27/35)**	**65.9% (29/44)**	**48.5% (16/33)**	**45.0% (18/40)**
Bovine herpesvirus-1	SVN	0.6% (3/537)	1.6% (2/129)	0.2% (1/407)	0.6% (3/469)	0.0% (0/66)	0.0% (0/51)	3.0% (3/100)	0.0% (0/57)	0.0% (0/32)	0.0% (0/45)	0.0% (0/51)	0.0% (0/49)	0.0% (0/45)	0.0% (0/107)
Bovine respiratory syncytial virus	IFA	39.3% (259/659)	**28.9% (37/128)**	**41.9% (222/530)**	39.9% (236/592)	34.8% (23/66)	**12.1% (8/66)**	**48.7% (38/78)**	**37.0% (30/81)**	**72.9% (43/59)**	**43.8% (21/48)**	**28.1% (18/64)**	**44.1% (26/59)**	**40.0% (30/75)**	**34.9% (45/129)**
Bovine viral diarrhea virus type-1	SVN	0.7% (4/541)	0.8% (1/129)	0.7% (3/411)	0.4% (2/472)	3.0% (2/67)	0.0% (0/51)	1.0% (1/101)	0.0% (0/57)	0.0% (0/32)	0.0% (0/45)	0.0% (0/54)	0.0% (0/49)	0.0% (0/45)	2.8% (3/107)
Bovine viral diarrhea virus type-2	SVN	0.0% (0/83)	0.0% (0/17)	0.0% (0/65)	0.0% (0/76)	0.0% (0/7)	0.0% (0/7)	0.0% (0/4)	NT	NT	0.0% (0/7)	0.0% (0/9)	0.0% (0/12)	0.0% (0/2)	0.0% (0/42)
Brucella ovis	ELISA	5.0% (23/459)	2.5% (3/118)	5.9% (20/340)	5.3% (21/399)	3.4% (2/59)	**0.0% (0/38)**	**0.0% (0/87)**	**0.0% (0/50)**	**0.0% (0/26)**	**5.3% (2/38)**	**16.3% (7/43)**	**10.9% (5/46)**	**0.0% (0/38)**	**9.7% (9/93)**

		Overall	Sex		Age		Recovery Region								
Pathogen	Test type		Female	Male	Lamb or yearling	Adult	San Jacinto Mtns	N. Santa Rosa Mtns	C. Santa Rosa Mtns	S. Santa Rosa Mtns	Coyote Canyon	N. San Ysidro Mtns	S. San Ysidro Mtns	Vallecito Mtns	Carrizo Canyon
Chlamydia spp.	CF	42.8% (199/465)	45.7% (53/116)	41.7% (145/348)	43.4% (179/412)	36.5% (19/52)	**48.8% (20/41)**	**17.0% (9/53)**	**71.4% (40/56)**	**12.9% (4/31)**	**38.1% (16/42)**	**30.8% (16/52)**	**42.6% (20/47)**	**42.2% (19/45)**	**56.1% (55/98)**
Leptospira spp.	MAT	12.1% (38/313)	10.5% (8/76)	12.7% (30/237)	12.7% (35/275)	8.1% (3/37)	**3.2% (1/31)**	**9.1% (6/66)**	**5.4% (2/37)**	**30.8% (8/26)**	**16.0% (4/25)**	**10.0% (3/30)**	**33.3% (8/24)**	**6.5% (2/31)**	**9.3% (4/43)**
Mycoplasma ovipneumoniae	PCR	12.0% (38/316)	13.5% (5/37)	11.9% (33/278)	11.8% (34/288)	14.3% (4/28)	**13.3% (4/30)**	**5.9% (2/34)**	**3.1% (1/32)**	**15.2% (5/33)**	**7.7% (2/26)**	**0.0% (0/30)**	**20.0% (6/30)**	**23.8% (10/42)**	**13.6% (8/59)**
	cELISA	60.2% (336/558)	63.0% (68/108)	59.5% (267/449)	60.3% (307/509)	58.3% (28/48)	55.6% (30/54)	71.2% (42/59)	58.1% (36/62)	55.8% (29/52)	48.9% (22/45)	56.7% (34/60)	50.0% (25/50)	71.0% (49/69)	64.5% (69/107)
Ovine progressive pneumonia virus	AGID	0.0% (0/186)	0.0% (0/48)	0.0% (0/138)	0.0% (0/170)	0.0% (0/14)	0.0% (0/26)	0.0% (0/36)	0.0% (0/9)	0.0% (0/12)	0.0% (0/13)	0.0% (0/17)	0.0% (0/21)	0.0% (0/18)	0.0% (0/34)
Orf virus	CF	71.8% (319/444)	77.2% (71/92)	70.4% (247/351)	**73.6% (295/401)**	**57.1% (24/42)**	**70.3% (26/37)**	**62.8% (49/78)**	**50.0% (23/46)**	**84.2% (32/38)**	**69.2% (27/39)**	**83.7% (36/43)**	**82.2% (37/45)**	**63.6% (28/44)**	**82.4% (61/74)**
Parainfluenza-3 virus	VI	11.4% (4/35)	8.3% (1/12)	13.0% (3/23)	4.5% (1/22)	23.1% (3/13)	0.0% (0/2)	20.0% (4/20)	NT	NT	NT	NT	NT	NT	0.0% (0/13)
	HI	21.2% (152/717)	20.7% (29/140)	21.4% (123/576)	22.2% (141/635)	12.5% (10/80)	**1.5% (1/66)**	**32.5% (38/117)**	**9.9% (8/81)**	**30.5% (18/59)**	**8.9% (5/56)**	**12.3% (8/65)**	**19.4% (12/62)**	**35.4% (29/82)**	**25.6% (33/129)**
Toxoplasma gondii	LA	18.0% (16/89)	**43.5% (10/23)**	**9.1% (6/66)**	18.5% (15/81)	14.3% (1/7)	**20.0% (4/20)**	**37.0% (10/27)**	**NT**	**NT**	**NT**	**5.9% (1/17)**	**7.7% (1/13)**	**NT**	**0.0% (0/12)**
Bluetongue virus	VI	3.3% (3/91)	0.0% (0/25)	4.5% (3/66)	1.4% (1/72)	11.1% (2/18)	0.0% (0/16)	5.0% (2/40)	NT	0.0% (0/4)	0.0% (0/5)	NT	NT	0.0% (0/5)	4.8% (1/21)
	cELISA	10.4% (60/576)	13.9% (15/108)	9.6% (45/467)	11.1% (58/523)	3.8% (2/52)	**8.0% (4/50)**	**5.2% (3/58)**	**3.7% (3/81)**	**8.5% (5/59)**	**5.4% (3/56)**	**22.9% (11/48)**	**12.3% (7/57)**	**18.3% (15/82)**	**10.6% (9/85)**
	VI	0.0% (0/22)	0.0% (0/8)	0.0% (0/14)	0.0% (0/12)	0.0% (0/10)	NT	0.0% (0/18)	NT	NT	NT	NT	NT	NT	0.0% (0/4)

		Overall	Sex		Age		Recovery Region								
Pathogen	Test type		Female	Male	Lamb or yearling	Adult	San Jacinto Mtns	N. Santa Rosa Mtns	C. Santa Rosa Mtns	S. Santa Rosa Mtns	Coyote Canyon	N. San Ysidro Mtns	S. San Ysidro Mtns	Vallecito Mtns	Carrizo Canyon
Epizootic hemorrhagic disease virus	SVN	24.4% (10/41)	36.4% (4/11)	20.0% (6/30)	29.2% (7/24)	17.6% (3/17)	**NT**	**18.9% (7/37)**	**NT**	**NT**	**NT**	**NT**	**NT**	**NT**	**75.0% (3/4)**
	AGP/ AGID (BTV cELISA neg)	5.3% (25/474)	5.8% (5/86)	5.2% (20/387)	5.8% (25/430)	0.0% (0/43)	**16.3% (7/43)**	**1.9% (1/54)**	**4.0% (2/50)**	**5.7% (3/53)**	**4.2% (2/48)**	**2.7% (1/37)**	**6.0% (3/50)**	**7.9% (5/63)**	**1.3% (1/76)**
Orbivirus spp.	AGP/ AGID	21.6% (145/670)	**29.0% (38/131)**	**19.9% (107/538)**	22.5% (134/595)	15.1% (11/73)	**30.6% (19/62)**	**31.9% (37/116)**	**7.5% (4/53)**	**12.1% (7/58)**	**9.8% (5/51)**	**21.5% (14/65)**	**17.7% (11/62)**	**20.3% (15/74)**	**25.6% (33/129)**
Mannheimia haemolytica betahemolytic	culture	85.0% (119/140)	80.6% (29/36)	86.5% (90/104)	83.3% (105/126)	100.0% (14/14)	70.0% (7/10)	85.7% (18/21)	87.0% (20/23)	100.0% (12/12)	90.0% (9/10)	83.3% (15/18)	82.4% (14/17)	76.5% (13/17)	91.7% (11/12)
Mannheimia haemolytica nonhemolytic	culture	23.6% (33/140)	22.2% (8/36)	24.0% (25/104)	23.8% (30/126)	21.4% (3/14)	0.0% (0/10)	23.8% (5/21)	34.8% (8/23)	25.0% (3/12)	10.0% (1/10)	33.3% (6/18)	17.6% (3/17)	11.8% (2/17)	41.7% (5/12)
Bibersteinia trehalosi betahemolytic	culture	12.1% (17/140)	2.8% (1/36)	15.4% (16/104)	12.7% (16/126)	7.1% (1/14)	**0.0% (0/10)**	**0.0% (0/21)**	**0.0% (0/23)**	**0.0% (0/12)**	**30.0% (3/10)**	**38.9% (7/18)**	**0.0% (0/17)**	**29.4% (5/17)**	**16.7% (2/12)**
Bibersteinia trehalosi nonhemolytic	culture	77.9% (109/140)	77.8% (28/36)	77.9% (81/104)	78.6% (99/126)	71.4% (10/14)	90.0% (9/10)	90.5% (19/21)	82.6% (19/23)	83.3% (10/12)	50.0% (5/10)	61.1% (11/18)	94.1% (16/17)	64.7% (11/17)	75.0% (9/12)

8. Figures

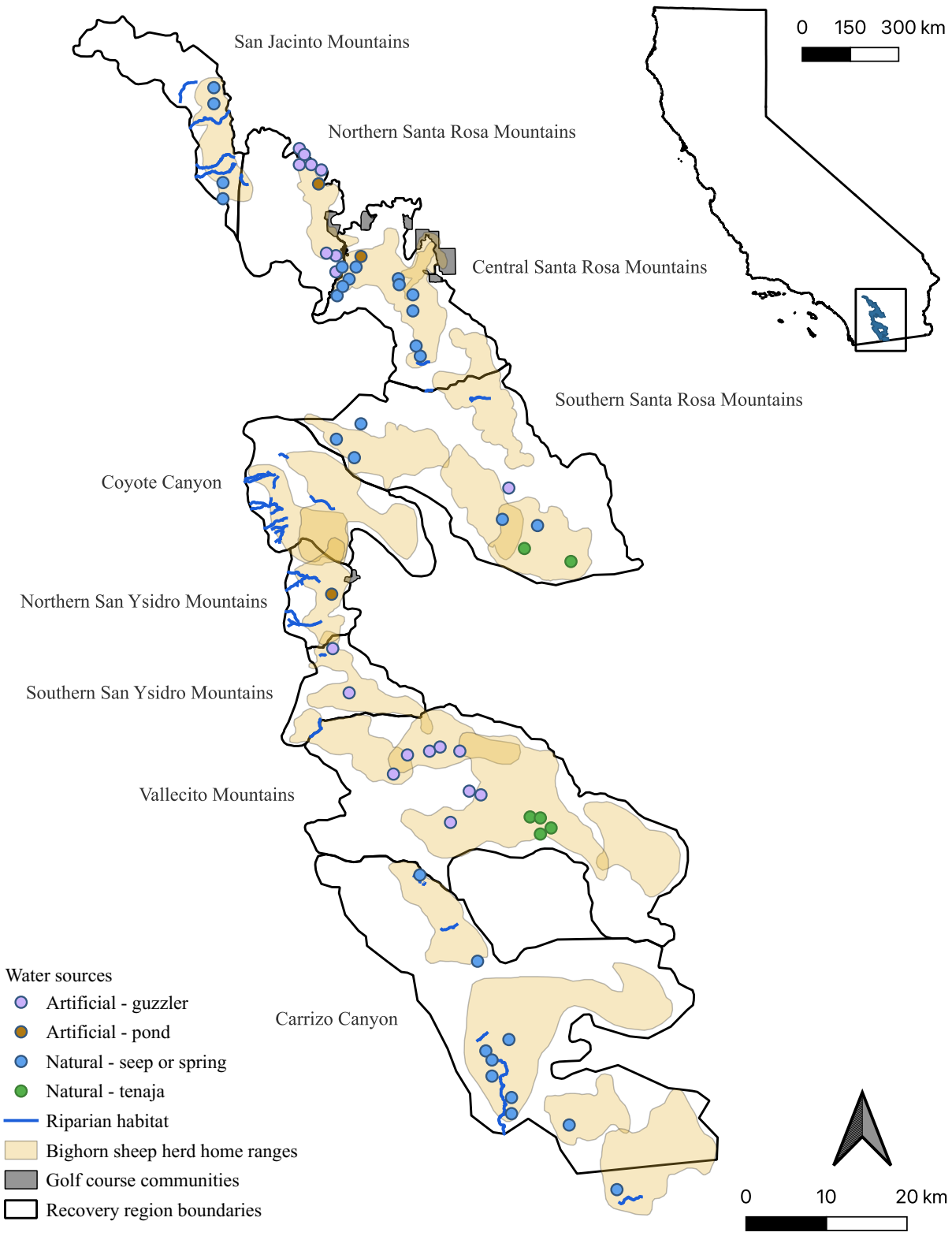
San Jacinto Mountains
Northern Santa Rosa Mountains
Central Santa Rosa Mountains
Southern Santa Rosa Mountains
Coyote Canyon
Northern San Ysidro Mountains
Southern San Ysidro Mountains
Vallecito Mountains
Carrizo Canyon
0 150 300 km
0 10 20 km
Water sources
Artificial - guzzler
Artificial - pond
Natural - seep or spring
Natural - tenaja
Riparian habitat
Bighorn sheep herd home ranges
Golf course communities
Recovery region boundaries

Figure 1. Map of the study area within the Peninsular Ranges of southern California, USA. Map depicts recovery region boundaries, bighorn sheep herd home ranges, golf course communities bordering or within bighorn sheep habitat, and major water sources. Major riparian areas have perennial or intermittent creeks and relatively large amounts of vegetation, including canopy cover and a dense understory. These areas are also utilized by deer and sometimes mountain lions. Artificial ponds and guzzlers provide year-round water through municipal sources or by collecting rainwater then delivering them to a drinking area. Guzzlers tend to be elevated, while ponds are at ground level and therefore vulnerable to contamination by rain run-off and/or animal excrement. Natural seeps and springs are small point sources of water at ground level that contain variable quantities and quality of water throughout the year. Tenajas are small rock depressions that hold water at the bottom of drainages, tend to be poor water quality, and are not dependable during the summer months. Golf course communities shown are those that bighorn sheep utilize on a regular basis; they are in urban areas where human-wildlife conflict is likely, but also have highly nutritious forage and many dependable water sources such as ponds, creeks, canals, reservoirs, and swimming pools.

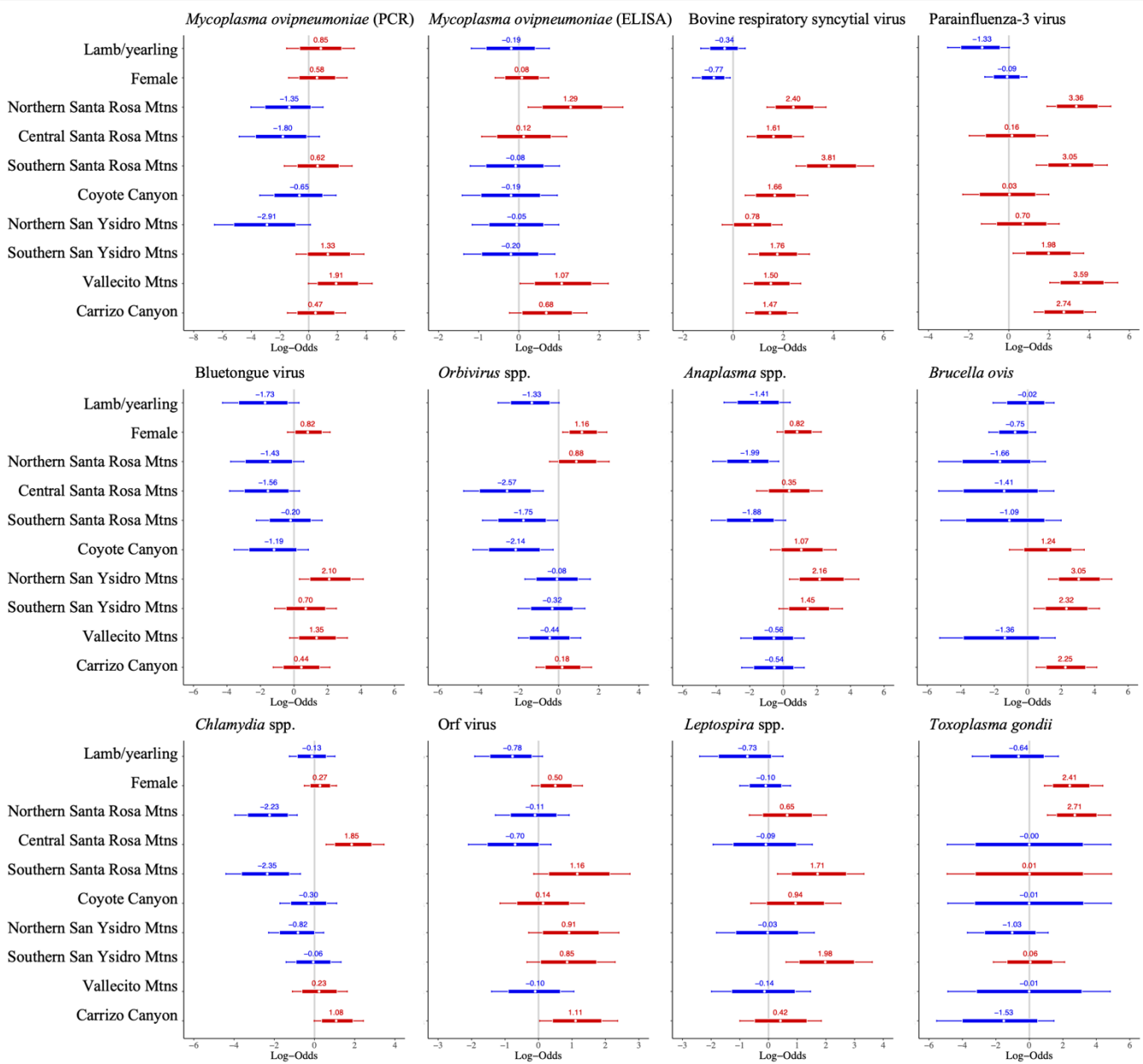
Mycoplasma ovipneumoniae (PCR)
Mycoplasma ovipneumoniae (ELISA)
Bovine respiratory syncytial virus
Parainfluenza-3 virus
Bluetongue virus
Orbivirus spp.
Anaplasma spp.
Brucella ovis
Chlamydia spp.
Orf virus
Leptospira spp.
Toxoplasma gondii
Lamb/yearling
Female
Northern Santa Rosa Mtns
Central Santa Rosa Mtns
Southern Santa Rosa Mtns
Coyote Canyon
Northern San Ysidro Mtns
Southern San Ysidro Mtns
Vallecito Mtns
Carrizo Canyon
Log−Odds

Figure 2. Forest plots demonstrating the relationship between pathogen status (positive, negative) and Peninsular bighorn sheep age (lamb/yearling, adult), sex (male, female), and recovery region (categorical, n = 9). Reference categories were adults (for age), males (for sex), and the San Jacinto Mountains (for recovery region). Numbers and white circle represent the log odds of testing positive for a pathogen, relative to a reference category, using Bayesian, multilevel, logistic regression models. Log odds <0 (blue) indicate a lower risk of testing positive for a given pathogen, and log odds >0 (red) indicate a higher risk of testing positive for a given pathogen, relative to the reference category. Thick bars represent the 80% credible interval, and thin bars represented the 95% credible interval. A covariate was a significant predictor of pathogen status if the 95% credible interval did not cross 0.

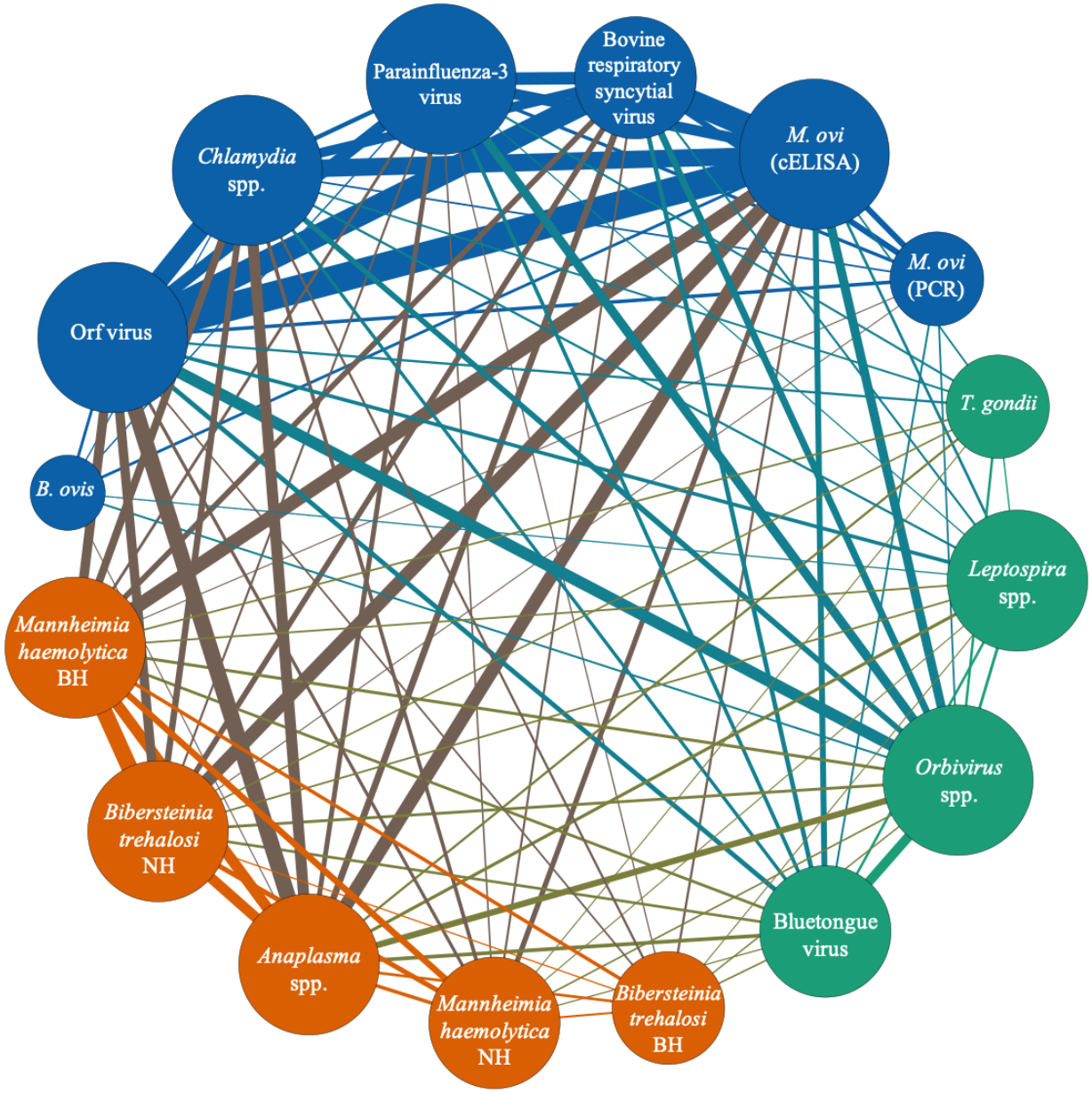

Figure 3. A weighted, undirected network of infectious pathogens that co-occurred together within an individual bighorn sheep. Node (circle) size is relative to the number of other pathogens that node is linked too (larger nodes are linked to more pathogens). Edge (lines) width is relative to the proportion of bighorn sheep samples which were positive for a pair of pathogens, given that both pathogens were tested for. The colors of the nodes identify which group a pathogen belongs to, based on network modularity. The color of the edges is determined by the group assignment of the nodes they connect. *B. ovis* = *Brucella ovis*, *M. ovi* = *Mycoplasma ovipneumoniae*, *T. gondii* = *Toxoplasma gondii*, cELISA = competitive enzyme-linked immunosorbent assay, PCR = polymerase chain reaction, BH = beta-hemolytic, NH = non-hemolytic.

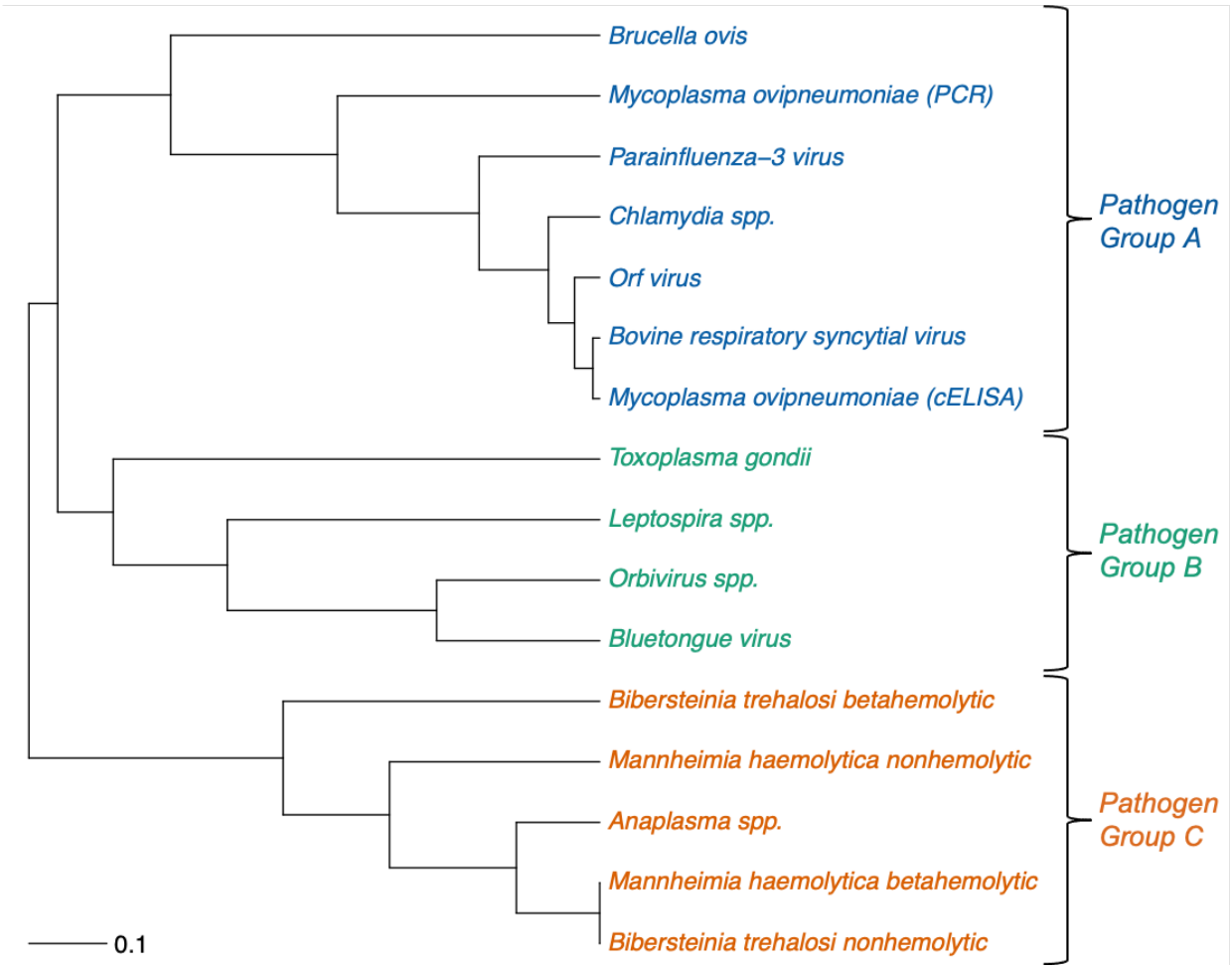

Figure 4. Dendrogram generated from a weighted, undirected network of infectious pathogens that co-occurred together within individual bighorn sheep. The branching structure of the tree was determined by the pair-wise weights between pathogens, calculated as the proportion of bighorn sheep samples that were positive for both pathogens, given that they were tested for both pathogens (larger weights result in shorter branch lengths between pathogens). The colors of the pathogen names designate which group a pathogen belongs to, as identified by network modularity.

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CONCLUSION

The research presented in this dissertation evaluates methods of surveillance, prevention, and control of disease across diverse host-pathogen systems. These systems represent different phases of pathogen spillover and spread, including the prevention of spillover into a novel host due to high-risk interactions, the subsequent epidemic in the new host population, and maintenance of disease in the reservoir and/or new host species (Figure 1). Each of these phases has unique challenges when it comes to the detection and control of disease due to the variation in the prevalence and distribution of disease as pathogens are introduced and spread within new hosts.

Major findings and recommendations

The animal-human interfaces identified at global animal markets will help us design targeted surveillance programs for pathogens with pandemic potential in the pre-emergence setting and guide local mitigation efforts to prevent spillover from occurring. The significance of education and concern about disease outbreaks in models of personal protective equipment (PPE) use is encouraging data to support the potential effectiveness of community outreach and training programs, both to increase PPE use and to reduce other behaviors which put people at risk for contracting zoonotic pathogens, such as housing live animals together. The global wildlife supply chain was examined in 23 countries, each with unique cultural preferences and traditions, taxa involved, and extent of their geographic impacts. The specific recommendations made must be adapted to these individual situations, in collaboration with local colleagues. However, some general recommendations that can be made from this study include: 1) reduce the presence of live wildlife within the supply chain; 2) eliminate the housing of live animals together, especially those from different taxa or geographic areas; 3) improve animal husbandry by disinfecting

cages and removing animal byproducts, especially in areas where there is a large degree of mixing of different species (i.e., point-of-sale nodes); and 4) educate hunters, supply chain workers, and consumers on the pathways for zoonotic spillover to encourage them to support behavior changes such as using PPE and modifying the way they handle animals.

We found that social distancing due to SARS-CoV-2 occurred prior to government issued guidelines, emphasizing the importance of timely and accurate public health communication. This result becomes even more important when combined with our finding that social distancing is likely to be most effective when done proactively at the start of an epidemic while case counts are still relatively low. Social distancing can be an effective epidemic response tool to slow disease transmission while developing other pandemic control measures that may not be readily available for a novel virus that has just spilled over into humans, including diagnostic testing, quarantine protocols, and vaccinations. The public showed that they can make behavioral modifications (such as social distancing) rapidly, even before official "lock-downs" were instituted, which demonstrates the importance of clearly communicating evolving research in a manner that is accessible to the public so they can participate in pandemic mitigation efforts.

The significance of recovery region as a risk factor for bighorn sheep pathogen exposure indicates that there are ecological factors that vary with geography and contribute to the continued exposure of bighorn sheep to pathogens which have become established post-spillover. Although using recovery region as the unit of analysis precluded further detailed investigation of these factors, it suggests that there is a spatial component to pathogen exposure which could be investigated with finer scale evaluation of bighorn sheep movements (e.g., using radio-collar location data) and the ecology of the region (e.g., the placement of artificial water sources). The trends we observed towards higher temperatures and lower precipitation over time, which can be

expected to worsen with climate change, also raise concerns about the role these factors will play as additional stressors compounding the effects of infectious disease. Systematic surveillance and monitoring of bighorn sheep pathogens, population performance, and environmental resources and stressors will be key to long-term bighorn sheep conservation, given the multifactorial nature of the threats to their population.

Data limitations and recommendations for future work

A lack of variability in the presence or absence of risk characteristics along the wildlife supply chain (Chapter 1) led to uncertainty around estimates due to small sample sizes in some model strata. Some risk variables were extremely common (or almost always absent) within certain strata of categorical model covariates (e.g., continent, node of the supply chain), leading to separation of data for regression modeling (i.e., the outcome variable is completely separated by a predictor variable, with the outcome only [or mostly] occurring within one level of the predictor). This lack of variation and small sample sizes among some strata resulted in wide confidence intervals of up to several hundred-fold difference between the upper and lower ends of the range. Surveying additional sites would address this problem by increasing the chances that we would observe uncommon circumstances, thereby increasing the sample size within each level of model covariates and resolving the separation of data between outcomes and predictors. Sampling additional sites would also improve how well our dataset represented the regions across which conclusions were being drawn from model results. Site characterization surveys were conducted at 1 – 28 sites in each country, and by visiting more sites per country, we can ensure that we are capturing the full range of risk interfaces to fully inform risk mitigation. Additionally, by categorizing a risk characteristic as present or absent based on whether the characteristic was observed at least once, we lost the ability to include information on how *often*

a variable was present or absent. Ensuring that each study site was visited multiple times (ideally the same number of times in both the wet and dry seasons) would allow us to calculate the proportion of visits in which a risk characteristic was observed and provide a finer scale of detail on the true occurrence of how frequently risky variables were present.

Differences among states in defining a SARS-CoV-2 case (Chapter 2) was a data limitation resulting from the uncoordinated efforts of individual states to rapidly respond to an emerging pandemic with limited testing resources. While most states reported cases as only those that were laboratory verified, a small subset included probable cases that fit the CDC case definition based on exposure and clinical signs. These differences in data definitions may have influenced some of the inter-state variation in estimates we measured. In addition, our study period ended on April 30, 2020 because some states began combining antibody and polymerase chain reaction test results in their reported case counts after this date. One of the lessons learned from the SARS-CoV-2 pandemic should be that early epidemic response requires coordinated efforts and transparent communication among jurisdictions regarding the methods used for basic data collection such as case counts. The prevalence of disease in one state can impact the burden of disease in other states, and effective epidemic response necessitates that each state is measuring and reporting their data in a consistent way. Ideally, this would include recording the most detailed data possible about patients, including the diagnostic test type and/or exposure history used to classify a person as a SARS-CoV-2 case.

Bighorn sheep (Chapter 3) are a long lived and wide-ranging species, and estimation of long-term population trends requires detailed sampling across multiple demographic, temporal, and geographic strata. The overall conservation efforts of Peninsular bighorn sheep have been managed by multiple agencies over the 36-year study period, which resulted in inconsistencies in

the data collected and sparse data across some strata, including: years where not all recovery regions were sampled, different numbers of sheep sampled from each region, and variable gaps of time between sampling events. These sampling inconsistencies led to small sample sizes in some model strata which resulted in wide confidence intervals and uncertainty around some estimates. Ideally, sampling would occur frequently enough to detect small outbreaks or introductions of disease and be stratified by recovery region in a systematic way, perhaps scaling the number of animals sampled by the population size of each region and obtaining a representative sample of animals from each herd within a region. By sampling the entire range frequently and on the same schedule, we would have more data points with which to evaluate the impact of factors that may affect disease prevalence or population performance, such as precipitation and other ecological variables. However, this level of monitoring is not often available for wildlife conservation projects, especially projects on species with such a large geographic range. The best approach could be combining intermittent range-wide disease sampling, targeted sampling of symptomatic or high-risk individuals, and continuous, longitudinal monitoring of marked animals. Range-wide sampling and testing for pathogens annually would be ideal to detect changes in pathogen prevalence on the same time scale as changes in survival, reproduction, or climatic and environmental variables. If surveys must be conducted less frequently, they could be supplemented with targeted sampling of symptomatic animals observed at year-round monitoring sites, such as water holes, to detect introductions or epidemics of disease between range-wide surveys. In addition, a subset of animals should be continuously radio-collared and monitored for estimation of survival rates and timely collection of mortalities for necropsy to identify causes of mortality. This multipronged approach would be

more labor and resource intensive, but also more likely to catch an introduction or epidemic of disease than less frequent, scattered sampling.

Summary

Together, these chapters provide novel insights into the maintenance, spillover, and control of pathogens across a range of host-pathogen and ecological systems. The research presented in this dissertation is the first to investigate associations between *Mycoplasma ovipneumoniae* exposure and shedding and the population health of bighorn sheep in the Peninsular Ranges, was among the first to quantify the relationship between social distancing and SARS-CoV-2 pandemic control, and assembled the largest dataset to date of human behavioral and ecological risk factors for pathogen spillover within the global wildlife trade network. The risk of pathogen spillover and spread involves a web of factors including host and pathogen biology, spatial distribution and contact rates among hosts, and landscape ecology. Quantifying these risk factors is complicated by the fact that they often vary across temporal and spatial scales, from the local distribution of bighorn sheep herds to the state-to-state differences in social distancing to the global diversity in the wildlife supply chain. This dissertation explores disease dynamics in the pre-spillover, initial epidemic, and post-spillover maintenance phases across three unique systems at different spatial scales, with relevance to both animal and human health. The challenges and risk factors identified can provide a foundation that will help inform the design of future surveillance systems and disease management efforts.

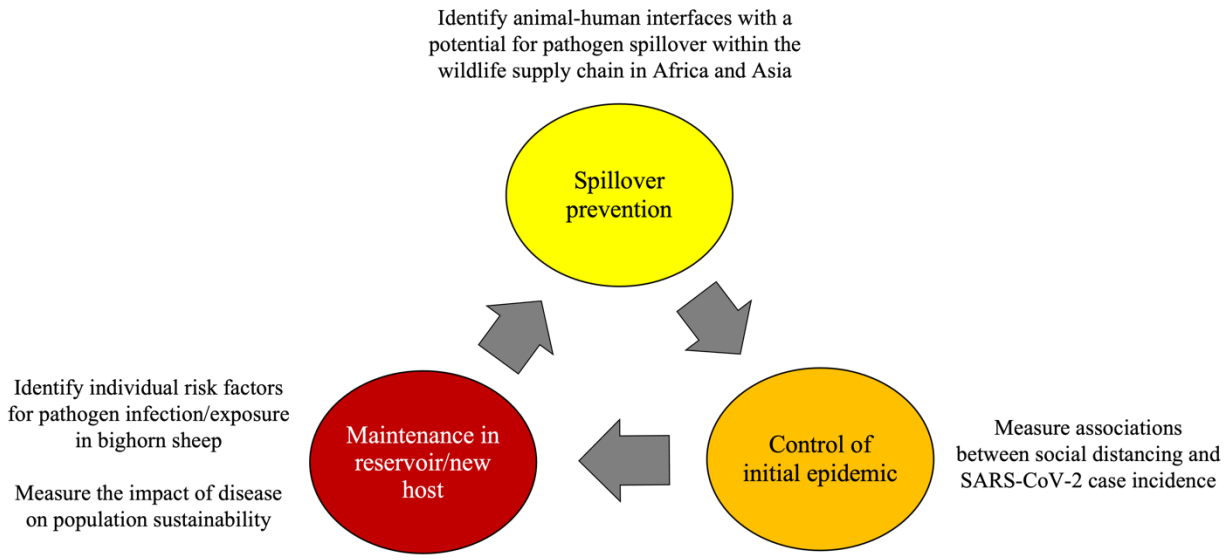

Figure 1: The process of spillover and subsequent spread of a pathogen in a new host population can include three phases, which are often circular: 1) spillover of disease from reservoir host to a novel host due to high-risk interactions (Chapter 1), and 2) rapid pathogen spread causing an epidemic in the new, immunologically naïve host population (Chapter 2), which can be followed by 3) maintenance of disease in the new host (Chapter 3).