Ecological Applications, 26(2), 2016, pp. 367–381

Pathogen exposure varies widely among sympatric populations of wild and domestic felids across the United States

Scott Carver,[1,2,16] Sarah N. Bevins,[3] Michael R. Lappin,[4] Erin E. Boydston,[5] Lisa M. Lyren,[5] Mathew Alldredge,[6] Kenneth A. Logan,[7] Linda L. Sweanor,[7] Seth P. D. Riley,[8] Laurel E. K. Serieys,[9] Robert N. Fisher,[10] T. Winston Vickers,[11] Walter Boyce,[11] Roy McBride,[12] Mark C. Cunningham,[13] Megan Jennings,[14] Jesse Lewis,[15] Tamika Lunn,[2] Kevin R. Crooks,[15] and Sue VandeWoude[1]

[1]*Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, Colorado 80523 USA*
[2]*School of Biological Sciences, University of Tasmania, Hobart, Tasmania 7005 Australia*
[3]*National Wildlife Disease Program, United States Department of Agriculture, Fort Collins, Colorado 80526 USA*
[4]*Department of Clinical Sciences, Colorado State University, Fort Collins, Colorado 80523 USA*
[5]*Western Ecological Research Center, U.S. Geological Survey, Thousand Oaks, California 91360 USA*
[6]*Colorado Parks and Wildlife Service, Fort Collins, Colorado 80526 USA*
[7]*Colorado Parks and Wildlife Service, Montrose, Colorado 81410 USA*
[8]*National Parks Service, Thousand Oaks, California 91360 USA*
[9]*Department of Ecology and Evolutionary Biology, University of California Los Angeles, Los Angeles, California 90095 USA*
[10]*Western Ecological Research Center, U.S. Geological Survey, San Diego, California 92101 USA*
[11]*Wildlife Health Center, University of California Davis, Davis, California 95616 USA*
[12]*Rancher's Supply, Ochopee, Florida 34141 USA*
[13]*Florida Fish and Wildlife Conservation Commission, Gainesville, Florida 32601 USA*
[14]*Institute for Ecological Monitoring and Management, San Diego State University, San Diego, California 92182 USA*
[15]*Department of Fish, Wildlife and Conservation Biology, Colorado State University, Fort Collins, Colorado 80523 USA*

Abstract. Understanding how landscape, host, and pathogen traits contribute to disease exposure requires systematic evaluations of pathogens within and among host species and geographic regions. The relative importance of these attributes is critical for management of wildlife and mitigating domestic animal and human disease, particularly given rapid ecological changes, such as urbanization. We screened >1000 samples from sympatric populations of puma (*Puma concolor*), bobcat (*Lynx rufus*), and domestic cat (*Felis catus*) across urban gradients in six sites, representing three regions, in North America for exposure to a representative suite of bacterial, protozoal, and viral pathogens (*B*artonella sp., *Toxoplasma gondii*, feline herpesvirus-1, feline panleukopenea virus, feline calicivirus, and feline immunodeficiency virus). We evaluated prevalence within each species, and examined host trait and land cover determinants of exposure; providing an unprecedented analysis of factors relating to potential for infections in domesticated and wild felids. Prevalence differed among host species (highest for puma and lowest for domestic cat) and was greater for indirectly transmitted pathogens. Sex was inconsistently predictive of exposure to directly transmitted pathogens only, and age infrequently predictive of both direct and indirectly transmitted pathogens. Determinants of pathogen exposure were widely divergent between the wild felid species. For puma, suburban land use predicted increased exposure to *Bartonella* sp. in southern California, and FHV-1 exposure increased near urban edges in Florida. This may suggest interspecific transmission with domestic cats via flea vectors (California) and direct contact (Florida) around urban boundaries. Bobcats captured near urban areas had increased exposure to *T. gondii* in Florida, suggesting an urban source of prey. Bobcats captured near urban areas in Colorado and Florida had higher FIV exposure, possibly suggesting increased intraspecific interactions through pile-up of home ranges. Beyond these regional and pathogen specific relationships, proximity to the wildland–urban interface did not generally increase the probability of disease exposure in wild or domestic felids, emphasizing the importance of local ecological determinants. Indeed, pathogen exposure was often negatively associated with the wildland–urban interface for all felids. Our analyses suggest cross-species pathogen transmission events around this interface may be infrequent, but followed by self-sustaining propagation within the new host species.

Key words: Bartonella *sp.; bobcat* (Lynx rufus); *cross-species transmission; disease exposure; domestic cat* (Felis catus); *feline calicivirus; feline herpesvirus-1; feline immunodeficiency virus; feline panleukopenea virus; puma* (Puma concolor); Toxoplasma gondii; *urbanization.*

Manuscript received 11 March 2015; accepted 8 June 2015; final version received 3 July 2015. Corresponding Editor: R. L. Knight.

[16]E-mail: scott.carver@utas.edu.au

Introduction

Pathogen exposure risk is dependent upon a wide array of host and pathogen traits and this risk is often complicated by landscape features, both natural and

anthropogenic, and the multi-host nature of many pathogens (Cleaveland et al. 2001, Bradley and Altizer 2007, Lloyd-Smith et al. 2009). There is a critical need for replicated spatially explicit studies across landscape gradients to test the relative impacts of host, pathogen, and anthropogenic landscape features on pathogen exposure among host species (Brearley et al. 2013). This is particularly true for secretive and difficult to study taxa, such as apex predators and other carnivores, which are ecologically pivotal organisms and frequently impacted by a variety of processes, including urbanization and disease (Crooks and Soulé 1999, Murray et al. 1999, Ripple et al. 2014). In this study, we address this critical knowledge gap, undertaking a large-scale systematic evaluation of a suite of pathogens within sympatric domestic and wild felid host species to test how natural and anthropogenic landscape features, as well as host and pathogen traits, shape patterns of exposure around urbanized areas.

In North America, puma (*Puma concolor*), bobcat (*Lynx rufus*), and domestic cats (*Felis catus*) occupy a continuum of natural and modified habitat types. Puma and bobcats are primarily associated with natural and domestic cats with anthropogenic landscape features, with sympatry frequently exhibited around the interface (Crooks 2002, Ordenaña et al. 2010, Carver et al. 2012). All three species can be infected by similar viral, parasitic, and bacterial pathogens, though risk of exposure varies widely because of differences in habitat, ecology, and species-specific behaviors (Riley et al. 2004, Bevins et al. 2009, 2012, Carver et al. 2012), and not all pathogens can move freely between host species (Parrish et al. 2008). Environmental determinants of pathogen exposure may be similar for the wild (nondomestic) felids, relative to domestic cats, owing to their landscape associations. The pile-up of their territories along the boundaries of natural and developed areas (Riley 2006, Riley et al. 2006), often termed the wildland–urban interface, may also influence pathogen exposure through exaggeration of intra- and interspecific contacts (Bevins et al. 2012). If pathogen transmission is more likely along the wildland–urban interface, then proximity to anthropogenic landscape features might be predictive of wild felid exposure, and proximity to natural habitat might be predictive of domestic felid exposure. Study of disease transmission among these species has broader relevance to human disease through analogous situations, such as human–primate interactions (Pedersen et al. 2005), and because the close association of humans with domestic cats can potentiate transmission of zoonotic agents to humans (Carver et al. 2012). Furthermore, by enhancing our understanding of exposure, this can inform management decisions aimed at mitigating intra- and interspecific transmission, such as through vaccination campaigns or selective removal of individuals.

To develop a deeper understanding of how natural and anthropogenic landscape features, along with host and pathogen traits, shape observed patterns of pathogen exposure, we sampled >1000 wild and domestic felids along urban gradients across multiple sites spanning southern California, Colorado, and southern Florida, USA (Fig. 1). We evaluated exposure to six pathogens that vary in classification, transmission characteristics, disease expression, environmental persistence, and host specificity (Table 1): *Bartonella* sp., *Toxoplasma gondii*, feline immunodeficiency virus (FIV), feline herpesvirus (FHV-1), feline panleukopenia virus (FPV), and feline calicivirus (FCV). *Bartonella* sp. and *T. gondii* infect all three species, are indirectly transmitted, and zoonotic (Bevins et al. 2012). Each felid species is typically infected via direct contact with conspecifics with species-adapted strains of FIV, thus this pathogen serves as a marker for intraspecific disease spread (VandeWoude et al. 2010). The three remaining directly transmitted viruses (FHV-1, FPV, and FCV) are thought to primarily reside in

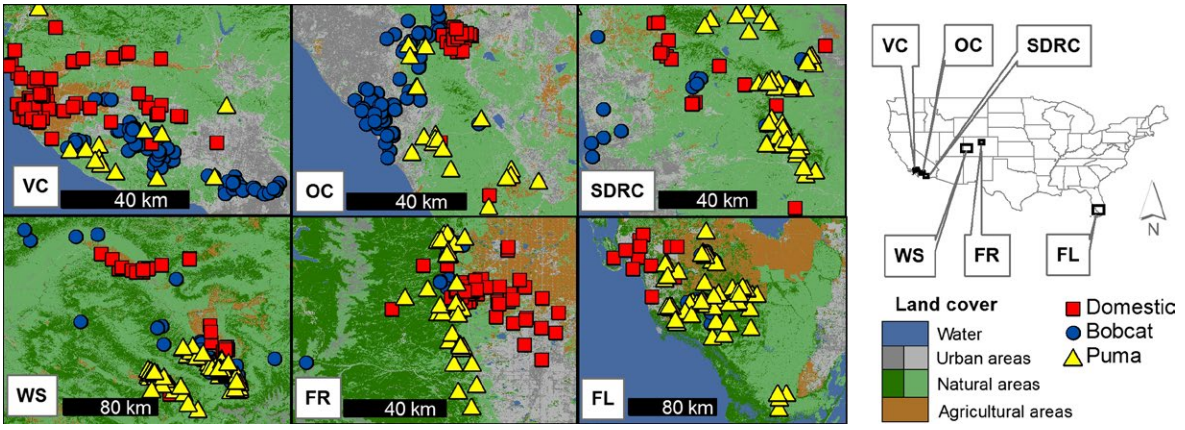

FIG. 1. Capture locations of wild and domestic felids screened for pathogens across study sites. California sites include Ventura County (VC), Orange County (OC), and San Diego and Riverside Counties (SDRC). Colorado sites include Western Slope (WS) and Front Range (FR). Southern Florida site marked FL. Dark and light green areas indicate forested and shrub and scrub areas respectively, brown indicates agricultural areas, gray represents urbanizing areas, and blue indicates water.

TABLE 1. Characteristics of pathogens evaluated in this study.

Pathogen	Classification	Zoonotic	Host specific	Transmission
T. gondii	Protozoan	Yes	No	Trophic and environmental
Bartonella sp.	Bacterium	Yes	No	Vector
FIV	Virus	No	Yes†	Direct
FHV-1	Virus	No	No	Direct
FCV	Virus	No	No	Direct
FPV	Virus	No	No	Direct and environmental‡

†Puma FIV detected in bobcats on rare occasions (Franklin et al. 2007*a*).
‡Treated predominantly as directly transmitted in analyses.

domestic cats, but can infect bobcats and puma as well (Eberle et al. 1991, Paul-Murphy et al. 1994, Nakamura et al. 1999, Riley et al. 2004, Allison et al. 2013, Foley et al. 2013). Probabilities of exposure may be less for the directly transmitted pathogens owing to dependence on direct interactions (Lelu et al. 2010, Bevins et al. 2012). In contrast, the indirectly transmitted pathogens may be more readily transmittable though multiple environmental and food-chain sources (*T. gondii*) and co-occurrence and social interaction leading to sharing of abundant vectors (*Bartonella* sp.; Lelu et al. 2010, Bevins et al. 2012). The probability of exposure to directly transmitted viruses is also often associated with host traits (being adult and male), owing to age- and sex-specific behaviors (Poulin 1996, Hudson et al. 2002, Zuk 2009, Tompkins et al. 2011), but these relationships are not always as consistent for indirectly transmitted pathogens (Hiestand et al. 2014).

We employed Bayesian hierarchical logistic regression approaches to model (1) how host species, pathogen species, and mode of pathogen transmission was related to the probability of exposure, and (2) how the probability of pathogen exposure within host species was related to host traits (sex and age) and natural and anthropogenic landscape features. We predicted: (1) prevalence of exposure would differ among host species and be greater for pathogens with indirect versus direct modes of transmission, (2) within each host species, traits of being male would positively predict exposure to directly transmitted pathogens, and being older would positively predict exposure generally, (3) landscape predictors of exposure would be more similar between the two wild felids, owing to their landscape associations, than with domestic cats, and (4) proximity to the wildland–urban interface would positively influence disease exposure in all three host species. Our extensive analyses revealed: (1) exposure risk differed among host species and mode of pathogen transmission, (2) within species, host traits were inconsistent predictors of pathogen exposure, (3) predictors of puma and bobcat exposure were highly divergent, and (4) proximity to the wildland–urban interface did not generally increase the probability of disease exposure in wild or domestic felids, emphasizing the importance of local ecological factors as drivers of disease exposure. Overall, our results may suggest relatively rare cross-species transmission events with self-sustaining propagation within the new host species when they do occur.

MATERIALS AND METHODS

Study populations, sample collection, and processing

Samples were derived from six sites spanning three study regions (Fig. 1).

California.—Ventura County (VC, also incorporating some of Los Angeles County) and Orange County (OC) sites represent highly urbanized landscapes surrounding the Santa Monica and Santa Ana Mountains north and south of Los Angeles, California respectively. Felids from the eastern portion of San Diego and Riverside Counties (SDRC) were also sampled, representing a more rural and exurban region bordering the Cleveland National Forest. All three Californian sites experience a warm, dry Mediterranean climate, with vegetation communities primarily comprising coastal California sage scrub, chaparral, riparian and coastal oak woodlands, and annual grasslands.

Colorado.—Sites included the western slope (WS) located around Montrose and Grand Junction, and the Front Range (FR) northwest of the highly urbanized Denver Metropolitan Area and immediately adjacent to Boulder, Colorado. These sites represent a primarily rural and exurban region for WS and an urban and exurban region for FR. Climates are cooler than Californian sites and semiarid with vegetation characterized by coniferous woodlands and forests primarily interspersed with aspens. Coniferous woodlands are dominated by pinyon–juniper communities at the WS and ponderosa–Douglas fir communities on the FR.

Florida.—The Florida (FL) site consists of a mixture of urban, exurban, and agricultural areas around Fort Myers and Naples and encompassing Okaloacoochee Slough State Forest, Florida Panther National Wildlife Refuge, Big Cypress National Preserve, Picayune Strand State Forest, Fakahatchee Strand Preserve State Park, Everglades National Park, and other public and private lands. Climate of this region is characterized as humid

subtropical or tropical savanna and vegetation communities consisting of pine flatwoods, south Florida rockland, cypress domes and strands, dwarf cypress, prairies, mixed hardwood swamps, hardwood hammocks, freshwater swamps, and mangroves.

Blood, serum, and oral swab samples from sympatric populations of wild and domestic felids were collected from each study site. Samples from bobcats and puma were obtained from collaborators performing ongoing research. Samples from domestic cats were collected from free-ranging individuals on admission to shelters, or through domestic cat trap, neuter, release programs. Within each site, samples were collected over a 2–3 yr period, with the majority of sample collection occurring from 2001 to 2012. Animal sex and location were recorded at the time of capture, and age (kitten <6 months, young 6 months to 2 yr, adult >2 yr) estimated based on size, weight, and dental wear (Logan and Sweanor 2001). Upon capture, wild felids were anesthetized using a variety of tranquilizers/sedatives (Logan and Sweanor 2001, Riley et al. 2004), sampled, and released. Thoracic fluid was collected from hunter-killed animals instead of serum from a subset of bobcats from WS (Carver et al. 2012). Blood and serum samples were initially stored in ethylenediaminetetraacetic acid and serum-separating tubes. Saliva samples were collected with a sterile swab. Samples were either refrigerated at 4°C or kept on ice until return from the field where they were temporarily frozen at −20°C, and later transferred to −80°C until screening for pathogen exposure. All procedures were performed after appropriate Institutional Animal Care and Use Committee (IACUC) approvals were obtained and with the permission of cooperating agencies.

Landscape characteristics

Based on georeferenced capture locations (Fig. 1), we calculated land cover types within a buffer surrounding each animal. Buffers were estimated based on published information on average home range size for each felid species, sex, and geographic location (Appendix S1); buffers were considered a relative measure of the extent to which each animal potentially comes into contact with surrounding landscape elements. Landscape elements were classified into 10 general categories that were present across all sites and represent four natural (water, forest, shrub and scrub, grassland) and six anthropogenic (agriculture, altered, exurban, suburban, urban, and highly developed) features. Altered landscape features represent parks, sports grounds, campgrounds, etc., and the urban features are defined as 0.1–10 houses/ha for exurban, 10–25 houses/ha for suburban, >25 houses/ha for urban, and city centers, industrial areas, etc. for highly developed. Home range buffers were created and the relative proportion of each land cover type within buffers calculated from 30 × 30 m pixel size rasters using the buffer and isectpolyrst tools in Geospatial Modeling Environment (Hawthorne 2012). Urban, altered, and highly developed land cover types were determined from the National Land Use Database raster (Theobald 2012), which provided detailed characterization of anthropogenic land use types. The remaining land cover types were calculated from the National Land Cover Database raster (Fry et al. 2011), which provided better coverage of natural features. As expected, land cover types surrounding domestic cat capture locations were dominated by urbanization, whereas land cover for wild felids was dominated by more natural landscape features, with site-specific variation (Appendix S2). In addition to land cover surrounding capture locations, we also calculated Euclidean distance of capture location to the nearest urban edge, as determined using the Urban Areas polygon layer from the US Census Bureau (Tiger/Line Shapefiles) and the Near tool in ArcGIS (v9.3.1; ESRI, Redlands, California, USA).

Pathogen screening

Samples were screened to determine exposure to pathogens using assays performed and interpreted following standardized protocols, as previously described (Lappin et al. 1991, 2002, Lappin and Powell 1991, Jensen et al. 2000, Troyer et al. 2005, Franklin et al. 2007*b*, Veir et al. 2008, Ruch-Gallie et al. 2011, Bevins et al. 2012, see Appendix S3). In the majority of cases, pathogen exposure was determined by serological analysis. A small number of domestic cats in this study may have been vaccinated against FCV, FHV-1, and FPV and, thus, serology was not utilized for these pathogens. FPV was not assessed for domestic cats, and FCV and FHV-1 were screened by PCR amplification of domestic cat oral swabs. PCR amplifies viral genomes, so represents individuals actively shedding FHV-1 and FCV, which is typically a characteristic of naturally acquired infection vs. vaccination. Given that domestic cat samples in this study were derived from feral individuals, and the general low prevalence that were PCR positive (see *Results*), it is highly likely that the majority of PCR-positive cats are harboring naturally acquired strains (Veir et al. 2008, Ruch-Gallie et al. 2011). The identity of *Bartonella* sp. serologic results were confirmed by performing PCR on a subset of matching blood samples (n = 609/921; Jensen et al. 2000), demonstrating two *Bartonella* sp. to be common among all three felid hosts (*B. henselae* and *B. clarridigea*; species that are predominantly vectored by the domestic cat flea, *Ctenocephalides felis*). Florida puma were the only wild felid population where some individuals receive vaccines (Cunningham et al. 2008) and, thus, analyses (described in following section) were restricted to unvaccinated individuals. Further, the spatial distribution of capture locations are inherently clumped among host species (predominantly urban for domestic cats and non-urban for the wild felids; Fig. 1) and, thus, we recognized spatial autocorrelation could play a role in pathogen exposure. In preliminary analyses we evaluated evidence of this (M. L. J. Gilbertson et al. *in review*),

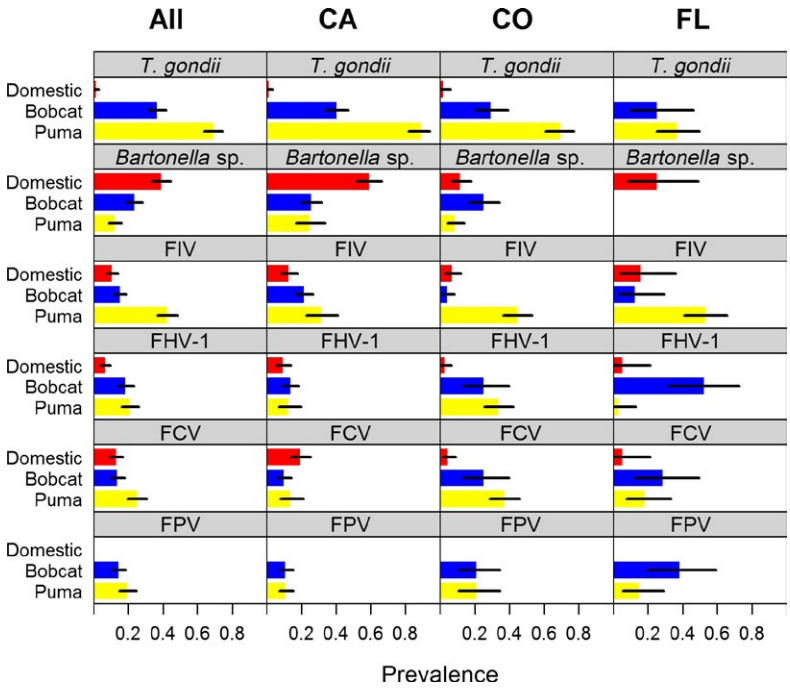

FIG. 2. Prevalence of exposure to pathogens for each host species for sites combined (All) and regions (California, Colorado, and Florida). Prevalence values are maximum likelihood estimates. Prevalence of FPV exposure in domestic cats not examined, due to potential assay cross-reaction if some individuals were vaccinated. See Table 1 for modes of pathogen transmission.

finding little supportive evidence and consequently omitted inclusion of spatially explicit capture location information in the analyses.

Analyses

Analyses reflect animals for which combined sex, age, and geographic location information existed (Appendix S4). To evaluate the effects of host species and mode of pathogen transmission on the probability of exposure we evaluated all pathogens except FPV, since analysis of this pathogen was not performed in domestic cats (Appendices C and D). In a small number of cases, extremely low or high pathogen prevalence (Figs. 2 and 3, Appendix S5) prevented analysis of host trait and landscape determinants of exposure.

Effects of host species and mode of transmission.—To evaluate common patterns of pathogen prevalence among host species and pathogen transmission modes, we evaluated how host species (domestic cat, bobcat, and puma) and mode of pathogen transmission (direct, vector-borne, and trophic and environmental) influenced the probability of pathogen exposure utilizing a Bayesian hierarchical logistic regression approach with varying intercepts and slopes among sites. Following preliminary explorations of the pathogen exposure data (see *Results*), we coded host species as 1, 2, and 3 for domestic cats, bobcats and puma, respectively, and mode of pathogen transmission as 1, 2, and 3 for direct (FIV, FHV-1, FCV), vector-borne (*Bartonella* sp.), and trophic and environmentally (*T. gondii*) transmitted pathogens, respectively. We assigned Y_{ij} as exposure status for individual $i = 1,\ldots,n_j$ at location $j = 1,\ldots,k$. Serostatus was assumed to have a Bernoulli distribution with parameter π_{ij}:

$$Y_{ij}|\pi_{ij} \sim \text{Bernoulli}(\pi_{ij})$$

where π_{ij} is the probability of individual i at location j being seropositive. We modeled the probability of being seropositive, π_{ij}, based on predictor variables. The probability of an individual being seropositive was modeled as

$$\text{logit}(\pi_{ij}) = \alpha_{ji} + \beta_{ji} x_i$$

where α and β are the model intercept and slope, respectively, for individual i varying by location j, and x was assigned as the predictor variable for individual i. Prior distributions for all model parameters in the hierarchy (study sites) were given with the goal of providing conjugate priors that contain little to no influence on the posterior distributions of all the model parameters. We assumed Normal prior distributions on slopes α, and intercepts β, with mean μ and variance σ^2:

$$\alpha_j \sim \text{Normal}(\mu_\alpha, \sigma^2_\alpha), \quad \text{for } j = 1,\ldots,k$$

$$\beta_j \sim \text{Normal}(\mu_\beta, \sigma^2_\beta), \quad \text{for } j = 1,\ldots,k$$

For the variance parameters, σ^2, we determined and utilized non-informative uniform prior hyperparameter distributions, specified as $\sigma^2 \sim \text{Uniform}(0, 100)$, which was

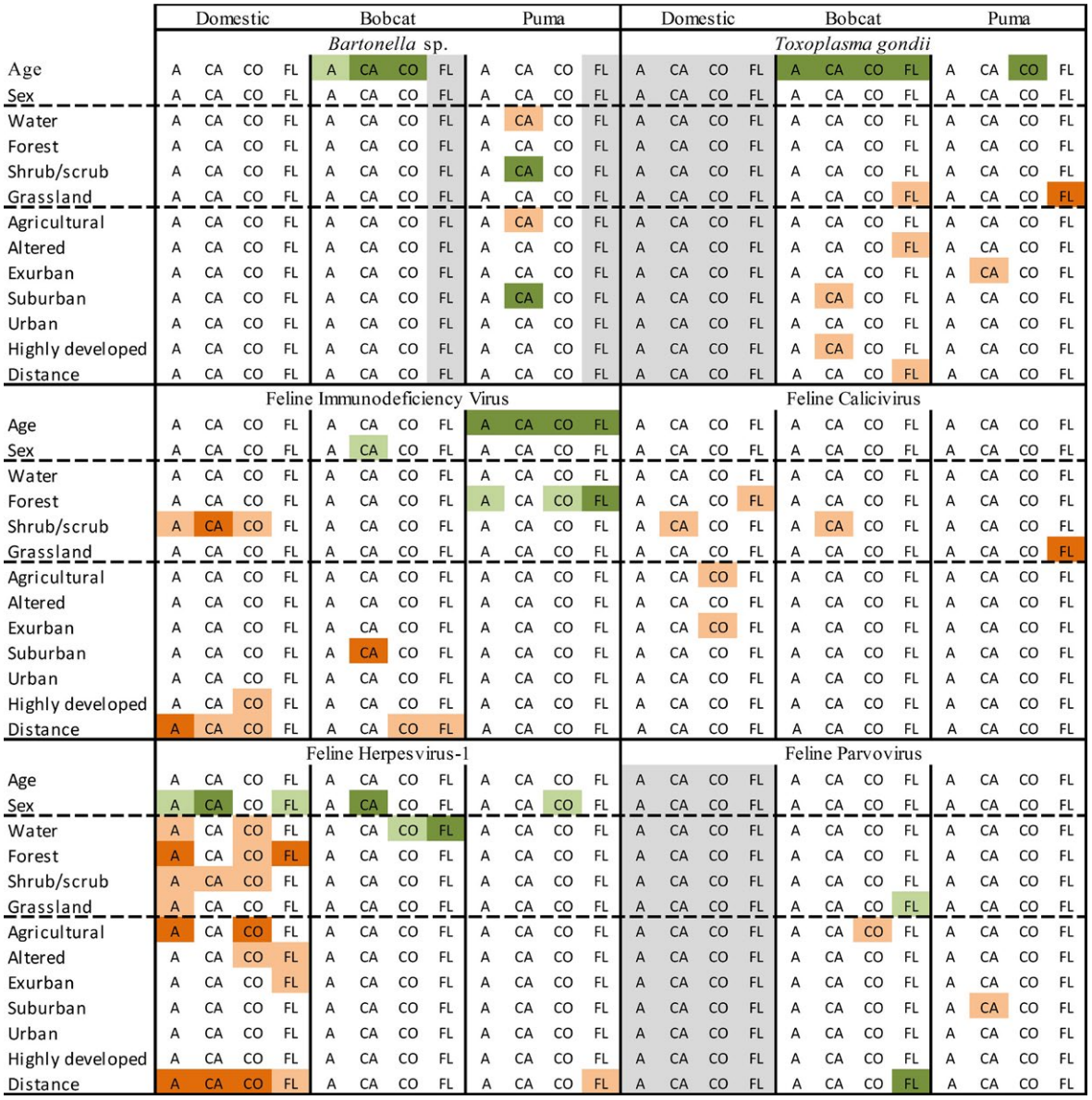

FIG. 3. Predictors of pathogen exposure among sites (all [A], California [CA], Colorado [CO], Florida [FL]) for domestic cat, bobcat, and puma. Dark and light green shading represents strong and trending positive relationships, and dark and light orange shading represents strong and trending negative relationships of predictors to exposure status. Strong and trending relationships denoted by credible intervals of coefficients not overlapping zero for 2.5–97.5% and 10–90%, respectively. Gray regions indicate pathogens and/or sites which were omitted. Florida puma and bobcats were not screened for *Bartonella* sp. (Appendix S4). *T. gondii* and FPV were also omitted for domestic cats owing to low prevalence (Fig. 2) and potential serological cross-reaction among vaccinated individuals (see *Methods*), respectively (see Appendix S4 and S8). Dashed lines demarcate host, natural, and anthropogenic predictor variables. See Appendix S8 for the full suite of coefficients among all sites.

used across all models. Hierarchical models were fit in R and WinBUGS (Speigelhalter et al. 2002), utilizing the R2WinBUGS package. The Markov chain Monte Carlo (MCMC) procedure was used to estimate posterior distributions. We initiated three chains with different points in parameter space, and ran these chains for 50 000 iterations after a burn-in period of 5000 iterations, ensuring convergence of model parameters. Convergence of the Markov chains was assessed following Gelman and Hill (2007). We summarized posterior distributions of model coefficients, β, by the Bayesian median and 95% credible intervals for study regions (CA, CO, and FL; see Appendix S6 for site-specific coefficients).

Because there were multiple directly transmitted pathogens, we also evaluated if the probability of exposure differed among these and if this had any impact on the

outcome of comparisons among transmission modes, again using a Bayesian hierarchical logistic regression structure (see Appendix S7 for site-specific coefficients). In preliminary analyses, FHV-1 and FCV did not differ and were thus lumped for comparison to FIV (see *Results*). Additionally, we evaluated the effects of host species within pathogen transmission modes independently (see justification in *Results*). For purposes of presentation estimates of prevalence (±95% credible interval [CI]) were made using maximum likelihood estimation using the stats4 package in R (v.12.14.1; R Development Core Team 2008).

Effects of host traits and landscape features for pathogen exposure within each host species

We evaluated how host traits (sex and age) and landscape features (proportion of buffered capture location for each land cover type and distance to urban edge) influenced the probability of pathogen exposure within each host species. A Bayesian hierarchical logistic regression approach, with varying intercepts and slopes among sites, was again utilized. We modeled all pairwise and single combinations of the 12 predictor variables (sex, age, water, forest, shrub and scrub, grassland, agriculture, altered, exurban, suburban, urban, highly developed, and distance to urban edge, resulting in 91 model combinations) to find the optimal predictors for π_{ij}. Single and pairwise model combinations enabled multi-model comparison and model averaging and avoided confounding effects of having more models than data (Burnham and Anderson 2002). We coded the sex effect to 1 for females and 2 for males. The age effect was coded as 1 for kitten, 2 for young, and 3 for adult. Prior to modeling effects of predictors on host exposure status, we evaluated relationships among predictors using Pearson correlation analyses, determining that correlations among predictors were acceptably low ($r < 0.7$) and did not warrant elimination of predictors or collapsing of predictors into principal components. We used the Deviance Information Criterion (DIC), a generalization of the Akaike Information Criterion (AIC; Burnham and Anderson 2002), to compare candidate models and calculate models weights (Speigelhalter et al. 2002), which is analogous to calculation of model weights from AIC (Burnham and Anderson 2002). We summarized posterior distributions of model coefficients, β, by the Bayesian median and 95% CI, which we model-averaged across single and pairwise model combinations as suggested by Burnham and Anderson (2002).

For all analyses, strong and trending predictors of exposure were classified as coefficients with 95% and 80% CI not overlapping zero.

Results

Effects of host species and pathogen mode of transmission

As predicted, the probability of pathogen exposure differed among host species (Table 2, Fig. 2). Exposure increased from domestic cat to bobcat to puma (coefficient and 95% CI: 0.606, 0.061–1.111). This relationship between host species and disease exposure was consistent among study regions (CA, 0.398, 0.151–0.636; CO, 1.037, 0.798–1.272; FL, 0.349, 0.077–0.629; see Appendices E–H for CA and CO specific sites). Also consistent with predictions, the probability of exposure corresponded to transmission mode, with increasing prevalence from directly transmitted (FIV, FHV-1, FCV) to vector-borne (*Bartonella* sp.) to trophic and environmentally transmitted (*T. gondii*) pathogens (Fig. 2, Table 2; 0.486, 0.003–0.928). This relationship was consistent in California (0.668, 0.457–0.862) and Colorado (0.463, 0.254–0.677), but not in Florida (−0.007, −0.282–0.270).

Because the directly transmitted pathogens composed multiple viruses, we evaluated if the probability of pathogen exposure differed among them (excluding FPV, see *Methods*). FIV exposure was greater than FHV-1 and FCV (Fig. 2, Table 2; mean 0.500, 0.205–0.813; CA, 0.465, 0.162–0.787; CO, 0.428, 0.103–0.710; FL, 0.705, 0.348–1.087). To determine if the differences among transmission modes were influenced by differences among the directly transmitted pathogens, we restricted the directly transmitted pathogen group to FIV (the highest prevalence of the directly transmitted pathogens) and re-ran the analysis. Again, consistent with our prediction, the probability of exposure increased from directly transmitted (FIV only) to vector-borne (*Bartonella* sp.) to trophic and environmentally transmitted (*T. gondii*) pathogens (Fig. 2, Table 2; 0.331, −0.020–0.731). This relationship was consistent in California (0.420, 0.206–0.645) and Colorado (0.374, 0.133–0.609), but not in Florida (−0.055, −0.370–0.246).

The effects of host species also differed among transmission modes (Fig. 2, Table 2) and, thus, we evaluated the effects of host species within each mode. The probability of host exposure increased from domestic cat to bobcat to puma for pathogens that were both directly transmitted (mean 0.560, −0.080–1.321; CA, 0.243, −0.085–0.568; CO, 1.083, 0.753–1.442; FL, 0.434, 0.110–0.742) and trophic and environmentally transmitted (mean 2.508, 1.627–3.564; CA, 2.933, 2.279–3.899; CO, 2.252, 1.757–2.771; FL, 1.739, 0.986–2.298). Differences in prevalence of exposure among host species was most pronounced for the trophic and environmentally transmitted pathogen, *T. gondii* (Fig. 2). In contrast, for the vector-borne *Bartonella* sp., the probability of exposure declined from domestic cat to bobcat to puma (mean −0.826, −1.889–0.114), particularly in California (−0.841, −1.336 to −0.369) and Florida (−1.881, −3.926 to −0.660), but not Colorado (−0.316, −0.794–0.214). FPV prevalence of exposure in wild felids was consistent with patterns for other directly transmitted viruses, potentially suggesting that this pathogen is commonly transmitted via direct contact (Table 1, Fig. 2).

Table 2. Summary of major findings associated with exposure risks.

Factor	Significant findings
Host species, pathogen species, and mode of transmission	Overall, prevalence differed among host species (puma > bobcat > domestic cat) and transmission modes (trophic and environmental [*T. gondii*] transmission > vector-borne [*Bartonella* sp.] > directly transmitted [viruses]). Exposure to directly transmitted and trophic and environmentally transmitted pathogens increased from domestic cat to bobcat to puma. Exposure to vector-borne transmitted decreased from domestic cat to bobcat to puma. Exposure varied among directly transmitted pathogens (FIV > FHV-1 and FCV), but this did not impact comparisons of transmission mode
Sex and age	Sex was a predictor of exposure (males > female) for directly transmitted pathogens only. But, overall, sex was not a common predictor among hosts, sites and pathogens. Age was positively predictive of puma FIV, and indirectly transmitted pathogens of bobcats (*Bartonella* sp. and *T. gondii*), but not predictive of domestic cat pathogen exposure
Similarity between wild felids	Divergent landscape and host predictors of pathogen exposure among bobcat and puma, despite more similar ecology than domestic cats
Urbanization and cross-species exposure	Urbanization positively predictive for *Bartonella* sp. exposure in California puma, implicating spillover from domestic cat fleas (*Ctenocephalides felis*). Florida puma near urban edges were more likely to be FHV-1 exposed suggesting possible exposure through domestic cat contact. Bobcat exposure to *T. gondii* greater near urban edges in Florida, implicating urban prey sources. Increased FIV exposure of bobcats near urban areas in CO and FL suggesting possible home-range pile-up. No other evidence of urban features associated with increased wild felid pathogen exposure, or natural landscape features associated with increased domestic cat exposure. Wild and domestic felid pathogen exposure often negatively associated with the wildland–urban interface

Effects of host trait and landscape features for pathogen exposure within host species

We predicted that the host trait of sex would be a consistent predictor of directly transmitted pathogens (FIV, FCV, FHV-1, and FPV) and age a general predictor of exposure, within each host species. Overall, sex (males > female) predicted the probability of host exposure to one (FHV-1) out of the three directly transmitted pathogens for domestic cats, but was not predictive of pathogen exposure among bobcat or puma (Fig. 3, Table 2). There was some site specificity for sex as a predictor for directly transmitted pathogens for all three host species. Sex was not predictive of exposure to vector-borne (*Bartonella* sp) or trophic and environmentally transmitted pathogens (*T. gondii*; Fig. 3, Table 2). Age was a consistent positive predictor among sites of one (FIV) out of the four directly transmitted pathogens of puma, but was not predictive of domestic cat or bobcat exposure to directly transmitted pathogens (Table 2). Age was also positively predictive of bobcat exposure to both vector-borne and trophic and environmentally transmitted pathogens. *T. gondii* exposure in puma was positively predicted by age in Colorado, but age was not predictive of domestic cat exposure to any pathogen (Fig. 3, Table 2). Overall, and in contrast to our prediction, host traits were inconsistent predictors of pathogen exposure within host species.

We expected that predictors of pathogen exposure would be more similar between bobcat and puma than with domestic cats. Consistent with this prediction, for both bobcats and puma, age was a common positive predictor of *T. gondii* exposure in Colorado and grassland a common negative predictor in Florida (Fig. 3). However, beyond these cases, strong or trending predictors of exposure were divergent between these hosts for all sites and pathogens (Table 2). Sex was a common positive predictor of FHV-1 exposure for domestic cats and bobcats in California. The probability of FIV exposure was also higher near urban areas in Colorado for both of these hosts. Domestic cats were otherwise also divergent in the strong and trending predictors from bobcat and puma (Fig. 3). Thus overall, contrary to predictions, variables that were predictive of pathogen exposure were highly divergent among host species.

A primary expectation was that proximity to boundaries of natural and anthropogenic areas may exacerbate pathogen exposure. We anticipated this would be evidenced by increased wild felid pathogen exposure associated with anthropogenic predictors, and increased domestic cat exposure associated with natural landscape predictors. For wild felids, we found pathogen- and region-dependent relationships between anthropogenic development and increased exposure. Our expectation was supported with regard to increased bobcat exposure to *T. gondii* exposure near urban edges in Florida and FIV exposure in Colorado and Florida (Fig. 3, Table 2). Our expectation was also supported for puma exposure to *Bartonella* sp., which increased with suburban areas in California, and FHV-1 exposure, which increased near urban edges in Florida (Fig. 3, Table 2). No other pathogens of puma or bobcats indicated increased exposure associated with anthropogenic features in these regions. Indeed, where predictors of pathogen exposure were related to urban and agriculture landscape types, these were otherwise negative, implying higher probability of exposure in natural habitats. For example, bobcat exposure to *T. gondii* was negatively related to suburban and highly developed landscape features in California (Fig. 3). Bobcat exposure to FPV was also negatively associated with agricultural areas in Colorado (Fig. 3). Similarly, in

California, puma exposure to *T. gondii* was negatively related to exurban areas, and exposure to FPV negatively related to suburban areas (Fig. 3). For natural landscape features, predictors of bobcat exposure to FPV exposure in Florida was higher associated with grassland and at greater distances from urban edges, and puma exposure to FIV overall (although most strongly in Colorado and Florida) was positively related to their known habitat preference of forest cover (Fig. 3).

Reciprocally, for domestic cats, results demonstrate that proximity to natural habitat does not generally result in increased pathogen exposure (in contrast to our prediction). Exposure to two pathogens (FIV and FHV-1) was generally lower in natural habitat, or near certain anthropogenic landscape features (highly developed, agriculture, altered, and exurban), where domestic cat densities are typically less than in urban and suburban areas (Fig. 3). Domestic cat exposure to FIV was negatively predicted by shrub/scrub, and FHV-1 was negatively predicted by water, forest, shrub/scrub, grassland, and agriculture landscapes. For both these pathogens the probability of exposure was higher near/within urban areas (as indicated by distance; Fig. 3). There were also region-specific relationships consistent with lower domestic cat pathogen exposure associated with natural or non-urban/suburban anthropogenic landscape features (Fig. 3). FCV exposure was negatively related to forest in Florida, shrub/scrub in California, and agriculture and exurban in Colorado, and exposure to FHV-1 was negatively related to exurban areas in Florida.

Discussion

Identifying determinants of host exposure to pathogens is advantageous for developing policies to manage wildlife, domestic animal, and human health, particularly where rapid ecological changes, such as urbanization, are occurring (Patz et al. 2004, Jones et al. 2008, Tompkins et al. 2015). To do so, there is a critical need for empirical studies that combine host, pathogen, and landscape determinants of pathogen exposure over spatially explicit gradients; particularly for multiple hosts, pathogens, and replicate sites (Brearley et al. 2013). Our study on the effects of landscape, host, and pathogen traits on exposure status for a suite of wild and domestic felid pathogens across multiple study regions represents an unprecedented attempt to evaluate if such generalizations are possible. Carnivores can play substantive roles in shaping ecological communities and pathogen transmission (Crooks and Soulé 1999, Estes et al. 2011, Levi et al. 2012, Ripple et al. 2014), and it is not uncommon for free-ranging felids to share pathogens with domestic congeners (Murray et al. 1999). However, there remain significant practical challenges with evaluating pathogen exposure and transmission among wild and domestic felid species. We conclude that differences in pathogen prevalence among host species and transmission modes are relatively evident, but within host species there is substantive divergence in patterns of host and landscape predictors among sites and pathogen species. Domestic cat pathogen exposure generally does not increase in relation to natural habitat and wild felid exposure exhibits pathogen and region dependent relationships.

Prevalence of exposure associated with differences in host species, pathogen species, and mode of pathogen transmission

Systematic evaluations of host and landscape traits on pathogen exposure generally derive from broad reviews spanning multiple host taxa and focus on pathogen richness (e.g., Murray et al. 1999, Cleaveland et al. 2001, Daszak et al. 2001, Nunn et al. 2003, Pedersen et al. 2005, Ezenwa et al. 2006, Bradley and Altizer 2007). This study complements prior studies, suggesting that in addition to richness, a suite of ecological traits associated with differences in host species may also be widely predictive of risk of exposure (Nunn et al. 2003, Ezenwa et al. 2006, Lindenfors et al. 2007). Specifically, we observed increasing prevalence with increasing host species mass (domestic cat to bobcat to puma) for a suite of pathogens (FIV, FHV-1, FCV, FPV, and *T. gondii*), although underlying mechanisms driving this pattern remain speculative. Greater home range size of larger felids (Beier et al. 2010, Riley et al. 2010, Horn et al. 2011) may also result in higher probabilities of exposure to trophic and environmentally transmitted pathogens owing to their mode of pathogen transmission and the likelihood of these pathogens existing somewhere in a larger home range. Puma and bobcats feed almost entirely on wild prey items (cervids, lagomorphs, rodents, etc.; Beier et al. 2010, Riley et al. 2010) and, thus, the diet of larger puma and bobcats may predispose individuals to greater exposure opportunities to *T. gondii* through food chain accumulation effects, relative to the smaller domestic cats. The exceedingly low prevalence of *T. gondii* in domestic cats (despite their samples originating from feral/semi-feral domestic cats) suggests that many of these individuals have at least a portion of their diet supplemented by scavenging in urban areas and provision by humans (Bevins et al. 2012) and consequently exposure through consumption of intermediate hosts is comparatively low. This finding also raises interesting questions about the roles of nondomestic felids in propagating the *T. gondii* sylvatic transmission cycle, and how these species indirectly contribute to human toxoplasmosis infections (e.g., Aramini et al. 1998). Finally, some studies have suggested that larger host species may also have a greater probability of pathogen exposure owing to their greater size providing physically greater niche space for pathogens to establish infections (Ezenwa et al. 2006, Lindenfors et al. 2007, Cooper et al. 2012).

The notable opposite relationship (prevalence for domestic cat > bobcat > puma) for the vector-borne *Bartonella* sp. suggests that domestic cats are the primary host species for this agent in the ecosystems examined. This relationship may also suggest mass-specific

allometries associated with host ecology (i.e., home range size, density, or domestication status) or interactive effects with mode of pathogen transmission. For example, densities of domestic cat populations are higher than bobcat populations, which in turn exceed puma (Cox et al. 2006, Dabritz et al. 2006, Burdett et al. 2010, Riley et al. 2010). The relatively higher densities of domestic cat populations are conducive to supporting abundant populations of *Ctenocephalides felis* vectors and an increased force of transmission for *Bartonella* sp. (Bevins et al. 2012). While little research on the abundance and spatial distribution of vectors in the environment exists, further research on this and associations with habitat utilization by felid hosts (or potential alternative host taxa) would be valuable.

Although unprecedented at this scale, we acknowledge this study is limited to only six pathogens, resulting in some limitations to inferences. For example, inclusion of more shared vector-borne, trophic, and environmentally transmitted pathogens would be valuable to further explore the effect of transmission mode on pathogen exposure. There was also some variation among directly transmitted pathogens in the probability of exposure (FIV was greater than FHV-1 and FCV), though this did not affect the relationship among transmission modes. Further research to study the generalities of exposure relationships in relation to interspecific host traits and modes of pathogen transmission on prevalence could also focus on other co-occurring host taxa (such as canids and cervids in North America), or experiments utilizing model organisms, such as rodents or invertebrates.

Host traits as common predictors of directly transmitted pathogens within host species

Being male and older are commonly assumed predictors of host exposure status for directly transmitted pathogens (e.g., Courchamp et al. 1998, Biek et al. 2006, Bevins et al. 2012). In support of our *a priori* prediction, being male was predictive, albeit inconsistently, of directly transmitted pathogens only. Our findings may also suggest an interaction between bobcat densities and sex specific risk of directly transmitted pathogen exposure. Bobcat densities, where they have been studied, are greater (and home ranges smaller) in California than Colorado or Florida (Karpowitz 1981, Jackson 1986, Wassmer et al. 1988, Riley et al. 2010), and being male positively predicted bobcat exposure to FIV and FHV-1 in California, but not the other study regions. This relationship may apply to other host taxa with variable densities across geographic ranges.

Also consistent with our predictions, age was predictive of both direct and indirectly transmitted pathogen exposures. Logically, the probability of exposure has a time/age-dependent component for any pathogen (Hudson et al. 2002). However, age was only infrequently detected as a predictor of direct and indirectly transmitted pathogen exposure (puma FIV, bobcat *Bartonella* sp., and *T. gondii*, not predictive for domestic cats). Positive age–prevalence relationships can often become obscured for a variety of reasons, for example where indirect pathogen exposure is highly heterogeneous or episodic either spatially or temporally, which is a characteristic of some indirectly transmitted pathogens (e.g., Stapp et al. 2009). Similarly, if exposure to indirectly transmitted pathogens is high in early life, then positive relationships between age and exposure may be obscured if animals are not sampled at a young enough age. Vertical transmission can also obscure age–prevalence relationships, but for the pathogens we investigated this is a relatively rare phenomenon. Further research on relationships between age and mode of pathogen transmission may benefit from expanding the range of hosts and pathogens evaluated and more precise estimates of host age than what was available in this study.

Similarity of predictors of pathogen exposure among wild felids, relative to domestic cats

There is little published systematic information contrasting predictors of pathogen exposure among similar host taxa (Brearley et al. 2013). Our results suggest that determinants of exposure were widely divergent between the wild felid species. Of the two host and 11 landscape predictors of exposure to the six pathogens evaluated for puma and bobcats, only one pathogen (*T. gondii*) had any consistent predictors of exposure, both of which were site specific; age positively predicted puma and bobcat exposure in Colorado and grassland negatively predicted such exposure in Florida. This broad incongruence among predictors of pathogen exposure is contrary to our *a priori* prediction that wild felids exposures would be similar owing to their landscape associations. Indeed, predictors of pathogen exposure differed for all three felid host species. The only similarity in predictors among domestic and wild felids was increased probability of FHV-1 exposure in male domestic cats and bobcats, and increased exposure of the host specific pathogen FIV near urban areas in Colorado.

Proximity to the wildland–urban interface and pathogen exposure

It has been suggested that increased intraspecific pathogen exposure may result from increased home range overlap, population densities, and contact of wild and domestic carnivores along anthropogenic boundaries (Riley 2006, Riley et al. 2006, Bevins et al. 2012, Lee et al. 2012). Such edges may also be a source of spillover events for pathogens able to infect multiple host species. Such events can have devastating impacts on wildlife; for example, canine distemper virus infection of African carnivores, and feline leukemia virus outbreaks in Iberian lynx and Florida panther have resulted in high morbidity and mortality in these threatened carnivores (Roelke-Parker et al. 1996, Cleaveland et al. 2000, Brown et al. 2008, Cunningham et al. 2008, Meli et al. 2009). Conversely, wildlife species are reservoirs of many diseases of domestic animals and humans, including West

Nile virus, Ebola, and influenza (Leroy et al. 2005, Vandegrift et al. 2010, Kilpatrick 2011, Shaman and Lipsitch 2013). Indeed, emerging infectious diseases of humans are strongly associated with mammals that utilize human-modified environments (McFarlane et al. 2012).

We thus predicted proximity to the wildland–urban interface would positively influence disease exposure for wild and domestic felids, and used multiple classifications of anthropogenic land use, as well as distance to urban edge, to test this hypothesis. This is an advance on other comparable studies, which generally consider all urbanization equal (Bradley and Altizer 2007, Brearley et al. 2013). In support of our prediction, urbanization (suburban development specifically) positively predicted puma exposure to *Bartonella* sp. in California, suggesting puma are exposed to increased *C. felis* vectors along urban edges, and this possibly represents vector-mediated cross-species transmission from sympatric domestic cats. This relationship may have been observed only in California owing to the high prevalence of *Bartonella* sp. exposure in domestic cats at this site, relative to Colorado and Florida (Bevins et al. 2012). Puma exposure to FHV-1 also increased near urban edges in Florida, suggesting possible increased exposure through increased intraspecific contact or home range overlap along the urban edge (Riley 2006, Riley et al. 2006), or interspecific contact with domestic cats (such as periodic predation events). Puma disease exposure (feline leukemia virus) through contact with domestic cats is known to have previously occurred in this region (Brown et al. 2008, Cunningham et al. 2008). Similarly, bobcat exposure to *T. gondii* in Florida and FIV in Colorado and Florida increased near urban edges. Increased *T. gondii* exposure near urban edges may reflect increased urban associated prey sources for bobcats in Florida, relative to California and Colorado. For FIV, cross-species transmission is improbable owing to the host-specific nature of this pathogen, and thus increased bobcat densities or home range overlap near urban edges may be the mechanism(s) to facilitate increased exposure in these regions (Riley 2006, Riley et al. 2006). Beside this result, we did not detect consistent positive signals of proximity to urbanization on pathogen exposure among wild felids or of proximity to natural habitat on pathogen exposure in domestic cats. We acknowledge that not all study sites are equal, and intensive studies at additional sites would further elucidate the context specific nature of the wildland–urban interface on pathogen exposure.

On the contrary, our results also demonstrate support for increased exposure to certain pathogens of wild felids in natural landscapes. For example, natural land cover predictors of puma exposure to FIV across sites and bobcats to FPV in Florida reflected known habitat preferences for forested areas and increased distance to anthropogenic development respectively (Crooks 2002, Ordenaña et al. 2010). Their increased exposure in these areas may be due to greater intraspecific contact (Cox et al. 2006, Burdett et al. 2010). The negative relationship of bobcat FIV and puma FPV exposure to suburban land cover in California may reflect altered behaviors and avoidance of urban areas (Crooks 2002, Ordenaña et al. 2010). Conversely, natural landscape features and anthropogenic landscape features where domestic cat abundances are generally low (e.g., agriculture, altered, exurban, and highly developed) were often a negative determinant of their pathogen exposure, likely reflecting their lower densities near these areas and reduced intraspecific force of infection.

Surprisingly, we did not observe positive relationships among urban predictors and domestic cat pathogen exposure status, even though their densities are higher in these areas. This potentially reflects the small home range size of domestic cats, resulting in limited variation in surrounding land cover types (Appendices A and B). Anthropogenic features dominated the landscape characteristics within most domestic cat home ranges, reducing the power to detect positive associations between pathogen exposure and urban variables. However, using Euclidean distance to urban edge we were able to overcome this potential problem, showing higher domestic cat exposure to FIV and FHV-1 within urban areas, consistent with their higher densities (Crooks 2002, Ordenaña et al. 2010). More broadly, higher resolution home range information of hosts (such as measured by radio-tracking or GPS collars) may increase the sensitivity to detect relationships between landscape and pathogen exposure than the buffered capture locations we used. Though individual home range measurements were logistically infeasible given the large scope of this study, this could be a feature of future site specific/intensive investigations.

It should also be acknowledged that this study took a relatively broad approach, considering pathogens capable of crossing between host species, with the exception of FIV. The pathogens we evaluated are well known infections of puma, bobcat, and domestic cats. However, further research on the extent to which these pathogens can freely infect from one host to the other without significant adaptation would be valuable. FIV, as mentioned previously, is most commonly a species-specific infection with transmission between wild felids rare, and no known transmission from domestic to wild felids (Lee et al. 2014). *Bartonella* sp. (*B. henselae* and *B. clarridigea* here) and *T. gondii* are generally considered to cross felid (and a wider host range for *T. gondii*) species barriers. Similarly, indistinguishable strains of FPV (at the VP2 locus) have been shown in domestic cat and puma compatible with onward viral transmission, and highly related strains between puma and bobcat are also known (Allison et al. 2013). Less is known about FHV-1 and FCV. Recently discovered feline species-specific gammaherpesviruses (Troyer et al. 2014) may suggest alphaherpesviruses (such as FHV-1) require adaptation for cross-felid transmission. On the other hand, reports in the literature suggest cross-felid transmission of FCV may be less restricted (Smith et al. 1998), but this remains to be specifically investigated.

Conclusions, implications, and future directions

We identified differences in pathogen prevalence that were associated with differences in host species and mode of pathogen transmission. However, within host species we noted substantive complexity in predictors of individual pathogen exposure, highlighting important challenges for future research. Indeed, we identified relatively few host and landscape predictors of pathogen exposure for the three felid species overall, demonstrating the difficulties associated with understanding disease exposure in these difficult to study taxa. Analysis of co-exposure and infection patterns could potentially contribute to a more nuanced appreciation of exposure patterns across the landscape. For example a behavior modifying pathogen, like *T. gondii*, may impact co-exposure to other agents through increased contacts with conspecifics and other organisms (Lafferty 2006, Al-Kappany et al. 2011, Flegr 2013). Similarly, additional research on contact rates within and among host species and genders might yield greater insight into mechanisms driving differences in pathogen exposures. Further, the pathogens evaluated here are not routinely considered highly pathogenic and threatening to wild or domestic felid populations, but this assumption remains largely unexplored, due to the impracticality of experimental studies on these carnivores, particularly the wild felids. Some pathogens may have mild to moderate direct or indirect impacts on felid survival, or influence the pathogenicity of other pathogens, such as may be caused by the immunosupressive FIV (Bendinelli et al. 1995, Roelke et al. 2006). Analytical approaches, such as age-based force-of-infection models (Heisey et al. 2006), may be a useful alternative to explore the impact of pathogens on wild felid, and other wild host, populations.

This study found pathogen and region dependent relationships in relation to the hypothesis that home-range pile-up is a risk for disease transmission in wild felids (Riley 2006, Riley et al. 2006). For both domestic and wild felids our findings also suggest that pathogen exposure patterns likely are most reflective of propagation in habitat that is most frequently used by each felid species. We thus conclude that pathogens evaluated in this study, primarily directly transmitted forms, appear commonly sustained by intraspecific interactions outside of the wildland–urban interface. This does not undercut the importance of the wildland–urban interface as a location of spillover for these felid hosts, or other wildlife, but rather suggests these events are likely rare and difficult to detect through evaluation of pathogen exposure patterns. Similarly, management to avoid such events may be best positioned to target reduction of the probability of interactions among domestic and wild felids, or vaccination of one of these groups around the wildland–urban interface (i.e., Cunningham et al. 2008). Further research considering phylogenetic and landscape genetic approaches would add valuable insight of pathogen transmission processes within and among these felid species (i.e., Lee et al. 2012). This study demonstrates that understanding intra- and interspecific pathogen transmission among wild and domestic felids, and other host species, remains a challenging and exciting frontier for disease ecologists and epidemiologists.

Acknowledgements

This study was supported by the National Science Foundation Ecology of Infectious Disease Research Program (NSF EF-0723676 and 1413925), and writing of the manuscript was facilitated by a University of Tasmania Visiting Scholarship to Sue VandeWoude. We thank K. Lafferty and two anonymous reviewers for comments and suggestions that helped improve this paper. Multiple humane societies and animal care centers helped with domestic cat samples, including the Montrose Animal Shelter, Ventura County Animal Shelter, Corona Animal Shelter, Escondido Animal Shelter, Pinegate Veterinary Center, Feral Cat Coalition, Boulder Humane Society, and Second Chance Humane Society. We acknowledge A. Griffith, M. Brewer, J. Hawley, A. Morris, V. Scorza, J. Bauer, E. York, M. Ehlbroch, M. Puzzo, G. Lee, and B. Millsap for assistance with sample collection and processing. We also thank Colorado Parks and Wildlife, U.S. Geological Survey, Florida Fish and Wildlife Conservation Commission, National Park Service, California Department of Fish and Wildlife, California State Parks, The Nature Conservancy, and The Anza Borrego Foundation for their collaboration and/or financial support for puma and bobcat capture and sampling. Any use of trade, product, or firm names is for descriptive purposes only and does not imply an endorsement by the U.S. Government.

Literature Cited

Al-Kappany, Y. M., M. R. Lappin, O. C. H. Kwok, S. A. Abu-Elwafa, M. Hilali, and J. P. Dubey. 2011. Seroprevalence of *Toxoplasma gondii* and concurrent *Bartonella* spp., feline immunodeficiency virus, feline leukemia virus, and *Dirofilaria immitis* infections in Egyptian cats. Journal of Parasitology 97:256–258.

Allison, A. B., D. J. Kohler, K. A. Fox, J. D. Brown, R. W. Gerhold, V. I. Shearn-Bochsler, E. J. Dubovi, C. R. Parrish, and E. C. Holmes. 2013. Frequent cross-species transmission of parvoviruses among diverse carnivore hosts. Journal of Virology 87:2342–2347.

Aramini, J. J., C. Stephen, and J. P. Dubey. 1998. Toxoplasma gondii in Vancouver Island cougars (*Felis concolor vancouverensis*): serology and oocyst shedding. Journal of Parasitology 84:438–440.

Beier, P., S. P. D. Riley, and R. M. Sauvajot. 2010. Mountain lions (*Puma concolor*). Pages 141–156 *in* S. D. Gehert, S. P. D. Riley, and B. L. Cypher, editors. Urban carnivores: ecology, conflict, and conservation. Johns Hopkins University Press, Baltimore, Maryland, USA.

Bendinelli, M., M. Pistello, S. Lombardi, A. Poli, C. Garzelli, D. Matteucci, L. Ceccherininelli, G. Malvaldi, and F. Tozzini. 1995. Feline immunodeficiency virus—an interesting model for AIDS studies and an important cat pathogen. Clinical Microbiology Reviews 8:87–112.

Bevins, S. N., et al. 2009. Wild felids as hosts for human plague, western United States. Emerging Infectious Diseases 15:2021–2024.

Bevins, S. N., et al. 2012. Three pathogens in sympatric populations of pumas, bobcats, and domestic cats: implications for infectious disease transmission. PLoS ONE 7:e31403.

Biek, R., T. K. Ruth, K. M. Murphy, C. R. Jr Anderson, M. Johnson, R. DeSimone, R. Gray, M. G. Hornocker, C. M. Gillin, and M. Poss. 2006. Factors associated with pathogen seroprevalence and infection in rocky mountain cougars. Journal of Wildlife Diseases 42:606–615.

Bradley, C. A., and S. Altizer. 2007. Urbanization and the ecology of wildlife diseases. Trends in Ecology & Evolution 22:95–102.

Brearley, G., J. Rhodes, A. Bradley, G. Baxter, L. Seabrook, D. Lunney, Y. Liu, and C. McAlpine. 2013. Wildlife disease prevalence in human-modified landscapes. Biological Reviews 88:427–442.

Brown, M. A., M. W. Cunningham, A. L. Roca, J. L. Troyer, W. E. Johnson, and S. J. O'Brien. 2008. Genetic characterization of feline leukemia virus from Florida panthers. Emerging Infectious Diseases 14:252–259.

Burdett, C. L., K. R. Crooks, D. M. Theobald, K. R. Wilson, E. E. Boydston, L. M. Lyren, R. N. Fisher, T. W. Vickers, S. A. Morrison and W. M. Boyce. 2010. Interfacing models of wildlife habitat and human development to predict the future distribution of puma habitat. Ecosphere 1:art4.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and inference: a practical information-theoretic approach. Spring-Verlag, New York, New York, USA.

Carver, S., A. V. Scorza, S. N. Bevins, S. P. D. Riley, K. R. Crooks, S. VandeWoude, and M. R. Lappin. 2012. Zoonotic parasites of bobcats around human landscapes. Journal of Clinical Microbiology 50:3080–3083.

Cleaveland, S., M. G. J. Appel, W. S. K. Chalmers, C. Chillingworth, M. Kaare, and C. Dye. 2000. Serological and demographic evidence for domestic dogs as a source of canine distemper virus infection for Serengeti wildlife. Veterinary Microbiology 72:217–227.

Cleaveland, S., M. K. Laurenson, and L. H. Taylor. 2001. Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. Philosophical Transactions of the Royal Society B 356:991–999.

Cooper, N., R. Griffin, M. Franz, M. Omotayo, and C. L. Nunn. 2012. Phylogenetic host specificity and understanding parasite sharing in primates. Ecology Letters 15:1370–1377.

Courchamp, F., N. G. Yoccoz, M. Artois, and D. Pontier. 1998. At-risk individuals in feline immunodeficiency virus epidemiology: evidence from a multivariate approach in a natural population of domestic cats (*Felis catus*). Epidemiology and Infection 121:227–236.

Cox, J. J., D. S. Maehr, and J. L. Larkin. 2006. Florida panther habitat use: new approach to an old problem. Journal of Wildlife Management 70:1778–1785.

Crooks, K. R. 2002. Relative sensitivities of mammalian carnivores to habitat fragmentation. Conservation Biology 16:488–502.

Crooks, K. R., and M. E. Soulé. 1999. Mesopredator release and avifaunal extinctions in a fragmented system. Nature 400:563–566.

Cunningham, M. W., et al. 2008. Epizootiology and management of feline leukemia virus in the Florida puma. Journal of Wildlife Diseases 44:537–552.

Dabritz, H. A., E. R. Atwill, I. A. Gardner, M. A. Miller, and P. A. Conrad. 2006. Outdoor fecal deposition by free-roaming cats and attitudes of cat owners and nonowners toward stray pets, wildlife, and water pollution. Journal of the American Veterinary Medical Association 229:74–81.

Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2001. Anthropogenic environmental change and the emergence of infectious diseases in wildlife. Acta Tropica 78:103–116.

Eberle, R., C. J. Baldwin, D. Black, A. A. Kocan, and R. W. Fulton. 1991. Feline herpesvirus infections in bobcats (*Lynx rufus*): disease in experimentally inoculated animals. Journal of Zoo and Wildlife Medicine 22:175–183.

Estes, J. A., et al. 2011. Trophic downgrading of planet Earth. Science 333:301–306.

Ezenwa, V. O., S. A. Price, S. Altizer, N. D. Vitone, and K. C. Cook. 2006. Host traits and parasite species richness in even and odd-toed hoofed mammals, Artiodactyla and Perissodactyla. Oikos 115:526–536.

Flegr, J. 2013. How and why *Toxoplasma* makes us crazy. Trends in Parasitology 29:156–163.

Foley, J. E., P. Swift, K. A. Fleer, S. Torres, Y. A. Girard, and C. K. Johnson. 2013. Risk factors for exposure to feline pathogens in California mountain lions (*Puma concolor*). Journal of Wildlife Diseases 49:279–293.

Franklin, S. P., J. L. Troyer, J. A. Terwee, L. M. Lyren, W. M. Boyce, S. P. D. Riley, M. E. Roelke, K. R. Crooks, and S. VandeWoude. 2007a. Frequent transmission of immunodeficiency viruses among bobcats and pumas. Journal of Virology 81:10961–10969.

Franklin, S. P., J. L. Troyer, J. A. TerWee, L. M. Lyren, R. W. Kays, S. P. D. Riley, W. M. Boyce, K. R. Crooks, and S. Vandewoude. 2007b. Variability in assays used for detection of lentiviral infection in bobcats (*Lynx rufus*), pumas (*Puma concolor*), and ocelots (*Leopardus pardalis*). Journal of Wildlife Diseases 43:700–710.

Fry, J., G. Xian, S. Jin, J. Dewitz, C. Homer, L. Yang, C. Barnes, N. Herold, and J. Wickham. 2011. Completion of the 2006 National Land Cover Database for the conterminous United States. Photogrammetric Engineering and Remote Sensing 77:858–864.

Gelman, A., and H. Hill. 2007. Data analysis using regression and multilevel/hierarchical models. Cambridge University Press, New York, New York, USA.

Hawthorne, B. L. 2012. Geospatial modelling environment. http://www.spatialecology.com/gme/.

Heisey, D. M., D. O. Joly, and F. Messier. 2006. The fitting of general force-of-infection models to wildlife disease prevalence data. Ecology 87:2356–2365.

Hiestand, S. J., C. K. Nielsen, and F. A. Jimenez. 2014. Epizootic and zoonotic helminths of the bobcat (*Lynx rufus*) in Illinois and a comparison of its helminth component communities across the American Midwest. Parasite 21:4.

Horn, J. A., N. Mateus-Pinilla, R. E. Warner, and E. J. Heske. 2011. Home range, habitat use, and activity patterns of free-roaming domestic cats. Journal of Wildlife Management 75:1177–1185.

Hudson, P. J., A. Rizzoli, B. T. Grenfell, H. Heesterbeek, and A. P. Dobson. 2002. The ecology of wildlife diseases. Oxford University Press, New York, New York, USA.

Jackson, D. H. 1986. Ecology of bobcats in east-central Colorado. Colorado State University, Fort Collins, Colorado, USA.

Jensen, W. A., M. Z. Fall, J. Rooney, D. L. Kordick, and E. B. Breitschwerdt. 2000. Rapid identification and differentiation of *Bartonella* species using a single-step PCR assay. Journal of Clinical Microbiology 38:1717–1722.

Jones, K. E., N. G. Patel, M. A. Levy, A. Storeygard, D. Balk, J. L. Gittleman, and P. Daszak. 2008. Global trends in emerging infectious diseases. Nature 451:990–993.

Karpowitz, J. T. 1981. Home ranges and movements of Utah bobcats with reference to habitat selection and prey base. Brigham Young University, Provo, Utah, USA.

Kilpatrick, A. M. 2011. Globalization, land use, and the invasion of West Nile virus. Science 334:323–327.

Lafferty, K. D. 2006. Can the common brain parasite, *Toxoplasma gondii*, influence human culture? Proceedings of the Royal Society B 273:2749–2755.

Lappin, M. R., and C. C. Powell. 1991. Comparison of latex agglutination, indirect hemagglutination, and ELISA techniques for the detection of *Toxoplasma gondii* specific antibodies in the serum of cats. Journal of Veterinary Internal Medicine 5:299–301.

Lappin, M. R., E. R. Jacobson, G. V. Kollias, C. C. Powell, and J. Stover. 1991. Comparison of serologic assays for the diagnosis of toxoplasmosis in nondomestic felids. Journal of Zoo and Wildlife Medicine 22:169–174.

Lappin, M. R., J. Andrews, D. Simpson, and W. A. Jensen. 2002. Use of serologic tests to predict resistance to feline herpesvirus 1, feline calicivirus, and feline parvovirus infection in cats. Journal of the American Veterinary Medical Association 220:38–42.

Lee, J. S., E. W. Ruell, E. E. Boydston, L. M. Lyren, R. S. Alonso, J. L. Troyer, K. R. Crooks, and S. VandeWoude. 2012. Gene flow and pathogen transmission among bobcats (*Lynx rufus*) in a fragmented urban landscape. Molecular Ecology 21:1617–1631.

Lee, J. S., et al. 2014. Evolution of puma lentivirus in bobcats (*Lynx rufus*) and mountain lions (*Puma concolor*) in North America. Journal of Virology 88:7727–7737.

Lelu, M., M. Langlais, M. L. Poulle, and E. Gilot-Fromont. 2010. Transmission dynamics of *Toxoplasma gondii* along an urban-rural gradient. Theoretical Population Biology 78:139–147.

Leroy, E. M., B. Kumulungui, X. Pourrut, P. Rouquet, A. Hassanin, P. Yaba, A. Delicat, J. T. Paweska, J. P. Gonzalez, and R. Swanepoel. 2005. Fruit bats as reservoirs of Ebola virus. Nature 438:575–576.

Levi, T., A. M. Kilpatrick, M. Mangel, and C. C. Wilmers. 2012. Deer, predators, and the emergence of Lyme disease. Proceedings of the National Academy of Sciences 109:10942–10947.

Lindenfors, P., C. L. Nunn, K. E. Jones, A. A. Cunningham, W. Sechrest, and J. L. Gittleman. 2007. Parasite species richness in carnivores: effects of host body mass, latitude, geographical range and population density. Global Ecology and Biogeography 16:496–509.

Lloyd-Smith, J. O., D. George, K. M. Pepin, V. E. Pitzer, J. R. C. Pulliam, A. P. Dobson, P. J. Hudson, and B. T. Grenfell. 2009. Epidemic dynamics at the human-animal interface. Science 326:1362–1367.

Logan, K. A., and L. L. Sweanor. 2001. Desert puma: evolutionary ecology and conservation of an enduring carnivore. Island Press, Washington, D.C., USA.

McFarlane, R., A. Sleigh, and T. McMichael. 2012. Synanthopy of wild mammals as a determinant of emerging infectious diseases in the Asian-Australasian region. EcoHealth 9:24–35.

Meli, M. L., et al. 2009. Feline leukemia virus and other pathogens as important threats to the survival of the critically endangered Iberian lynx (*Lynx pardinus*). PLoS ONE 4:e4744.

Murray, D. L., C. A. Kapke, J. F. Evermann, and T. K. Fuller. 1999. Infectious disease and the conservation of free-ranging large carnivores. Animal Conservation 2:241–254.

Nakamura, K., Y. Ikeda, T. Miyazawa, N. T. P. Nguyen, D. D. Duong, K. H. Le, S. D. Vo, L. V. Phan, T. Mikami, and E. Takahashi. 1999. Comparison of prevalence of feline herpesvirus type 1, calicivirus and parvovirus infections in domestic and leopard cats in Vietnam. Journal of Veterinary Medical Science 61:1313–1315.

Nunn, C. L., S. Altizer, K. E. Jones, and W. Sechrest. 2003. Comparative tests of parasite species richness in primates. American Naturalist 162:597–614.

Ordenaña, M. A., et al. 2010. Effects of urbanization on carnivore species distribution and richness. Journal of Mammalogy 91:1322–1331.

Parrish, C. R., E. C. Holmes, D. M. Morens, E.-C. Park, D. S. Burke, C. H. Calisher, C. A. Laughlin, L. J. Saif, and P. Daszak. 2008. Cross-species virus transmission and the emergence of new epidemic diseases. Microbiology and Molecular Biology Reviews 72:457–470.

Patz, J. A., et al. 2004. Unhealthy landscapes: policy recommendations on land use change and infectious disease emergence. Environmental Health Perspectives 112:1092–1098.

Paul-Murphy, J., T. Work, D. Hunter, E. McFie, and D. Fjelline. 1994. Serologic survey and serum biochemical reference ranges of the free-ranging mountain lion (*Felis concolor*) in California. Journal of Wildlife Diseases 30:205–215.

Pedersen, A. B., S. Altizer, M. Poss, A. A. Cunningham, and C. L. Nunn. 2005. Patterns of host specificity and transmission among parasites of wild primates. International Journal for Parasitology 35:647–657.

Poulin, R. 1996. Sexual inequalities in helminth infections: a cost of being a male? American Naturalist 147:287–295.

R Development Core Team. 2008. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. www.r-project.org.

Riley, S. P. D. 2006. Spatial ecology of bobcats and gray foxes in urban and rural zones of a national park. Journal of Wildlife Management 70:1425–1435.

Riley, S. P. D., J. Foley, and B. Chomel. 2004. Exposure to feline and canine pathogens in bobcats and gray foxes in urban and rural zones of a national park in California. Journal of Wildlife Diseases 40:11–22.

Riley, S. P. D., J. P. Pollinger, R. M. Sauvajot, E. C. York, C. Bromley, T. K. Fuller, and R. K. Wayne. 2006. A southern California freeway is a physical and social barrier to gene flow in carnivores. Molecular Ecology 15:1733–1741.

Riley, S. P. D., E. E. Boydston, K. R. Crooks, and L. M. Lyren. 2010. Bobcats (*Lynx rufus*). Pages 121–140 *in* S. D. Gehert, S. P. D. Riley, and B. L. Cypher, editors. Urban carnivores: ecology, conflict, and conservation. Johns Hopkins University Press, Baltimore, Maryland, USA.

Ripple, W. J., et al. 2014. Status and ecological effects of the world's largest carnivores. Science 343:1241484.

Roelke, M. E., J. Pecon-Slattery, S. Taylor, S. Citino, E. Brown, C. Packer, S. VandeWoude, and S. J. O'Brien. 2006. T-lymphocyte profiles in FIV-infected wild lions and pumas reveal CD4 depletion. Journal of Wildlife Diseases 42:234–248.

Roelke-Parker, M. E., et al. 1996. A canine distemper virus epidemic in Serengeti lions (*Panthera leo*). Nature 379: 441–445.

Ruch-Gallie, R. A., J. K. Veir, J. R. Hawley, and M. R. Lappin. 2011. Results of molecular diagnostic assays targeting feline herpesvirus-1 and feline calicivirus in adult cats administered modified live vaccines. Journal of Feline Medicine and Surgery 13:541–545.

Shaman, J., and M. Lipsitch. 2013. The El Niño-Southern Oscillation (ENSO)–pandemic influenza connection: coincident or causal? Proceedings of the National Academy of Sciences USA 110:3689–3691.

Smith, A. W., D. E. Skilling, N. Cherry, J. H. Mead, and D. O. Matson. 1998. Calicivirus emergence from ocean reservoirs: zoonotic and interspecies movements. Emerging Infectious Diseases 4:13–20.

Speigelhalter, D. J., A. Thomas and N. G. Best. 2002. WinBUGS. User manual. Version 1.4. MRC Biostatitics Unit, Cambridge, UK.

Stapp, P., D. J. Salkeld, H. A. Franklin, J. P. Kraft, D. W. Tripp, M. F. Antolin, and K. L. Gage. 2009. Evidence for the involvement of an alternate rodent host in the dynamics of introduced plague in prairie dogs. Journal of Animal Ecology 78:807–817.

Theobald, D. M. 2012. National Land Use Database. http://www.nrel.colostate.edu/ftp/theobald/.

Tompkins, D. M., A. M. Dunn, M. J. Smith, and S. Telfer. 2011. Wildlife diseases: from individuals to ecosystems. Journal of Animal Ecology 80:19–38.

Tompkins, D. M., S. Carver, M. E. Jones, M. Krkošek, and L. F. Skerratt. 2015. Emerging infectious diseases of wildlife: a critical perspective. Trends in Parasitology 31:149–159.

Troyer, J. L., et al. 2005. Seroprevalence and genomic divergence of circulating strains of Feline Immunodeficiency virus among Felidae and Hyaenidae species. Journal of Virology 79:8282–8294.

Troyer, R. M., et al. 2014. Novel gammaherpesviruses in North American domestic cats, bobcats and pumas: identification, prevalence and risk factors. Journal of Virology 88:3914–3924.

Vandegrift, K. J., S. H. Sokolow, P. Daszak, and A. M. Kilpatrick. 2010. Ecology of avian influenza viruses in a changing world. Annals of the New York Academy of Sciences 1195:113–128.

VandeWoude, S., J. Troyer, and M. Poss. 2010. Restrictions to cross-species transmission of lentiviral infection gleaned from studies of FIV. Veterinary Immunology and Immunopathology 134:25–32.

Veir, J. K., R. Ruch-Gallie, M. E. Spindel, and M. R. Lappin. 2008. Prevalence of selected infectious organisms and comparison of two anatomic sampling sites in shelter cats with upper respiratory tract disease. Journal of Feline Medicine and Surgery 10:551–557.

Wassmer, D. A., D. D. Guenther, and J. N. Layne. 1988. Ecology of the bobcat in south-central Florida. Bulletin of the Florida State Museum, Biological Sciences 33:159–228.

Zuk, M. 2009. The sicker sex. PLoS Pathogens 5:e1000267.

Supporting Information

Additional supporting information may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1890/15-0445.1/suppinfo