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Is pathogen exposure spatially autocorrelated? Patterns of pathogens in puma (*Puma concolor*) and bobcat (*Lynx rufus*)

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Abstract. Understanding pathogen spread in wildlife has important implications for conservation and management efforts. This is particularly the case for taxa that are susceptible to disease spillover events resulting in outbreaks and rapid population declines, such as carnivores. However, assessment of the spatial structure of pathogen exposure (pathogen spatial autocorrelation) is relatively rare for these kinds of taxa. Structure in pathogen exposure may reflect a number of important features, including host traits, pathogen traits, and detection methods utilized. The relatively wide-ranging nature of many carnivores may lead to rapid pathogen spread and obfuscate any spatial autocorrelation being detectable, but this has not yet been explicitly evaluated. Here, we tested for evidence of spatial structuring of pathogen exposure and coexposures for puma (*Puma concolor*) and bobcat (*Lynx rufus*), both mobile and wide-ranging felid species. The study included 440 puma and 639 bobcat from six study regions (one in Florida, two in Colorado, and three in California), as well as each animal's capture location and exposure status for up to eight pathogens. This allowed a thorough examination of spatial patterns of pathogen exposure across different pathogen transmission types, different habitats, and different host ecology. We tested for spatial autocorrelation for each pathogen in each host species at each site, as well as both host species combined. In addition, we tested for coexposure between all pathogens in the study, and for those pathogens that were correlated, we tested for spatial clusters of coexposure. We detected spatial autocorrelation in exposure status for approximately 2% and 10% of examined cases for puma and bobcats, respectively, and spatial clustering in approximately 17% of cases where pathogen coexposures were detected. These results suggest that wide-ranging species, such as puma and bobcat, may rapidly disseminate pathogens across their populations, precluding substantive detection of autocorrelation in pathogen exposure by traditional serological and infection detection methods. Thus, targeted pathogen surveillance or control might focus on individual host characteristics, and advances in understanding pathogen spread in these secretive felids may necessitate examinations of spatial structure in both pathogen and host genetics.

Key words: carnivore; cluster; coexposure; *Lynx rufus;* pathogen exposure; *Puma concolor;* serology; spatial autocorrelation.

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Introduction

Understanding patterns of pathogen spread is a significant issue for wildlife conservation, owing to the importance virulent pathogens can have on populations (Tompkins et al. 2015). An appreciation of such information has powerful consequences for intervention strategies, particularly in determining priorities for wildlife vaccination or treatment. However, it can be challenging to empirically assess likely patterns of virulent pathogen spread a priori, while assessing spread following outbreaks is generally too late to implement intervention strategies. Accordingly, one strategy is to utilize nonvirulent pathogens as surrogates for virulent congeners. By studying the spatial structure of relatively benign pathogens (particularly across multiple pathogens and transmission modes), it is possible to begin building generalized pictures of how virulent pathogens may spread among host populations given invasion and outbreak.

One approach for understanding how an introduced pathogen may spread in a host population is to study the spatial structure of existing pathogens through analysis of spatial autocorrelation. Spatial autocorrelation is the concept that subjects close together in space are more likely to be similar for a particular factor and subjects distant to each other are more likely to be dissimilar for that factor (Sokal and Oden 1978). Spatial autocorrelation is relevant in epidemiology as individuals that are close in space may be more likely to interact with each other and transmit pathogens than individuals that are far apart (Bates et al. 2001, McCallum et al. 2001), thereby leading to spatial structure in patterns of pathogen infection or infection history. For example, one of the best predictors of Dengue virus infection is the presence of an infected individual in the same household (Kuno 1995, Gubler 1998). Conversely, pathogen spread may be less spatially structured (and therefore autocorrelation not evident) where hosts are highly mobile causing disease spread to be more homogeneous.

We might also expect different levels of spatial autocorrelation to depend on the length of the infectious period and whether pathogen exposure is determined via molecular detection (representing active infection) or via serological method (representing past and/or current infection). For example, for pathogens with short infectious periods and where spread is measured based on active infection, evidence of spatial autocorrelation should reflect contemporary pathogen dynamics. For chronic infections, and for pathogen exposure based on serological method, evidence of spatial autocorrelation might reflect longer-term transmission dynamics. Thus, it might be expected that the probability of detecting spatial autocorrelation is greater for pathogens with short infectious periods and for pathogens that can be determined via molecular detection, while the presence of spatial autocorrelation for chronic infections (or when assessing exposure) could indicate comparatively slower spread. However, assessment of spatial autocorrelation of individual pathogens is rare in the literature, and thus, there is a need for empirical testing of patterns. Indeed, existing studies evaluating spatial autocorrelation of pathogens focus on pathogen communities. For example, Davies and Pedersen (2008) found that if different primate species shared a geographic range, they were also more likely to share pathogens, and Poulin (2003) found that the similarity of parasite communities decays with Euclidean distance within some mammalian and fish host species. Because some pathogens can influence host susceptibility to other infections (Telfer et al. 2010), evaluating patterns of coexposure, and how coexposures are spatially structured, also furthers understanding of how pathogens spread through populations.

In this study, we evaluate evidence of pathogen spatial autocorrelation and coexposure clustering using data from chronic infections or antibody evidence of exposure (Table 1) in populations of puma (*Puma concolor*) and bobcat (*Lynx rufus*). In North America, puma and bobcat exist across a range of natural and anthropogenically impacted habitats (Gehrt et al. 2010). Both of these felids are relatively solitary in behavior and wide ranging. Accordingly, it might be hypothesized that spatial patterns of pathogens should be diffuse (not spatial autocorrelated), but this has not previously been tested. There are notable differences in species traits between puma and bobcats that may influence the likelihood of detecting spatial autocorrelation of pathogens. Bobcats are much smaller than puma, with smaller and generally more distinct home ranges, relative to larger and less distinct home ranges of puma (Hansen 2007,

Table 1. Pathogens included in this study, with infection period and primary mode of transmission.

Pathogens	Infection period	Primary mode of transmission	Source
Bartonella sp. (Bart)	Generally chronic infection with relapsing bacteremia	Arthropod vector (primarily *Ctenocephalides felis* fleas)	1, 2, 3, 4
Feline Calicivirus (FCV)	May be lifelong carriers	Direct contact (saliva and nasal secretions)	4, 5, 6, 7
Feline Herpes Virus (FHV)	Lifelong (latently infected carriers with intermittent reactivation)	Direct contact (saliva and nasal secretions)	2, 6
Feline Immunodeficiency Virus (FIV)	Lifelong	Direct contact (biting, fighting)	1, 4, 8
Feline Panleukopenia Virus (FPV)	Acute, self-limiting	Direct or indirect contact	4, 7, 9
Mycoplasma spp. (Mhm, Mhf.tc)	May be lifelong carriers	Direct contact (biting, fighting); Arthropod vector (fleas, ticks)	10, 11, 12
Toxoplasma gondii (*T. gondii*)	Lifelong (chronic tissue phase of infection)	Consumption of infected prey	1

Note: Sources are as follows: (1) Bevins et al. (2012); (2) Breitschwerdt (2008); (3) Chomel et al. (2006); (4) Greene (2006); (5) Filoni et al. (2006); (6) Foley et al. (2013); (7) Hofmann-Lehmann et al. (1996); (8) Biek et al. (2006*a*); (9) Steinel et al. (2001); (10) André et al. (2011); (11) Tasker (2010); (12) Willi et al. (2007).

Hornocker and Negri 2010). Both felids are also susceptible to many of the same or similar pathogens, and in some cases, they may also share these pathogens with other host species (Bevins et al. 2012, Carver et al. 2016).

Here, we tested the spatial structure of pathogens for 440 individual pumas and 639 individual bobcats across six study regions (three in California, two in Colorado, and one in Florida). We focus on six directly transmitted pathogens (feline calicivirus [FCV], feline herpes virus [FHV], feline immunodeficiency virus [FIV], feline panleukopenia virus [FPV], and the hemotropic *Mycoplasma* spp. *Candidatus M. haemominitum* [Mhm] and nondifferentiated diagnosis for *M. haemofelis* or *Candidatus M. turicensis* [Mhf.tc]), one vectorborne pathogen (*Bartonella* sp. [Bart]), and one trophic and environmentally transmitted pathogen (*Toxoplasma gondii*). These pathogens largely cause chronic infections in affected animals (Table 1), but Bart, FCV, FHV, and FPV may have shorter infectious periods and, as a result, exhibit a greater likelihood of spatial structure in detection among hosts.

We hypothesize that the spatial patterns of pathogens in bobcat and puma should be relatively diffuse (lacking structure and spatial autocorrelation). However, if any patterns of pathogen spatial autocorrelation do occur, they will be more commonly detected in bobcat populations and for pathogens with shorter infectious periods. We (1) evaluate evidence of spatial autocorrelation in pathogen exposure for puma, bobcat, and both host species combined; (2) evaluate whether there is evidence of coexposure among any pairwise combinations of pathogens; and (3) for pathogen pairs exhibiting coexposure, test for evidence of spatial clustering.

Methods

Study area

Our study included six study regions: one in Florida, two in Colorado, and three in California (Bevins et al. 2012, Lagana et al. 2013, Troyer et al. 2014, Carver et al. 2016). The Florida (FL) study region encompassed the Fort Meyers and Naples areas, as well as Okaloacoochee Slough State Forest, Florida Panther National Wildlife Refuge, Big Cypress National Preserve, Picayune Strand State Forest, and Fakahatchee Strand Preserve State Park. The two Colorado study regions included the Western Slope (WS) region around Montrose and Grand Junction and the Front Range (FR) region extending from the vicinity of Denver and Boulder, north to Fort Collins. The three California study regions included Ventura County (VC), Orange County (OC), and San Diego/Riverside Counties (SDRC). The FL, VC, and OC study regions contained highly fragmented habitat patches, bounded almost entirely by urban development, agricultural areas, or ocean. The SDRC, FR, and WS study regions were less fragmented, with the landscape of the WS study region having the least amount of anthropogenic modification.

Within these six study regions, individual felids were captured and biological samples

collected as part of ongoing research, according to protocols previously described (Bevins et al. 2012, Troyer et al. 2014, Carver et al. 2016). Some individuals were captured multiple times during the course of the study. Because recaptured animals were more likely to be captured closer to their previous capture location, only the data from initial captures were included for analysis. Ultimately, 440 individual pumas and 639 individual bobcats were evaluated in this study, although not all individuals had data for each pathogen.

Size, weight, and dental wear were used to estimate ages of study animals (Bevins et al. 2012). Ages were categorized as "kitten" for individuals under 6 months of age, "young" for individuals between 6 months and 2 years of age, or "adult" for individuals greater than 2 years of age. Because of the risk of passive transfer of maternal antibodies causing the detection of false positives for pathogen antibodies in felids up to 4–5 months of age (Pu et al. 1995, MacDonald et al. 2004, Munson et al. 2010), "kittens" were removed from the analysis.

Pathogen screening

Exposure to Bart, FCV, FHV, FIV, FPV, and *T. gondii* was estimated by measuring serum antibodies according to protocols previously described (Bevins et al. 2012, Carver et al. 2016). As there are no serological tests for hemoplasmas, amplification of specific DNA from blood by polymerase chain reaction assay was used to estimate infection by Mhm and Mhf.tc. All positive cases (infection or serological detection method) are referred to as "exposures," as such terminology is the most conservative representation of infection status, although terms such as "infection history" would also be appropriate (Gilbert et al. 2013). Because most pathogens in this study form chronic infections, exposure may represent active infection, but cannot distinguish how recent an infection event occurred (see also *Discussion*). Exposure status for each pathogen was recorded in binary format, with "0" for negative and "1" for positive. Any individual that lacked location or exposure data for the pathogen in question was removed from analysis. Depending on the study region and species, samples were collected over a range of sampling periods ranging from less than 2 months to over 14 years (Table 2), and ultimately allowed for the sampling of an unprecedented number of animals (Bevins et al. 2012, Troyer et al. 2014).

Analyses

Spatial autocorrelation.—To evaluate spatial autocorrelation, we used Mantel tests, which enabled us to evaluate whether pathogen exposure status was related to capture distance between hosts. We selected this approach as it is a relatively sensitive test to detect evidence of spatial autocorrelation in pathogen exposure (Guillot and Rousset 2013). While there is a possibility that this test may on occasion inflate type I statistical error, we deemed this unlikely in the present study owing to (1) extensive visualization of spatial patterns of exposure to check statistical findings, (2) our relatively minimal a priori expectations of spatial

Table 2. Duration of sample collection for each species in each study region.

Species	Region	First sample	Last sample	Total span (yr)
Puma	FL	29 June 2004	10 February 2010	5.5
	FR	30 November 2001	27 January 2011	9
	OC	28 June 2004	20 April 2010	5.7
	SDRC	28 March 2001	16 February 2010	8.8
	VC	30 January 1998	13 February 2008	9.9
	WS	7 January 2005	26 January 2012	7
Bobcat	FL	8 March 2010	5 May 2010	0.2
	FR	31 October 2007	8 December 2011	4
	OC	12 December 2002	9 July 2009	6.5
	SDRC	23 November 2008	1 January 2012	3.1
	VC	30 September 1996	9 February 2011	14.2
	WS	7 January 2007	18 November 2009	2.8

Notes: This table reflects the sampling dates from animals that were used in the analysis. Columns represent the species, study region, date of first sample collection, date of last sample collection, and the resulting time span in years.

structuring, and (3) our results largely supporting this expectation (see *Results*).

These tests were conducted for each pathogen, in each study region, for pumas and bobcats separately. Mantel tests were also conducted with both host species combined to examine whether spatial patterns in pathogen exposure were different when testing sympatric felid species together, rather than separately. These combined host tests were completed for all pathogens except FIV, as strains of this pathogen are species specific (VandeWoude and Apetrei 2006, Lagana et al. 2013), with cross-species transmission rare. In all cases, Mantel tests were conducted by constructing a "predictor matrix," a pairwise Euclidean distance matrix between the capture locations of all individuals in the data set. A "dependent distance matrix" was created with the binary exposure status of all pairs of individuals in the data set for each pathogen. A pair of individuals that were either both seropositive or both seronegative had a distance of "0," whereas a pair of individuals that had one seropositive individual and one seronegative individual had a distance of "1." Distance matrices were calculated and Mantel tests conducted using the stats and ade4 packages in R (R Core Team 2014). Mantel test results include *P*-values (*P*), ±SE, and the observed correlation (ρ).

Evaluation of coexposure.—To test for correlations in exposure for any two pathogens, chi-square tests ("*N* − 1") were conducted on 2 × 2 contingency tables for every pairwise combination of pathogens in each species, in each study region (Campbell 2007). In addition, the exposure status for each pathogen was combined to include all study regions, and chi-square tests conducted for every pairwise combination of pathogens in each species. The chi-square tests were computed using the R v3.1.0 stats package (R Core Team 2014); the threshold for significance was set to $P \leq 0.05$. Any 2 × 2 contingency tables with a cell count of "0" were excluded from analysis, as the chi-square test is not recommended for use with cell counts less than 1 (Campbell 2007).

Spatial clustering of coexposure.—Pairwise pathogen combinations with significant correlations in coexposure were then used to test for spatial clustering. This cluster analysis was completed via the SaTScan v9.3 program (Kulldorf and Information Management Services Inc. 2009), using the multinomial scan statistic (Jung et al. 2010), a circular window, and a maximum spatial cluster size of 50% of the population at risk. The categories for the multinomial test were defined based on coexposure status. For example, if testing for clusters with pathogens "A" and "B," the SaTScan analysis treated exposure status as one of four categories: A−/B−, A+/B−, A−/B+, or A+/B+. Significant clusters would therefore show greater or lesser risk than expected for any of these categories.

Results

Spatial autocorrelation

There was little evidence of spatial autocorrelation in pathogen exposure for puma. Of 48 possible pathogen and study region combinations, only *T. gondii* in the SDRC study region was found to be spatially autocorrelated (Table 3). The *T. gondii* data set for SDRC, however, was limited to clustering of two seronegative individuals, compared with 31 seropositive individuals. FCV in the WS study region and FIV in the FL study region exhibited trending patterns of spatial autocorrelation ($P = 0.056$ and $P = 0.051$, respectively), suggesting that there may be weak spatial factors for these study region and pathogen combinations.

There was more evidence of spatial autocorrelation in pathogen exposure for bobcat. Five (~10%) of the 48 bobcat pathogen and study region combinations were found to be spatially autocorrelated (Table 3): FHV in the FL study region; Mhm in the FR, SDRC, and VC study regions; and *T. gondii* in the SDRC study region. Additionally, FCV in the FR study region trended toward spatial autocorrelation ($P = 0.058$), although in this case the trending result was likely an artifact of a single seropositive spatially distinct individual who was located far from the rest of the seronegative individuals in the data set.

There was little evidence of spatial autocorrelation when assessing pathogen exposure in pumas and bobcats together. Only four (~10%) of the 42 pathogen and study region combinations exhibited spatial autocorrelation (Mhm in SDRC and VC and *T. gondii* in SDRC and WS; Table 4). Of these four, three pathogen–study region combinations were spatially autocorrelated in at least one felid species in the separate-species analyses (Mhm in bobcat in SDRC and VC and *T. gondii*

Table 3. Mantel test results for spatial autocorrelation of pathogen exposure in puma and bobcat.

		Pumas			Bobcats		
Pathogen	Region	*P*	ρ	*n* (pos.)	*P*	ρ	*n* (pos.)
Bart	FL	NA	NA	48 (0)	NA	NA	22 (0)
	FR	0.14 ± 0.01	0.09	61 (6)	0.66 ± 0.01	−0.1	19 (2)
	OC	0.83 ± 0.01	−0.04	22 (10)	0.64 ± 0.02	−0.01	70 (19)
	SDRC	0.18 ± 0.01	0.09	33 (6)	0.15 ± 0.01	0.1	20 (2)
	VC	NA	NA	2 (0)	0.95 ± 0.007	−0.04	130 (41)
	WS	0.17 ± 0.01	0.03	54 (3)	0.70 ± 0.01	−0.04	20 (7)
FCV	FL	0.30 ± 0.01	0.003	33 (6)	0.19 ± 0.01	0.04	23 (6)
	FR	0.70 ± 0.01	−0.01	61 (29)	0.058 ± 0.007	0.8	18 (1)
	OC	0.99 ± 0.004	−0.2	22 (2)	0.65 ± 0.02	−0.02	70 (4)
	SDRC	0.12 ± 0.01	0.1	33 (6)	0.49 ± 0.02	−0.06	15 (3)
	VC	NA	NA	2 (0)	0.78 ± 0.01	−0.04	130 (10)
	WS	0.056 ± 0.007	0.05	54 (17)	0.95 ± 0.007	−0.06	20 (9)
FHV	FL	0.25 ± 0.01	0.01	31 (1)	**0.018 ± 0.004**	**0.2**	**23 (12)**
	FR	0.15 ± 0.01	0.05	61 (20)	NA	NA	18 (0)
	OC	0.56 ± 0.02	−0.04	22 (4)	0.73 ± 0.01	−0.03	70 (11)
	SDRC	NA	NA	34 (0)	0.94 ± 0.007	−0.09	15 (6)
	VC	NA	NA	2 (0)	0.29 ± 0.01	0.03	130 (14)
	WS	0.99 ± 0.004	−0.04	54 (17)	0.51 ± 0.02	−0.03	20 (9)
FIV	FL	0.051 ± 0.007	0.05	51 (29)	0.73 ± 0.01	−0.07	23 (3)
	FR	0.67 ± 0.01	−0.02	62 (25)	0.43 ± 0.02	−0.09	21 (3)
	OC	0.66 ± 0.01	−0.07	21 (4)	0.95 ± 0.007	−0.06	90 (19)
	SDRC	0.29 ± 0.01	0.02	34 (13)	0.70 ± 0.01	−0.05	22 (6)
	VC	NA	NA	2 (1)	0.15 ± 0.01	0.05	140 (24)
	WS	0.27 ± 0.01	0.01	61 (25)	0.48 ± 0.02	0.00	20 (2)
FPV	FL	0.28 ± 0.01	0.01	34 (5)	0.76 ± 0.01	−0.05	23 (8)
	FR	0.46 ± 0.02	−0.001	61 (14)	0.98 ± 0.004	−0.1	18 (3)
	OC	0.89 ± 0.01	−0.2	22 (4)	0.41 ± 0.02	0.01	70 (11)
	SDRC	NA	NA	34 (0)	NA	NA	15 (0)
	VC	NA	NA	2 (0)	0.30 ± 0.01	0.02	130 (10)
	WS	0.81 ± 0.01	−0.02	54 (17)	0.11 ± 0.01	0.07	20 (5)
Mhm	FL	NA	NA	1 (0)	0.33 ± 0.01	0.04	19 (16)
	FR	0.47 ± 0.02	−0.01	31 (16)	**0.026 ± 0.005**	**0.3**	**18 (6)**
	OC	0.21 ± 0.01	0.05	11 (5)	0.97 ± 0.006	−0.08	20 (12)
	SDRC	NA	NA	3 (0)	**0.001 ± 0.001**	**0.7**	**18 (11)**
	VC	0.12 ± 0.01	0.1	13 (5)	**0.025 ± 0.005**	**0.03**	**126 (59)**
	WS	0.65 ± 0.02	−0.03	30 (17)	0.73 ± 0.01	−0.04	20 (6)
Mhf.tc	FL	NA	NA	1 (0)	0.47 ± 0.02	−0.01	19 (3)
	FR	0.12 ± 0.01	0.1	31 (1)	NA	NA	18 (0)
	OC	NA	NA	11 (0)	NA	NA	20 (0)
	SDRC	NA	NA	3 (0)	0.97 ± 0.005	−0.1	18 (1)
	VC	NA	NA	13 (0)	0.12 ± 0.01	0.05	126 (6)
	WS	NA	NA	30 (0)	0.24 ± 0.01	0.04	20 (2)
Toxoplasma gondii	FL	0.67 ± 0.01	−0.02	48 (18)	0.92 ± 0.009	−0.09	23 (5)
	FR	0.11 ± 0.01	0.02	61 (33)	0.84 ± 0.01	−0.1	19 (5)
	OC	0.19 ± 0.01	0.2	22 (21)	0.568 ± 0.02	−0.01	70 (50)
	SDRC	**0.039 ± 0.006**	**0.2**	**33 (31)**	**0.006 ± 0.002**	**0.3**	**19 (11)**
	VC	NA	NA	2 (2)	0.29 ± 0.01	0.02	130 (30)
	WS	0.37 ± 0.02	0.004	54 (41)	0.81 ± 0.01	−0.05	20 (12)

Notes: For each pathogen tested and study area, we have reported the *P*-value results (*P*), ±SE, the observed correlation (ρ), the sample size (*n*), and the number of positive individuals in each test (pos.). Significant results are highlighted in boldface and represent data sets with spatial autocorrelation in pathogen exposure. In some cases, the Mantel test results are listed as "NA" for "not applicable." This result occurs when a data set has individuals that all have the same serostatus (e.g., all seronegative) or when there are ≤ 2 individuals in a data set.

Table 4. Mantel test results for spatial autocorrelation of pathogen exposure in puma and bobcat combined.

Pathogen	Region	Pumas and bobcats		
		P	ρ	*n* (pos.)
Bart	FL	NA	NA	70 (0)
	FR	0.19 ± 0.01	0.07	80 (8)
	OC	0.39 ± 0.02	0.01	92 (29)
	SDRC	0.17 ± 0.01	0.09	53 (8)
	VC	0.97 ± 0.006	−0.05	132 (41)
	WS	0.15 ± 0.01	0.04	123 (22)
FCV	FL	0.41 ± 0.02	−0.004	56 (12)
	FR	0.16 ± 0.01	0.03	79 (30)
	OC	0.62 ± 0.02	−0.03	92 (6)
	SDRC	0.29 ± 0.01	0.04	48 (9)
	VC	0.79 ± 0.01	−0.04	132 (10)
	WS	0.77 ± 0.01	−0.02	79 (24)
FHV	FL	0.38 ± 0.02	−0.01	54 (13)
	FR	0.23 ± 0.01	0.04	79 (20)
	OC	0.958 ± 0.006	−0.08	92 (15)
	SDRC	0.889 ± 0.01	−0.1	49 (6)
	VC	0.33 ± 0.01	0.01	132 (14)
	WS	0.83 ± 0.01	−0.03	79 (25)
FPV	FL	0.65 ± 0.02	−0.04	57 (13)
	FR	0.54 ± 0.02	−0.01	79 (17)
	OC	0.63 ± 0.02	−0.03	92 (15)
	SDRC	NA	NA	49 (0)
	VC	0.40 ± 0.02	0.01	132 (10)
	WS	0.28 ± 0.01	0.01	79 (22)
Mhm	FL	0.28 ± 0.01	0.1	20 (16)
	FR	0.46 ± 0.02	−0.01	49 (22)
	OC	0.22 ± 0.01	0.02	31 (17)
	SDRC	**0.001 ± 0.001**	**0.3**	**21 (11)**
	VC	**0.029 ± 0.005**	**0.03**	**139 (64)**
	WS	0.56 ± 0.02	−0.008	55 (25)
Mhf.tc	FL	0.56 ± 0.02	−0.02	20 (3)
	FR	0.075 ± 0.008	0.2	49 (1)
	OC	NA	NA	31 (0)
	SDRC	0.98 ± 0.004	−0.1	21 (1)
	VC	0.18 ± 0.01	0.05	139 (6)
	WS	1.0 ± 0	−0.09	55 (2)
Toxoplasma gondii	FL	0.70 ± 0.01	−0.03	71 (23)
	FR	0.22 ± 0.01	0.006	80 (38)
	OC	0.61 ± 0.02	−0.02	92 (71)
	SDRC	**0.001 ± 0.001**	**0.5**	**52 (42)**
	VC	0.076 ± 0.008	0.06	132 (32)
	WS	**0.001 ± 0.001**	**0.3**	**123 (62)**

Notes: For each pathogen tested and study area, we have reported the *P*-value results (*P*), ±SE, the observed correlation (ρ), the sample size (*n*), and the number of positive individuals in each test (pos.). Significant results are highlighted in boldface and represent data sets with spatial autocorrelation in pathogen exposure. In some cases, the Mantel test results are listed as "NA" for "not applicable." This result occurs when a data set has individuals that all have the same serostatus (e.g., all seronegative) or when there are ≤ 2 individuals in a data set.

in both puma and bobcat in SDRC). One pathogen–study region combination tested as spatially autocorrelated, without having previously been identified in the species-specific analyses: *T. gondii* in the WS study region.

Coexposure correlations

Few pathogen combinations (9 of 168 for puma; 15 of 168 for bobcat) were correlated in chi-square tests for pathogen coexposure (significant results shown in Table 5). The most notable of the coexposure correlations was FHV × FPV, which was strongly correlated in the FR study region for puma and in the OC and VC study regions for bobcat. In addition, we evaluated coexposure correlations with all study regions grouped together and found FHV × FPV to be strongly correlated for each species (for puma: $\chi^2 = 29$, $P < 0.001$; for bobcat: $\chi^2 = 32$, $P < 0.001$).

Coexposure clusters

We tested for spatial clustering of the 24 pairwise pathogen combinations that exhibited significant coexposure correlations (of 336 total pairwise pathogen combinations) using SaTScan. Four of these pathogen combinations exhibited spatial clustering (highlighted in Table 5). Three of the four pathogen combinations with spatial clusters included *T. gondii* in bobcats in the VC study region, and the fourth combination featured FHV and FPV for puma in the FR study region. For *T. gondii* in the VC study region, there was an increased risk of being positive for *T. gondii* exposure and negative for the second pathogen (FIV, FPV, or Mhf.tc), or positive for both *T. gondii* exposure and the second pathogen (FIV, FPV, or Mhf.tc) (Table 6). For FR puma, there were two significant clusters, one with increased risk of being negative for both FHV and FPV exposure and the other with increased risk of being positive for FHV and FPV exposure.

Discussion

How pathogens spread through animal populations is a major issue in wildlife conservation and management, yet this is a difficult phenomenon to directly measure. One approach is to evaluate the spatial structure of pathogens in a host population for multiple pathogens, multiple

Table 5. Significant results from "$N - 1$" chi-square tests, and corresponding results from subsequent SaTScan multinomial analysis.

				Chi-square test		SaTScan clusters	
Species	Region	Pathogens	n	χ^2	P	# Clusters	P
Puma	OC	Bart × Mhm	11	4.9	0.03	0	
	WS	FCV × FIV	54	6.0	0.01	0	
	WS	FCV × *Toxoplasma gondii*	54	4.6	0.03	0	
	FR	**FHV × FPV**	**61**	**13**	**<0.01**	**2**	**0.001, 0.005**
	FR	FHV × Mhm	30	5.2	0.02	0	
	WS	FHV × *T. gondii*	54	4.6	0.03	0	
	WS	FIV × Mhm	30	5.6	0.02	0	
	WS	FIV × *T. gondii*	54	4.6	0.03	0	
	WS	FPV × *T. gondii*	54	4.6	0.03	0	
Bobcat	OC	Bart × FHV	70	5.0	0.02	0	
	OC	Bart × FPV	70	5.0	0.02	0	
	FL	FCV × *T. gondii*	23	4.0	0.05	0	
	OC	FCV × FHV	70	3.8	0.05	0	
	OC	FCV × *T. gondii*	70	4.5	0.03	0	
	VC	FCV × FHV	130	4.2	0.04	0	
	OC	FHV × FIV	70	4.5	0.03	0	
	OC	FHV × FPV	70	15	<0.01	0	
	VC	FHV × FPV	130	9.7	<0.01	0	
	FL	FIV × *T. gondii*	23	4.3	0.04	0	
	OC	FIV × FPV	70	8.6	<0.01	0	
	VC	FIV × Mhf.tc	118	5.5	0.02	0	
	VC	**FIV × *T. gondii***	**128**	**5.5**	**0.02**	**1**	**0.02**
	VC	**FPV × *T. gondii***	**130**	**4.5**	**0.03**	**1**	**0.03**
	VC	**Mhf.tc × *T. gondii***	**119**	**4.8**	**0.03**	**1**	**0.01**

Notes: Only those pathogen combinations that were correlated in chi-square analysis are displayed, as these are the only combinations that underwent SaTScan analysis. Pathogen combinations with significant spatial clusters are highlighted in boldface. Columns represent species, study region, the pathogen combination in question, the sample size (n), the chi-square results (χ^2), the chi-square P-value results (P), the number of significant clusters from the SaTScan analysis, and P-values for significant clusters in SaTScan (P). SaTScan cluster P-values are only displayed for the pathogen combinations that exhibited significant clusters.

hosts, and multiple sites, and in doing so begin to build a picture of how pathogen exposures may or may not be spatially structured, including phenomena such as isolation by distance. Surprisingly, few studies evaluate spatial autocorrelation of pathogen exposure, and here, we addressed that knowledge gap, focusing on puma and bobcats, for which we hypothesized their wide-ranging ecologies may obfuscate evidence of spatial structuring of pathogen exposure and coexposure. We included eight pathogens spanning six sites, providing a substantive array of

Table 6. Relative risk for pathogen exposure in significant SaTScan clusters.

Species	Study region	Pathogen A	Pathogen B	A−/B−	A−/B+	A+/B−	A+/B+
Puma	FR	FHV	FPV	2.71	0	0	0
				0.44	0.39	2.75	Inf
Bobcat	VC	FIV	*Toxoplasma gondii*	0.47	3.29	0	6.11
		FPV	*T. gondii*	0.44	3.88	1.05	6.30
		Mhf.tc	*T. gondii*	0.45	3.00	0	Inf

Notes: Columns indicate species, study region, and the two pathogens in a cluster. "A−/B−" represents the relative risk for an individual testing negative for both "Pathogen A" and "Pathogen B" for each cluster; "A−/B+" represents the relative risk for an individual testing negative for "Pathogen A" and positive for "Pathogen B," etc. A relative risk of "Inf" means risk was calculated to be "infinitely high." The combination of FHV and FPV in puma in the FR study region had two significant clusters; therefore, we report two sets of relative risks for this pathogen combination.

pathogens, hosts, and environments to test for spatial autocorrelation, particularly for carnivores. Overall, we found limited evidence of spatial autocorrelation in examined cases for puma (~2%), bobcats (~10%), and when these hosts were combined for analysis (~10%). Evidence of clusters of coexposure among the pathogens was also limited, with 1% of total possible coexposure combinations and 17% of the pathogens that exhibited coexposure exhibiting evidence of clustering. We find support for our hypothesis and conclude that this study generally suggests that pathogens (at least for the agents examined) may spread relatively rapidly across bobcat and puma populations, precluding detection of spatial autocorrelation by the serological and molecular detection methods employed.

We also provide tentative support to our prediction that patterns of spatial autocorrelation may be more commonly detected in bobcats than in puma, owing to greater population density and smaller and more distinctive home ranges (Biek et al. 2006*b*, Lee et al. 2012). Bobcat home ranges can be an order of magnitude smaller than puma (Gehrt et al. 2010). Although spatial autocorrelation in pathogen exposure was limited for both species, we did detect evidence more often for bobcats (5 of 48 pathogens) compared with pumas (1 of 48). While increased density is expected to elevate contact rates and disease transmission in some systems (Davis et al. 2014), further research to study intraspecific contacts in puma and bobcats would advance understanding of the mechanisms and rates of pathogen transmission in these hosts (Franklin et al. 2007, Lewis et al. 2015). Further studies examining spatial autocorrelation in pathogen exposure for other carnivore host species across a range of home range sizes and densities, and controlling for other biological differences, would also be valuable to understand whether more general patterns of spatial structure associated with host density or home range size emerge.

Puma and bobcat share most of the pathogens evaluated in the study. Indeed, five of the eight pathogens are considered feline specific, with the remaining three being species specific (FIV) or with a wider host range beyond felines (Bart, *T. gondii*). For this reason, we also evaluated patterns of spatial structure in pathogen exposure for both host species combined. However, doing so did not result in appreciably greater evidence of spatial autocorrelation. The absence of an increase in identification of spatial autocorrelation when assessing both puma and bobcat together could indicate a limited rate of disease transmission between host species, in addition to rapid pathogen dissemination. Recent genetic evidence from FIV supports this interpretation (Lee et al. 2014). It is also likely that the limited spatial autocorrelation of pathogen exposure within species restricted our ability to detect such autocorrelation when combining species.

In the process of testing for clusters of pathogen coexposure, we first evaluated pathogen coexposure between all pairwise combinations of pathogens, finding that relatively few pathogens (9% of bobcat–pathogen combinations and 5% of puma–pathogen combinations) are commonly coexposed with others. However, we do note that for the coexposure relationships that were detected, the majority (92%; 22 of 24) of the pathogen pairs consisted of one or both being viruses. Evaluation of clusters of significant coexposures found little evidence of spatial clustering in pathogen coexposure relationships. Three of the four clustered coexposures were for bobcats with *T. gondii* in VC (a highly restricted site by surrounding urbanization) and two of the four (one for puma and one for bobcat) involve FPV. It is noteworthy that all of the coexposure pathogens with spatial clustering involved pathogens with environmental routes of transmission. Potentially, this may suggest that hotspots of these pathogens can occur in the environment (Afonso et al. 2008, Hampson et al. 2011).

With the exception of the mycoplasmas, this study utilized serological methods to detect pathogen exposure. Serologic surveys for antibody prevalence are an often-used method for unraveling infection dynamics in wildlife, especially as a "first-pass" technique. The utility of serological tests comes from the fact that antibodies to a pathogen typically persist longer in a host than the antigen itself, thereby allowing a better snapshot of infection history in a population. In addition, antibodies are typically easier to detect than antigens, and for pathogens where the antigen is not easily sampled by field techniques (e.g., not present in blood, urine, feces, or mucosal secretions), serologic tests do not require lethal sampling of wildlife (Gilbert

et al. 2013). This is particularly of importance for threatened or endangered species and subspecies, such as the Florida panther subpopulation of puma included in this study. While serologic data therefore provide many practical benefits when studying wildlife pathogens, particularly as a first-pass technique for making initial discoveries and elucidating future directions, we note that this method does not always provide information about active vs. latent or recovered infection, time since original exposure, or genetic information about the pathogen (Munson et al. 2010). Because puma and bobcat are wide-ranging species, and because serological analysis does not provide information about the time-point or original location of exposure, an animal could have been exposed to a pathogen distant from where it was later captured and sampled. To mitigate this limitation, we can evaluate whether there is greater evidence of spatial autocorrelation in study areas with high degrees of habitat fragmentation (FL, VC, and OC) and with short sampling windows (bobcat in FL, SDRC, and WS). Habitat fragmentation can limit dispersal of wide-ranging species, minimizing the range of locations at which an animal was originally exposed (Gehrt et al. 2010). In addition, study areas with a shorter sampling window may give a better "snapshot" of pathogen exposure and be more reflective of the state of spatial autocorrelation. Because spatial autocorrelation was also not significantly evident in study regions that are highly fragmented or had a short sampling window, this further supports our conclusion of limited structure and spatial autocorrelation of pathogen exposure, even with the limitation of serological data.

We have therefore used serological analysis as the first step of assessing infection dynamics in bobcat and puma, which allows us to generate new hypotheses for future work. For example, it would be ideal to conduct a longitudinal study focusing on juvenile individuals in these populations, assessing seroconversion over time in association with habitat use, dispersal, and contact structure. This information would provide powerful information about transmission events in bobcat and puma, but would, of course, be cost and labor intensive. To mitigate these costs, it may be possible to utilize serological data from young or juvenile animals to test for exposure dynamics, thereby limiting the temporal window and refining our results. This work was not possible in the current study due to low sample sizes of young or juvenile individuals, but may be possible if future sampling targets these age classes as priorities for sampling. Another clear future direction of this study is to utilize pathogen genetics to provide information about pathogen relatedness relative to host relatedness and geographic location. This would allow us to refine the scale of our methods for more fine-scale assessments of infection dynamics in these populations.

Here, we assessed patterns of pathogen exposure in puma and bobcats in order to advance our general understanding about the spatial structure and potential spread of pathogens in their populations, with applications to other wildlife, particularly large carnivores. Relatively few studies have examined spatial autocorrelation of pathogen exposure or coexposure, and thus, this study addressed a knowledge gap in epidemiological patterns in wildlife. Our findings suggest that there is limited evidence of spatial structure in pathogen exposure (based on serological and molecular detection) or coexposure clustering for puma and bobcat, even with our extensive sampling for eight different pathogens across six different sites. We conclude that pathogens may disseminate relatively rapidly throughout populations of these felids, precluding substantive detection of spatial structure by routine diagnostic techniques, and justifying a need for more detailed pathogen and host genetic analyses. Ultimately, this study provides insight about the spatial patterns of pathogen exposure in wide-ranging carnivores in multiple locations with varying levels of anthropogenic modification.

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