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Monitoring Seroprevalence of Infectious Diseases in the Florida Panther (*Puma concolor coryi*)

Desiree Walton,[1,7] Marie Gilbertson,[2] Mark Cunningham,[3] Dave Onorato,[4] Joshua Ringer,[3,5] and Meggan Craft[6]

[1] Department of Fisheries, Wildlife, and Conservation Biology, University of Minnesota, 2003 Upper Buford Circle, Saint Paul, Minnesota 55108, USA
[2] Wisconsin Cooperative Wildlife Research Unit, Department of Forest and Wildlife Ecology, University of Wisconsin–Madison, 1630 Linden Dr., Madison, Wisconsin 53706, USA
[3] Fish and Wildlife Research Institute, Florida Fish and Wildlife Conservation Commission, 1105 SW Williston Road, Gainesville, Florida 32601, USA
[4] Fish and Wildlife Research Institute, Florida Fish and Wildlife Conservation Commission, 298 Sabal Palm Road, Naples, Florida 34114, USA
[5] Foothills Wildlife Research Facility, Colorado Parks and Wildlife, 4330 Laporte Avenue, Fort Collins, Colorado 80521, USA
[6] Department of Ecology, Evolution, and Behavior, University of Minnesota, 1987 Upper Buford Circle, St. Paul, Minnesota 55108, USA
[7] Corresponding author (email: walton.dj27@gmail.com)

ABSTRACT: Infectious diseases can have detrimental effects on wildlife populations, particularly those that persist at small sizes, have low genetic diversity, and are affected by fragmented habitat. One such example is the endangered Florida panther (*Puma concolor coryi*), which has been intensively managed since the early 1980s, with the current population ranging between 120 and 230 individuals. For more than three decades, panthers have been captured, demographics recorded, and blood samples collected to monitor for multiple infectious diseases; however, an updated comprehensive study of many of these pathogens has not occurred since 1991. Our goal was to identify temporal patterns and spatial clustering in seroprevalence; determine if the pathogens of interest tend to co-occur; and describe relationships between an individual's genetic assignment (admixed or canonical) and seropositivity. We analyzed serology data for eight pathogens representing different modes of transmission (direct, indirect, vector borne) and infection duration (acute, chronic) from 232 panthers collected between 1992 and 2017. Panthers held consistently high seropositivity for feline calicivirus (62.3%) and panleukopenia virus (79.7%) throughout the study, whereas feline herpesvirus and feline leukemia virus were at lower prevalence (3.1% and 2.4%, respectively), although neither had been noted prior to 1992. Panthers were frequently seropositive for canine distemper virus and feline immunodeficiency virus, and seroprevalence fluctuated through time. West Nile virus seropositivity increased over the study period following its introduction in North America in 1999. Panthers were consistently negative for feline coronavirus, which causes feline infectious peritonitis. Genetics and demographics (sex and age) had little influence on serostatus, and coexposure among pathogens did not tend to occur. Both feline immunodeficiency virus and feline leukemia virus appeared to have spatial clusters of seropositive individuals. Our findings enhance the understanding of pathogen exposure in panthers, informing and supporting ongoing surveillance efforts for timely detection and management of potential disease threats in vulnerable populations.

Key words: Conservation, epidemiology, felids, serosurvey, wildlife health.

INTRODUCTION

Infectious disease monitoring in wildlife populations is an important aspect of species management plans, particularly for populations that are small and have low genetic diversity. Understanding disease dynamics in wildlife populations can support management strategies for conservation efforts, such as vaccination and treatment (Gilbertson et al. 2016). Seroprevalence, the proportion of individuals with detectable antibodies in serum, is commonly used for disease monitoring because antibodies are easier to detect and persist longer in the blood than do infectious agents (Gilbert et al. 2013). Monitoring seroprevalence may inform management strategies, for example by evaluating spillover risk from domestic animals to wildlife or determining efficacy of vaccinations (Gilbert et al. 2013).

Florida panthers (*Puma concolor coryi*), a subspecies of puma, face threats from habitat

loss, fluctuations in prey populations, human-wildlife interactions, and loss of genetic variation. Their small population size heightens vulnerability to infectious diseases (Roelke et al. 1993a; Cunningham et al. 2008, 2021; Onorato et al. 2010). In 1995, with only about 20 individuals remaining, inbreeding characteristics such as cryptorchidism and atrial septal defects were documented (Roelke et al. 1993b; Johnson et al. 2010). Genetic rescue via the release of female Texas pumas (*P. concolor stanleyana*) in 1995 resulted in an increased population size of 120–230 adult and subadult panthers (McBride et al. 2008; FWC 2021). Additionally, panthers with admixed heritage, <90% pre–genetic-rescue heritage, displayed significant improvements in correlates of inbreeding (e.g., increased percentage normal sperm, decreased frequency of kinked tails) compared with those with canonical heritage, ≥90% pre–genetic rescue heritage; panthers typically descended from the inbred individuals in the 1970s–early 1990s (Johnson et al. 2010; Onorato et al. 2024).

Despite panther health improvements since 1995, infectious diseases remain a concern, as evidenced by the feline leukemia virus (FeLV) outbreaks in 2002–04 and 2010–16, even after a limited vaccination campaign began in 2003 (Cunningham et al. 2008; Chiu et al. 2019). Furthermore, nine documented cases of a novel disorder known as feline leukomyelopathy have occurred since 2017, the cause of which remains unknown (FWC 2023), highlighting the need for ongoing monitoring. Additionally, because of their lack of genetic diversity (Roelke et al. 1993b), canonical panthers may exhibit some level of immunosuppression when compared with admixed panthers. Disease susceptibility differences between ancestral groups still need additional research.

The only comprehensive analysis of seroprevalence focused on Florida panthers (hereafter, panthers) included capture years from 1978 to 1991 (Roelke et al. 1993a). Although various studies have explored the epidemiology and ecology of specific pathogens or diseases, such as FeLV (Cunningham et al. 2008; Chiu et al. 2019), pseudorabies (Cunningham et al. 2021), and feline immunodeficiency virus (FIV; Malmberg et al. 2019; Gilbertson et al. 2022a), none have broadly examined the spatial and temporal patterns of a wide range of pathogens following genetic rescue. Our goal was to provide an update on the seroprevalence of multiple pathogens (Table 1) within the panther population, including panthers sampled pre– and post–genetic rescue. Our objectives were to 1) assess seroprevalence across years and temporal changes while identifying associations between seropositivity and panther demographics; 2) identify possible coexposure among the eight pathogens, including canine distemper virus (CDV), feline calicivirus (FCV), feline herpesvirus (FHV), FIV, feline coronavirus (FCoV, the cause of feline infectious peritonitis), FeLV, feline panleukopenia virus (FPV), and West Nile virus (WNV); 3) determine spatial distribution of seropositivity; and 4) determine if genetic heritage and expression of correlates of inbreeding were associated with higher seroprevalence.

MATERIALS AND METHODS

Panther captures and sample and data collection

The Florida Fish and Wildlife Conservation Commission (FWC) and National Park Service collected samples used in this study during annual live captures in southern Florida, US, from 1992 to 2017. Panther captures (described in McCown et al. 1990; Cunningham et al. 2008; van de Kerk et al. 2019) followed FWC agency guidelines for the immobilization and handling of wild panthers, designed to follow the American Society of Mammalogists' guidelines for the use of wild mammals in research (Sikes and Animal Care and Use Committee of the American Society of Mammalogists 2016). While immobilized, panthers were examined by veterinarians; blood and tissue samples were collected for genetic and health assessments; and a VHF or GPS radio collar was fitted for location monitoring. The presence or absence of a cowlick, cryptorchidism, and/or a kinked tail (hereafter, *correlates of inbreeding*) were also recorded. Age at time of capture was either known because of handling as kittens at natal dens or estimated

TABLE 1. Transmission routes, acute vs. chronic designation, and the diagnostic tests used to test for antibodies and antigens of feline pathogens in Florida panthers (*Puma concolor coryi*) captured in southern Florida, USA, 1992–2017 by the Florida Fish and Wildlife Conservation Commission and the National Park Service. Panthers were tested for canine distemper virus (CDV), feline coronavirus (FCoV; responsible for feline infectious peritonitis [FIP]), feline calicivirus (FCV), feline herpesvirus (FHV), feline immunodeficiency virus (FIV), feline leukemia virus (FeLV), feline panleukopenia virus (FPV), and West Nile virus (WNV).

Pathogen	Mode of transmission[a]	Acute vs. chronic	Diagnostic test
CDV	Direct contact	Acute	Serum neutralization (antibodies)
FCoV (causing FIP)	Direct and indirect contact	Both	ELISA (antibodies)
FCV	Direct contact	Acute	Serum neutralization (antibodies)
FHV	Direct contact	Both	Serum neutralization (antibodies)
FIV	Direct contact	Chronic	Western blot (antibodies)
FeLV	Direct contact	Both	Antigen ELISA (SNAP in field)
FPV	Direct and indirect contact	Acute	Hemagglutination (antibodies)
WNV	Indirect contact (vector borne)	Acute	Serum neutralization (antibodies)

[a] Transmission of CDV is direct; it is considered airborne and transmissible via coughing and sneezing (AVMA 2023). Modes of transmission for FCV, FHV, FIV, and FPV were referenced from Gilbertson et al. (2016). In domestic cats, FCoV is transmissible via the direct fecal-oral route (Hartmann 2005); FeLV is directly transmitted via saliva (bite wounds) but can be transmitted via other bodily secretions such as urine and feces (Cornell University College of Veterinary Medicine 2017). The main transmission route of WNV is via mosquito bites (CDC 2021).

from dental wear and gum recession characteristics (Ashman and Greer 1976). Genetic heritage (*canonical* or *admixed* panthers) was determined using genotypes from microsatellite loci (described in van de Kerk et al. 2019).

Captured panthers of at least 4 mo of age were often vaccinated against FCV, FHV, FPV (Fel-O-Vax PCT, Fort Dodge Animal Health, Fort Dodge, Iowa, USA), and FeLV (Fel-O-Vax Lv-K or Fevaxyn FeLV; Schering-Plough Animal Health Corporation, Omaha, Nebraska, USA; Cunningham et al. 2008). Because vaccination vs. natural infection could not be differentiated, these four pathogens were analyzed only in unvaccinated individuals, generally at their initial capture, prior to vaccination.

Diagnostics

Cornell Animal Diagnostic Laboratories (Cornell University, Ithaca, New York, USA) analyzed all serum samples for eight pathogens of interest (Table 1) between 2000 and 2017. Starting in 2003, Idexx SNAP Feline Combo tests (IDEXX Laboratories, Westbrook, Maine, USA) were used in the field to detect FeLV antigens at capture (Cunningham et al. 2008). Both FeLV antigens and antibodies against FCoV were identified using ELISAs. The FeLV ELISAs specifically detect free group-specific antigens in the bloodstream (Cornell University College of Veterinary Medicine 2017). Serum neutralization assays determined antibody presence in the blood against CDV, FCV, FHV, and WNV (Gauger and Vincent 2014), Exposure to FPV was assessed with a hemagglutination inhibition assay, detecting antibody levels through red blood cell agglutination. The titer value represents the highest dilution at which agglutination is observed (Tankeshwar 2014). Testing for FIV used both ELISA and western blot; we used the western blot test results for analysis because the ELISA was highly sensitive but not very specific (Supplementary Table S1); thus, it is most appropriate as a screening test (as in domestic felines; Little et al. 2020). Additionally, FIV was the only diagnostic test that resulted in equivocal results; because only confident positive results are considered seropositive, we treated equivocal results as negatives for our analysis.

Statistical analysis

To test for relationships between pathogen seropositivity (binary response variable) and predictor variables, we fit generalized linear models with and without random effects for each pathogen in R 4.2.2 (R Core Team 2022). Our predictor variables included age (continuous variable), sex (binary categorical variable), year of capture (random effect), heritage (binary categorical variable), and the presence or absence of any correlates of inbreeding

TABLE 2. Mean pathogen seroprevalence in Florida panthers (*Puma concolor coryi*) from our 1992–2017 study, with 95% confidence intervals (CIs), and with comparison with the 1978–91 mean seroprevalence. Overall seropositivity remained similar for feline panleukopenia virus (FPV), feline calicivirus (FCV), feline immunodeficiency virus (FIV), and feline coronavirus (FCoV). Changes were noted in detection of feline leukemia virus (FeLV) antigens and in and (feline herpesvirus (FHV) seroprevalence. Note that West Nile virus (WNV) and canine distemper virus (CDV) were not tested for before 1992.

Pathogen	Individuals sampled	No. positive	Seroprevalence in this study (%) (95% CI)[a]	Seroprevalence 1978–91 (%)
FPV	133	106	79.9 (72.9–86.5)	78.0
WNV	75	56	74.7 (64.8–84.5)	NA[b]
FCV	129	81	62.3 (54.0–70.6)	56.0
FIV	148	67	45.2 (37.2–53.2)	37.0
CDV	132	51	38.6 (30.3–46.9)	NA
FHV	129	4	3.1 (0.10–6.10)	0
FeLV	208	5	2.4 (0.30–4.40)	0
FCoV	123	0	0 (0)	0

[a] For feline leukemia virus, detection of antigens in serum.
[b] NA = not tested in the 1978–91 study.

(binary categorical variable), all of which we expected a priori to have an effect on seropositivity. We selected models using Akaike information criterion and confirmed logistic regression assumptions (linearity, multicollinearity, etc.) with the "performance" package in R (Lüdecke et al. 2021). However, small sample sizes for some pathogens precluded robust inference with logistic regression (e.g., FeLV, with only five positive cases). In these cases, we performed hypothesis-driven Fisher exact tests for key predictor variables and used Bonferroni corrections when making multiple comparisons. For example, we hypothesized that inbreeding depression would increase susceptibility to FeLV (Petch et al. 2022); therefore, a Fisher test was used to test specifically for a relationship between FeLV cases and the presence or absence of correlates of inbreeding.

Coexposure between the diseases of interest were determined via Fisher exact tests between each pair of pathogens, where the null hypothesis was that the relative proportion of one pathogen is independent of the relative proportion of the other pathogen.

We used SatScan v10.1.1 (Kulldorff 2023) to assess spatial clustering of seropositive panthers for each individual pathogen, treating positive panthers as cases and negatives as controls. Panthers caught multiple times were deemed positive if they tested positive at any capture event; the first positive event was used to calculate a median coordinate location for cluster analysis. Median locations were calculated using location data from radio-collared panthers; specifically, we calculated the median x and y coordinates (Universal Transverse Mercator) for each panther, spanning from the day of capture to 1 yr postcapture. We used a circular window (maximum cluster size=50% of the population at risk) with a Bernoulli model, to identify significant spatial clustering. We ran all spatial clustering tests with multiple different maximum cluster sizes to ensure that results were reliable. Visualization was performed in R using the "sf" package (Pebesma and Bivand 2023).

RESULTS

We evaluated serology results from 232 individual panthers (120 males, 112 females) sampled from 1992 to 2017. Of those panthers with known ancestry, 164/218 (75%) were classified as having admixed heritage and 54/218 (25%) canonical. The mean age at capture was 3.68 yr (SD, 2.68 yr). Supplementary Table S2 summarizes pathogen seroprevalence by age and sex.

Mean seroprevalence for each of the eight pathogens is reported in Table 2 and temporal trends are shown in Figure 1. A mean seroprevalence of 2.4% (95% confidence interval [CI], 0.30–4.40%) for FeLV was noted from five positive individuals sampled between

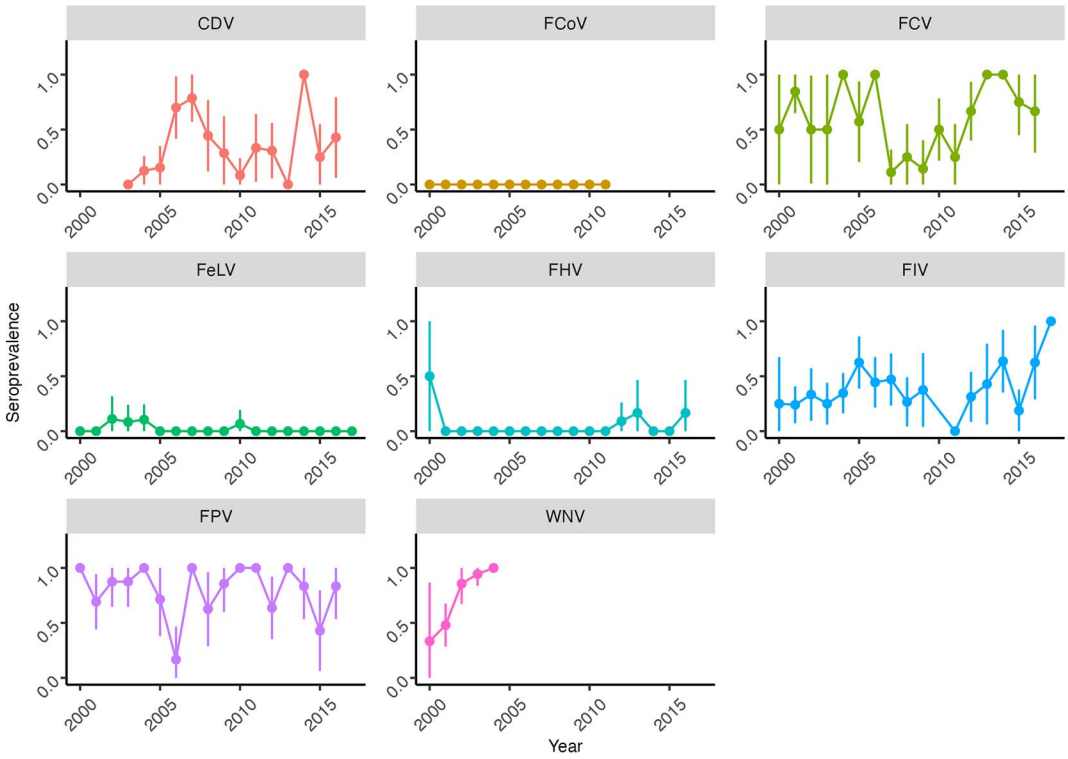

FIGURE 1. Temporal trends in seroprevalence by year for eight pathogens of interest among Florida panthers (*Puma concolor coryi*) in Florida, USA. Samples from 1992–2001 were all negative for feline leukemia virus antigens; results prior to 2000 are therefore omitted here for visualization purposes. Vertical bars on the points are the 95% confidence intervals. CDV = canine distemper virus; FCV = feline calicivirus; FeLV = feline leukemia virus; FHV= feline herpesvirus; FCoV = feline coronavirus, which causes feline infectious peritonitis (FIP); FIV = feline immunodeficiency virus; FPV = feline panleukopenia virus; WNV = West Nile virus.

2002 and 2004. Fisher test results indicated that canonical panthers were more likely to test positive for FeLV than admixed panthers (odds ratio=5.44), as were individuals with correlates of inbreeding (odds ratio=4.31); however, neither of these results achieved statistical significance (heritage: P=0.07; 95% CI, 0.60–67.2; inbreeding correlates: P=0.20; 95% CI, 0.42–215.3). The mean seropositivity for FHV was 3.1% (95% CI, 0.10–6.10%) from four positive individuals; however, seroprevalence was 0% in most years. Because of the lack of cases for FHV, no further analysis was attempted.

We found consistently high FCV, FPV, and WNV mean seroprevalence throughout the study period: FCV 62.3% (95% CI, 54.0–70.6%; seropositive=81), FPV 79.9% (95% CI, 72.9–86.5%; seropositive=106), WNV 74.7% (95% CI, 64.8–84.5%; seropositive=56). Seroprevalence of FCV and FPV fluctuated between years but generally remained high, whereas WNV seroprevalence showed an overall rising trend, starting at approximately 50% when samples were first tested in 2000 and increasing to 100% prevalence in 2004. There were no significant differences in seroprevalence between males and females for FCV, FPV, or WNV. However, the probability of testing positive for FPV increased 1.7 times with each additional year of age (P=0.002). We did not observe any relationship between inbreeding (either heritage or presence of correlates of inbreeding) and seropositivity (Supplementary Tables S3–S5).

Mean seroprevalences for CDV and FIV were 38.6% (95% CI, 30.3–46.9%; seropositive=51) and 45.2% (95% CI, 37.2–53.2%; seropositive=67),

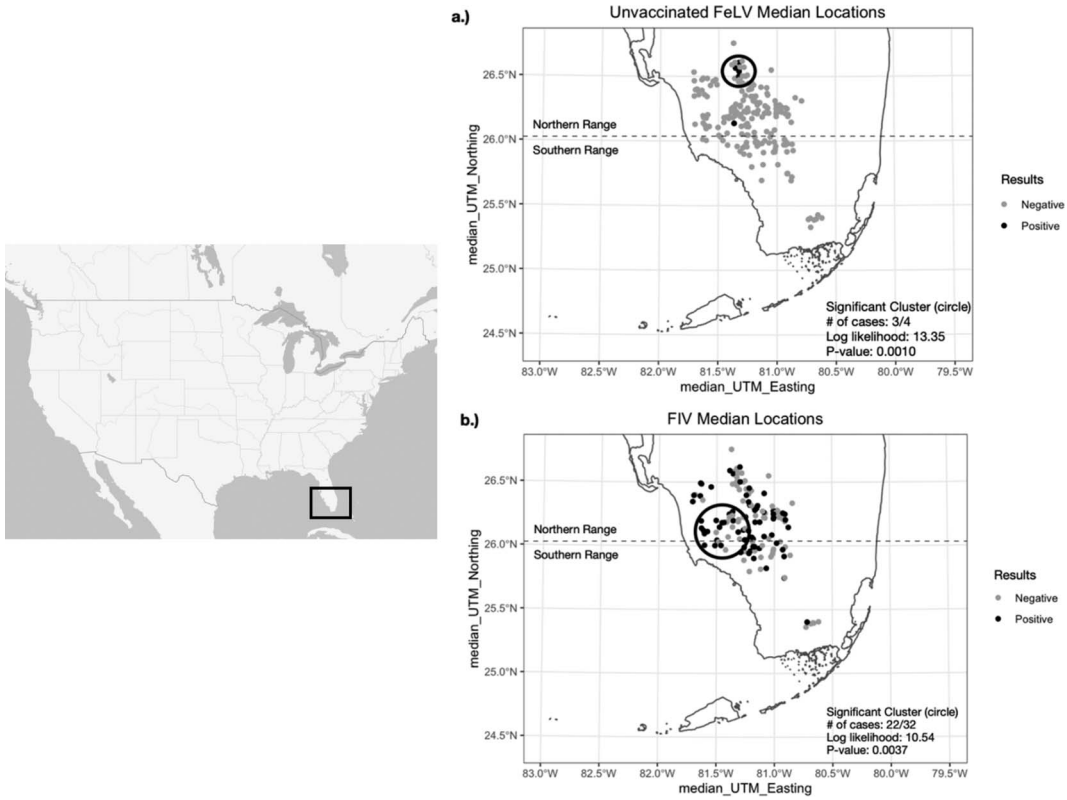

FIGURE 2. Map of southern Florida, USA, showing the calculated median locations (x and y coordinates, Universal Transverse Mercator) using data from radio-collared Florida panthers (*Puma concolor coryi*) tested for (a) feline leukemia virus antigens (FeLV), 1992–97 and (b) feline immunodeficiency virus antibodies (FIV). Gray points represent negative results and black represent positive results, with the significant spatial cluster noted within the black circle. The black dashed line indicates the approximate location of Interstate Highway 75.

respectively. Seroprevalence fluctuated for both, but FIV appeared to have an overall rise in prevalence through time; nevertheless, we did not observe a significant effect of capture year on FIV seropositivity. Our model for CDV found a significant random effect of capture year ($\tau 00=3.09$), suggesting substantial variability in the probability of panthers testing positive for CDV across different years. Additionally, as panther age increased, we observed a 1.25 times higher chance of being seropositive ($P=0.024$; Supplementary Table S6). There was no evidence to support that age and sex influenced seropositivity to FIV (Supplementary Table S7). Seroprevalence for FCoV remained at zero throughout our study period.

The Fisher exact tests conducted between each pair of pathogens suggested that infection with one pathogen did not affect susceptibility to another in the Florida panther population (Supplementary Fig. S1). One spatial cluster of cases was identified for FeLV in the northern part of panther range (Fig. 2a). This statistically significant cluster ($P=0.001$) was characterized by observation of three cases in a radius of 4.3 km, which was 50 times higher than expected. Additionally, FIV showed a significant spatial cluster of cases along Highway 75, which bisects the northern and southern portions of primary panther range (Fig. 2b). This significant cluster ($P=0.003$) included 22 cases in a radius of 23.8 km, which was two times higher than expected. For all other pathogens, seroprevalence appeared to be spatially random.

DISCUSSION

Our study found evidence that exposure to FCV and FPV was consistently high, exposure to WNV rose from low prevalence to high prevalence in a matter of a few years, and CDV and FIV exposure fluctuated. Conversely, FeLV, FHV, and FCoV exposures were rare or absent. Age, sex, genetic heritage, and correlates of inbreeding were infrequently associated with seropositivity for various pathogens. No significant associations between pairs of pathogens were detected, and only FeLV and FIV showed significant spatial clustering.

Seroprevalence patterns and influential factors

The seroprevalence dynamics in this study offer insights into infectious diseases within the panther population, revealing potential endemic and epidemic patterns. We defined *endemic* as a pathogen that remains at a relatively high and consistent seroprevalence through time and an *epidemic* pathogen as one that is characterized by alternating periods of high and low seroprevalence. Both FCV and FPV displayed generally consistent prevalence throughout the study period, which is consistent with expectations for endemic diseases. We also determined that seroprevalence of FCV and FPV have remained relatively stable within the panther population since initial screening in the late 1980s and early 1990s (Roelke et al. 1993a).

We observed low detection of FeLV throughout our study period, with periodic influxes indicative of an epidemic pathogen. Notably, earlier studies did not detect FeLV (Roelke et al. 1993a). Unlike the other pathogens in our study, for FeLV infection detection we relied on an ELISA antigen, not antibody, test. In domestic cats this detects progressive infections but fails to detect abortive and possibly regressive infections (Hartmann 2012). Regressive infections can be detected by PCR testing, and both abortive and regressive infections with antibody testing; however, these methods were absent from our dataset. Progressive infections are expected to result in death in panthers; because we lacked postmortem data, our detection of FeLV in the panther population is likely to be an underestimate, especially compared with studies using PCR and postmortem analyses (Chiu et al. 2019). The increase and fluctuation of FeLV in our study does align with the 2002–04 outbreak in Florida panthers, suggesting transmission from domestic cats to panthers (Brown et al. 2008) followed by panther-to-panther transmission (Cunningham et al. 2008). The near absence of FeLV after vaccination efforts might indicate the effectiveness of these strategies, although the relative rarity of spillover events and the high pathogenicity in panthers play important roles (Cunningham et al. 2008). Nevertheless, recent research (Chiu et al. 2019) identified multiple FeLV strains in the panther population and an increase in FeLV-positive panthers after 2010 (detected by PCR). This is probably the result of higher panther densities, especially along the urban-wildlife interface, and a growing human and panther population. Efficacy of FeLV vaccine against these strains is unknown (Gilbertson et al. 2022b); therefore, multiple strains might introduce more complexities with these current management strategies. Given the presence of multiple strains, epidemic dynamics, and increased exposure, FeLV remains a significant concern for the contemporary panther population, necessitating continued monitoring and evaluation of vaccination efforts (Chiu et al. 2019; Gilbertson et al. 2022b; Petch et al. 2022).

Although FHV seroprevalence also fluctuated through time, there was only one positive case noted in each year a positive test was established. Therefore, this pathogen is unlikely to be acting as an epidemic or endemic disease. Instead, intermittent positives may be related to low circulating prevalence or false positives during testing, particularly because small sample sizes in previous studies noted no seroprevalence of FHV (Roelke et al. 1993a).

Antibodies against CDV also displayed temporal trends consistent with epidemic dynamics. Temporally varying seroprevalence might be related to prey consumption (e.g., raccoons,

Procyon lotor) that have been known to experience epidemic-like temporal trends of CDV prevalence (Taylor et al. 2021). Following genetic restoration, panther diets have diversified from primarily wild hog (*Sus scrofa*) and white-tailed deer (*Odocoileus virginianus*) to include more meso-mammals (Caudill et al. 2019). This shift may influence the prevalence of diseases such as CDV, which occur frequently in prey populations. Canine distemper virus has caused clinical illness, including respiratory, gastrointestinal, central nervous system disease, and death, in populations of *Panthera* spp.; however, few studies have examined CDV in *Puma* spp. Historically, panthers have not succumbed to CDV infections but have exhibited minor respiratory or gastrointestinal signs (Deem et al. 2000). Reportedly, CDV exposure is low in California and Utah panthers (Foley et al. 2013; Roug et al. 2023); given the high fatality rate in canids and mustelids, this low seroprevalence might be due to disease-induced mortality (Foley et al. 2013). Given the vulnerable status of the Florida panther population, its proximity to urban areas (Caudill et al. 2019; Chiu et al. 2019), and the subsequent increase in consumption of prey that can serve as hosts for CDV (Caudill et al. 2019), future studies should continue to monitor panthers for clinical illness or mortality from CDV infections.

Both FIV and WNV showed increasing trends over time. Although tested for only 5 yr, WNV seroprevalence aligns with its introduction to Florida in 1999, suggesting the establishment of a new endemic pathogen. Further testing is required to confirm this hypothesis, such as testing archived samples from before 1999, and testing recent samples for contemporary seroprevalence. Consistent with Malmberg et al. (2019), seroprevalence of FIV displayed slight fluctuations over time, but overall trends indicate an ongoing increase in prevalence of this disease in the population. This is also supported by the higher mean prevalence in our study compared with initial testing in 1978–91. Although prevalence was relatively high and rising during our study, FIV has not been known to cause clinical illness in this species (Carver et al. 2016). Nevertheless, a new FIV strain, probably introduced by Texas pumas during genetic restoration, rapidly spread through the population (Malmberg et al. 2019). Despite the apparent lack of significant clinical disease in this population, its increasing prevalence underscores the risk of disease spread from wildlife translocation (Malmberg et al. 2019).

Age, sex, and annual variation were rarely, if ever, associated with seropositivity. Age and sex are often significant predictors of FIV exposure in *P. concolor*, with males and older individuals showing higher seroprevalence (Carver et al. 2016; Reynolds et al. 2019; Fountain-Jones et al. 2022; Gilbertson et al. 2022a). However, these relationships vary across populations. For instance, sex was predictive of FIV exposure in California pumas but not in Colorado or Florida (Carver et al. 2016). In addition, older Florida panthers have been linked to increased FIV exposure or transmission in some studies (Carver et al. 2016; Gilbertson et al. 2022a), whereas others found that younger male panthers had a higher FIV force of infection (the rate at which susceptible individuals acquire infection; Reynolds et al. 2019). These findings suggest that FIV exposure risk is nuanced and complex, and perhaps it is unsurprising that we failed to detect age and sex as predictors with our relatively small sample sizes. Age was positively related to seropositivity for FPV, which is consistent with findings in Roelke et al. (1993a), which noted that panther kittens under 6 mo old may be at risk for high mortality rates (similar to domestic kittens). Therefore, it may be that panther kittens more frequently succumb to FPV than adults, with the observed high adult seropositivity linked to lifelong immunity or continuous exposure in the environment (Roelke et al. 1993a). Seroprevalence of CDV increased with age, as expected for a morbillivirus such as distemper with long-lasting immunity. We might expect that the longer the panther is on the landscape, the more likely it is to be exposed to CDV, resulting in increased

seropositivity as panthers age. Conversely, research on California mountain lions (*P. concolor*) found no significant associations between CDV seroprevalence and age (Foley et al. 2013).

Coexposure of pathogens

Reduced fitness from endemic infections, as seen with FIV in domestic cats and lions (*Panthera leo*), can increase susceptibility to other pathogens (Roelke et al. 2009). We did not find significant pairwise relationships, which is in agreement with previous studies (Gilbertson et al. 2016; Reynolds et al. 2019). Nevertheless, the lack of detected coexposure relationships does not necessarily preclude immune impacts of infection, but may add evidence to support limited clinical impact of FIV in panthers.

Spatial clustering of seropositive individuals

Our detection of significant spatial clustering of FeLV and FIV cases is supported by previous findings where weak clustering or spatial structuring has been noted for both pathogens (Chiu et al. 2019; Gilbertson et al. 2022a). Panther habitat is divided into northern and southern regions by Highway 75, with the northern section being closer to human activity (i.e., east of Fort Myers and areas north of public conservation lands). Exposure to FIV may increase when panthers are farther away from developed urban areas, because of heightened intraspecific conflict (Carver et al. 2016). This virus is directly transmitted and is partly predicted by spatial proximity, meaning that panthers closer together are more likely to spread the virus (Gilbertson et al. 2022a). Our significant cluster of FIV cases, centered on I-75 between the northern and southern ranges, aligns with panther home ranges, supporting intraspecific conflict and spatial structuring. Clustering of FeLV in the northern portion of their habitat is consistent with a documented historical outbreak (Cunningham et al. 2008) and is probably indicative of FeLV spillover from domestic cats, attributed to increased proximity of panthers to human populations. Similar intraspecific transmission patterns are exhibited by FeLV and FIV, potentially explaining why these pathogens displayed spatial clustering, unlike other panther pathogens that appeared randomly distributed.

Canonical vs. admixed and correlates of inbreeding

The panther population, historically constrained as small and isolated with low genetic diversity, has experienced positive impacts from genetic introgression efforts, including population growth and improved overall fitness (Penfold et al. 2022). Admixed panthers, a result of these efforts, have shown higher survival rates compared with canonical panthers (Hostetler et al. 2010; van de Kerk et al. 2019). Nevertheless, concerns persist regarding the introduction of new genetic variation, with potential trade-offs and uncertainties about long-term impacts. For example, there is risk of incorporating deleterious alleles (Ochoa et al. 2022) and of introducing new pathogens or variations of pathogen strains, as with FIV (Malmberg et al. 2019).

We did not find any evidence to suggest that canonical panthers were more susceptible to exposure than admixed panthers or that the presence of inbreeding characteristics were linked to higher susceptibility. Nevertheless, there are multiple studies indicating that genetics influence the overall fitness of the panther (Hostetler et al. 2010, 2013; Johnson et al. 2010; van de Kerk et al. 2019; Penfold et al. 2022). This might suggest that other factors outside of this study (e.g., prey consumption or interactions, environmental conditions) may exert greater influence when determining serostatus. It is important to note that the higher percentage of admixed panthers or small sample sizes in this study may have contributed to this conclusion.

Limitations

Serology testing in wildlife has limitations, as it only indicates exposure through antibody detection, not clinical illness or active infection (Gilbert et al. 2013). Using protocols

designed for domestic animals or livestock may introduce complexities in investigating disease dynamics within wildlife populations (Miller et al. 2006; Stallknecht 2007; Jia et al. 2020). For example, FIV testing in wild felids may produce inconsistent results because of lower antibody levels and strain differences (e.g., puma lentivirus vs. FIV) compared with domestic felids (Miller et al. 2006). These challenges underscore the importance of cautiously interpreting serology results in the context of wildlife research. Despite these challenges, serology studies may provide valuable insights into wildlife disease dynamics, including gathering data on incidence and prevalence to infer temporal or spatial infection dynamics (Gilbert et al. 2013), infection timing (Packer et al. 1999), and force of infection (Reynolds et al. 2019).

Infectious diseases continue to threaten small, isolated populations such as the Florida panther (Roelke et al. 1993a; Cunningham et al. 2008, 2021; Onorato et al. 2010). Continued monitoring is crucial, especially with the ongoing potential for FeLV outbreaks (Chiu et al. 2019) and the emergence of diseases such as CDV, which showed high seroprevalence in our dataset compared with other puma populations (Deem et al. 2000; Foley et al. 2013; Roug et al. 2023). Careful consideration of diagnostic methods, such as combining FeLV PCR, antibody, and antigen testing, may better identify and characterize types of FeLV infections in panthers (Hartmann 2012). A larger dataset would enable further research on how genetic heritage influences susceptibility to infectious diseases, especially for a population limited by small sample size. We recommend future research on waning vaccine immunity, particularly the benefits of revaccinating individuals who may lose protection over time (Gilbertson et al. 2022b). Although we lacked sufficient recapture data, analyzing temporal changes in titer values for recaptured individuals might reveal long-term vaccination effectiveness, informing strategies for panthers and other endangered felids under vaccination management (e.g., Iberian lynx (*Lynx pardinus*); López et al. 2009).

Overall, this study has provided valuable insights into the seroprevalence dynamics of infectious diseases in the panther population, highlighting endemic and epidemic trends. It provides our foundational exploration of seroprevalence for multiple diseases in this endangered species and contributes to understanding the evolving pathogen threats to the panther population over recent decades. Further data collection and analysis are needed to draw definitive conclusions, especially for diseases with limited samples and potential for significant morbidity and mortality, such as FeLV. Our study enhances our understanding of how evolving pathogen threats interact with wildlife populations in their natural habitats and should support informed conservation strategies that consider genetics and the environment, which may ensure the long-term survival of the Florida panther.

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SUPPLEMENTARY MATERIAL

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