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FACTORS ASSOCIATED WITH PATHOGEN SEROPREVALENCE AND INFECTION IN ROCKY MOUNTAIN COUGARS

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ABSTRACT: Serological and genetic material collected over 15 years (1990–2004) from 207 cougars (Puma concolor) in four populations in the Rocky Mountains were examined for evidence of current or prior exposure to feline immunodeficiency virus (FIV), feline parvovirus (FPV), feline coronavirus (FCoV), feline calicivirus (FCV), canine distemper virus (CDV), feline herpesvirus (FHV), and Yersinia pestis. Serologic data were analyzed for annual variation in seroconversions to assess whether these pathogens are epidemic or endemic in cougars, and to determine whether family membership, age, sex, or location influence risk of exposure. FIV and FPV were clearly endemic in the studied populations, whereas exposure to FCoV, FCV, CDV, and Y. pestis was more sporadic. No evidence was found for FHV. Age was the most consistent predictor of increased exposure risk, often with no other important factors emerging. Evidence for transmission within family groups was limited to FIV and FCoV, whereas some indication for host sex affecting exposure probability was found for FIV and Y. pestis. Overall, cougar populations exhibited few differences in terms of pathogen presence and prevalence, suggesting the presence of similar risk factors throughout the study region.

Key words: Canine distemper virus, feline calicivirus, feline coronavirus, feline herpesvirus, feline immunodeficiency virus, feline parvovirus, feline pathogens, Puma concolor, Yersinia pestis.

INTRODUCTION

Infectious diseases have received particular attention in the context of large carnivores, a group that includes many species of conservation concern and that has provided some of the best-documented examples of disease-induced die-offs (Murray et al., 1999; Funk et al., 2001). However, our understanding of disease epidemiology in wild carnivores is still very limited compared to domestic species and captive populations. More effective conservation and management of carnivores clearly requires a better understanding of the natural history of these diseases (Murray et al., 1999; Cleaveland et al., 2002; Haydon et al., 2002).

Cougars (Puma concolor) are the most widely distributed carnivore species in the Americas, occupying a range that stretches from southern Alaska to Patagonia. Among the New World felines they are second in size only to the jaguar (Panthera onca). In North America, cougars have been extirpated from the east almost entirely, but occur in most of the western states and provinces, where numbers are thought to have increased in recent decades, likely as a consequence of regulated hunting and an increased prey base (Logan and Sweanor, 2001). Recovery of western populations has also led to eastward expansions, as evidenced by individual animals recently appearing in several midwestern and eastern states where cougars had not been recorded for decades (Tischendorf and Johnson, 2003). Despite their wide distribution, possibly increasing abundance, and important ecological role,
information about pathogen dynamics in cougars is limited and virtually absent for the Rocky Mountain region.

In the present study, we use extensive serologic and genetic data collected from four cougar (Puma concolor) populations in the central part of the Rocky Mountains to examine patterns of variation in antibody prevalence and exposure risk for six viruses and one bacterial agent. Predictions about the occurrence and dynamics of infectious diseases can be made based on cougar life history, which is similar to that of other solitary large carnivores. Typically, male home ranges overlap with those of several females and contact among individuals is limited to territorial fights, mating, and family groups, consisting of females and their offspring (Logan and Sweanor, 2001). Low contact rates would predict that few infectious diseases are endemic in cougars, whereas family groups should offer greater opportunities for transmission. Based on these predictions, our aim was to determine: 1) which pathogens can be classified as endemic or epidemic in Rocky Mountain cougar populations; 2) whether individuals within family groups share similar serologic status for particular pathogens; and 3) which additional factors (age, sex, and population) predispose individuals to higher risk of exposure.

METHODS

We collected 277 blood samples from 207 cougars from four study sites in Montana and Wyoming (Table 1 and Fig. 1) as part of detailed population studies (Murphy, 1998; Anderson, 2003). The largest number of samples (n=150) came from the Northern Yellowstone ecosystem, involving two consecutive cougar studies, referred to in the following as Yellowstone I (1990–94) and Yellowstone II (1998–2004).

Kittens (<1 yr) and most yearlings (1–2 yr) were aged within one day to one month based on denning behavior of females, denning dates predicted from male–female associations, or like adults (≥2 yr; estimated to the nearest year), based on morphologic characteristics such as pelage and tooth characteristics (Ashman et al., 1983). Among our study sites, sport hunting was the primary source of cougar mortality in the Garnet and Snowy Range, whereas hunting was largely absent in Yellowstone and the Teton populations. None of the sampled cougar populations exhibited conspicuous levels of disease-related or unexplained mortality during the course of the study.

Six viruses and one bacteria, which are considered important in wild and domestic felines (Murray et al., 1999; Packer et al., 1999), were included in this study. Polymerase chain reaction (PCR) using DNA extracted from blood samples was used to test for feline immunodeficiency virus (FIV) infection (Biek et al., 2003). Serologic testing for feline parvovirus (FPV), feline coronavirus (FCoV), feline calicivirus (FCV), canine distemper virus (CDV), and feline herpesvirus (FHV) was performed at the Washington Disease Diagnostic Lab (WADDL) in Pullman, Washington, using assays and criteria from Biek et al. (2002) or at Tufts or Cornell University using equivalent assays. For all viruses, labs yielded similar proportions of positive cases as indicated by a chi-square analysis (P>0.304) and results were pooled for further analysis. All tests for Yersinia pestis were performed at the Wyoming State Diagnostic Laboratory in Laramie, Wyoming as described (Biek et al., 2002).

Because we employed mainly serologic assays, it is most appropriate to interpret positive results as evidence of exposure rather than of past or current infection. The only exception to this was FIV, for which a PCR assay was used that provided evidence for current infection. For consistency however, we use the terms “exposure” throughout this manuscript, even when referring to FIV.

Temporal variation in exposure patterns was examined based on five years of data from the Snowy Range and eleven years in Yellowstone. Because the presence of antibodies in older individuals by itself provides little information about the actual time of exposure, this analysis focused on recent seroconversions only. For each year (1 July–30 June), we estimated the proportion of susceptible individuals that had recently seroconverted and tested for significant deviations in the proportion of new cases among years. Susceptible individuals were defined as those that were either born within the last two years or that had been seronegative for a given pathogen at a previous capture (usually 1–2 yr prior). This analysis was conducted for all pathogens except FIV, which causes chronic infections and is endemic in North American cougars in most areas (Carpenter et al., 1996).
Potential effects of family groups on exposure were examined in two ways. First, we looked at possible transmission between mothers and offspring by testing whether seropositive mothers were more likely to have at least one seropositive offspring in their litters (n = 44 recorded litters). Over 90% of litters were sampled when kittens were ≤4 months so that maternal antibodies were unlikely to be present, based on data from domestic cats (Ueland and Nesse, 1992; MacDonald et al., 2004). Second, we used a binomial distribution to assess correlation in serologic status among littermates by comparing the observed distribution of seropositive offspring in litters with more than one kitten to an expected distribution based on combined prevalence of seropositive kittens and yearlings.

Differences in serologic status among statistical groups (years, family groups) were evaluated using a chi-square or Fisher’s exact test (P < 0.05) with Bonferroni correction for multiple tests. In cases where the number of groups became too large for the exact calculation, or when cells in contingency tables had expected numbers <5, Monte Carlo approximations were employed.

Logistic regression and model selection based on sample size adjusted Akaike’s Information Criterion (AIC$_C$; Burnham and Anderson, 2002; Johnson and Omland, 2004) were used to identify factors that best explained differences in exposure probability (Table 2). Support for a particular factor was determined based on its Akaike weight, $w_i$ (Burnham and Anderson, 2002), which ranges from zero (no support) to one (maximum support relative to other factors considered). Where more than one factor received considerable support (here defined as $w_i$ > 0.4), parameter estimates and variances were derived through model averaging (Burnham and Anderson, 2002), based on all appropriate models within four AIC$_C$ units of the best model. Full details regarding the candidate set of models and the model selection results can be found in a complimentary paper, focusing on the effects of FIV on cougar fitness and pathogen susceptibility (Biek et al., 2006b).

**RESULTS**

Positive serologic or PCR results were found for all selected pathogens except FHV; antibodies to FHV were not detected in any of 158 tested individuals (Fig. 2). Results indicated that all four cougar populations had been exposed to FIV, FPV, and FCoV. Prevalence was consistently high for FPV (antibody prevalence, 58–69%) and FIV (% positive by PCR, 19–50%); all other pathogens had an antibody prevalence of ≤ 28%. We found no seropositive cases of FCV in the Snowy Range, CDV in the Teton Range, or Y. pestis in the Garnet and Teton Ranges. Given low antibody prevalence for these pathogens (average: 9–17%), nondetection due to sampling error is a possibility for small samples (n < 20 for Garnet and Teton Range) but probably does not explain why we did not find antibodies to FCV in the more extensively sampled Snowy Range (n = 58).

Antibody prevalence data do not provide information to determine whether

<table>
<thead>
<tr>
<th>Factor</th>
<th>Number of categories</th>
<th>Categories</th>
<th>Model notation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>3</td>
<td>Kittens: 1–12 mo, yearlings: 13–24 mo, adults: &gt;24 mo</td>
<td>Age(3)</td>
</tr>
<tr>
<td></td>
<td>continuous</td>
<td>Age in years as individual covariate</td>
<td>Age(cov)</td>
</tr>
<tr>
<td>Sex</td>
<td>2</td>
<td>male, female</td>
<td>Sex</td>
</tr>
<tr>
<td>Population</td>
<td>5</td>
<td>Garnet Range, Yellowstone I, Yellowstone II, Teton Range, Snowy Range</td>
<td>Pop(5)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Garnet Range, Yellowstone, Teton Range, Snowy Range</td>
<td>Pop(4)</td>
</tr>
</tbody>
</table>
cougars came into contact with a pathogen at a constant basis or whether contact was sporadic. For two populations with long-term data, Yellowstone and Snowy Range, we therefore estimated the annual proportion of susceptibles that seroconverted. Seroconversions for FPV were observed in most years; however, the proportion of new cases tended to decrease following years of high incidence (Fig. 2a). New cases in most years were also characteristic for FCoV in Yellowstone, but not for the Snowy Range, where seroconversions were only detected in one out of six years (Fig. 2b). Exposure to CDV, FCV, and \( Y. pestis \) was sporadic, and noticeable only for short periods of time (Fig. 3a–c). Apart from FCV in Yellowstone, differences in the number of seroconversions were not significant among years after correcting for multiple tests (all \( P > 0.123 \)).

Among mothers and their offspring, antibody test results for FCoV were significantly correlated (\( P < 0.001 \)). All FIV-infected kittens were offspring of infected mothers (\( P < 0.001 \)). For all remaining pathogens, there was no correlation between serologic status of mothers and offspring (all \( P > 0.091 \)). Antibody test results for littermates were not significantly correlated for any of the pathogens.

All best models for explaining exposure risk contained “age” as a factor, either in the form of age as a continuous variable (FIV, CDV, FCV, \( Y. pestis \); Table 2) or as age groups (FPV, FCoV; Table 3). Differences in exposure risk among populations were supported for FIV (Table 2) and FCoV (Table 3). In the case of FIV, a strong difference for males and females was apparent in the Yellowstone population, where exposure probability rose quickly for males but much less so for females (Fig. 4b). In comparison, exposure probability increased in a much more similar fashion for both sexes in other populations (Fig. 4a, c, d). There was also some indication for differences in exposure risk between males and females for \( Y. pestis \) but model support for this effect was weak (\( w_i = 0.42 \), Table 2).

**DISCUSSION**

High FPV prevalence, also reported from cougar populations in California and Florida (Roelke et al., 1993; Paul-Murphy et al., 1994), likely reflects the virus’ transmission mode. This virus remains infectious in the environment for months and exposure occurs through contact with feces of virus-shedding individuals (Barker and Parrish, 2001). Indirect transmission can therefore account for high levels of exposure even in a solitary species. Observed seroconversions for FPV in most years (Fig. 2a) further support that this virus is endemic in cougar populations. The fluctuations in the frequency of new cases are thereby suggestive of a cyclic pattern of FPV exposure, possibly associated with the changing proportion of susceptibles over time (Packer et al., 1999).

Despite its lability in the environment, FIV can reach higher prevalence levels by causing persistent infections and by ex-
ploiting the close association of mothers and kittens for frequent vertical transmission. All other pathogens evaluated in this study cause acute infections, have short infectious periods outside the host (hours or days), and require direct or close contact for transmission. Their lower seroprevalence might thus reflect the relative infrequency of cougar-to-cougar contact. Although FCoV appeared to be endemic in Yellowstone, based on frequent seroconversions (Fig. 2b), antibodies were observed too infrequently to considered FCoV endemic in the Snowy Range. The dynamics of FCoV infections could be complicated by the occasional occurrence of persistently infected individuals (Kennedy et al., 2001), which would provide a mechanism for the virus to persist independently of the number of susceptibles.

The sporadic nature of CDV, FCV, and *Y. pestis* in these populations suggests that these pathogens are either maintained in other host species or are able to persist as a metapopulation, where the pathogen might be locally absent from some cougar populations but not from all simultaneous-

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### Table 2. Model selection results and parameter estimates of pathogen exposure probability in cougars. Shown are four pathogens for which probability of exposure increased continuously with age. Only factors supported by Akaike weights (*w*<sub>i</sub>) >0.4 are shown. Also shown are the best model and its AIC<sub>C</sub> difference from the next best model (∆<sub>i</sub> next). See Methods for further details and Table 1 for factors included in candidate models.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Factor</th>
<th><em>w</em>&lt;sub&gt;i&lt;/sub&gt;</th>
<th>Best model</th>
<th>∆&lt;sub&gt;i&lt;/sub&gt; next</th>
<th>Odds ratio of exposure probability per year of age (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIV&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Age</td>
<td>1.00</td>
<td>Age(cov)×&lt;sub&gt;Sex&lt;/sub&gt;Pop(4)×</td>
<td>3.02</td>
<td>GR: Males 1.91 (0.36–10.03), Females 2.46 (0.91–6.66)&lt;sup&gt;b&lt;/sup&gt;, YE: Males 8.04 (1.99–32.51), Females 1.32 (1.07–1.63)</td>
</tr>
<tr>
<td></td>
<td>Population</td>
<td>0.99</td>
<td>Sex</td>
<td></td>
<td>TR: Males 1.45 (0.78–2.68), Females 1.42 (0.87–2.33), SR: Males 1.62 (0.84–3.13), Females 1.86 (1.14–3.05)</td>
</tr>
<tr>
<td></td>
<td>Sex</td>
<td>0.74</td>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDV</td>
<td>Age</td>
<td>0.99</td>
<td>Age(cov)</td>
<td>1.49</td>
<td>1.36 (1.15–1.60)</td>
</tr>
<tr>
<td>FCV</td>
<td>Age</td>
<td>0.98</td>
<td>Age(cov)</td>
<td>1.95</td>
<td>1.24 (1.07–1.43)</td>
</tr>
<tr>
<td><em>Y. pestis</em></td>
<td>Age</td>
<td>0.99</td>
<td>Age(3)</td>
<td>0.75</td>
<td>Males 0.88 (0.43–1.76), Females 1.16 (0.98–1.38)</td>
</tr>
<tr>
<td></td>
<td>Sex</td>
<td>0.42</td>
<td>Sex</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Because PCR assay was used for detection of FIV, exposure in this case represents actual infection.

<sup>b</sup> GR = Garnet Range, YE = Yellowstone, TR = Teton Range, SR = Snowy Range.
in their offspring. This could be due to frequent transmission among individuals within family groups or due to spatial autocorrelation (a higher risk resulting from time spent in the same area). The short infective period for coronavirus outside the host makes exposure from direct contact among individuals more likely. For FIV, all positive kittens were associated with infected mothers, consistent with infection through vertical transmission (Biek et al., 2003).

That serologic status of littermates was uncorrelated for all pathogens tested was surprising, given that littermates lived in close proximity for several months and thus likely were nonindependent in their history of pathogen exposure. In addition to sampling issues (kittens from the same litter were not always sampled at the same time), occasional persistence of maternal antibodies might have obscured such relationships.

Much of the variation in exposure among cougars could be explained by age (Table 2, Table 3). Age increases the probability of experiencing a local outbreak in the case of epidemic pathogens or might correlate with entering new life stages that are associated with higher exposure risk due to physiologic or behavioral changes. For example, FPV exposure probability more than doubled from about 0.42 for kittens and yearlings to 0.88 for adults (Table 3).

We found a much higher predisposition of males to FIV exposure in Yellowstone (Fig. 4b) but this strong bias was not
repeated in other populations (Fig. 4a, c, d; see below). Among the remaining pathogens, only *Y. pestis* showed some model support for an effect of sex \((w_i = 0.42; \text{Table 2})\). Closer examination revealed that only two of nineteen individuals positive for plague were males, aged 1.6 and 2.0 yr. Consequently, the estimated increase in exposure probability with age only pertained to female cougars, 35% of which were plague positive as adults. Logan and Sweanor (2001) report three confirmed deaths, all females, caused by plague in a New Mexico population. Higher exposure risk in females could reflect a larger reliance on small prey compared to males, but we are unaware of any data to support or refute this.

Support for an effect of location on exposure risk was only found for FCoV and FIV. For the former, the evidence was in fact weak, given that confidence intervals for all populations were overlapping widely (Table 3). Spatial differences were more pronounced for FIV, largely due to exposure probability in Yellowstone rising particularly fast for males but much more slowly in females (Table 2 and Fig. 4). Genetic differences in susceptibility of cougars or transmission ability among virus strains could account for this observation. However, both seem unlikely because cougars throughout the study area show little genetic distinction and genetic population structure in the virus does not correspond to high and low prevalence populations (Anderson et al., 2004; Biek et al., 2006a).

**Table 3.** Model selection results and parameter estimates of pathogen exposure probability in cougars. Shown are two pathogens for which probability of exposure increased with age group (kitten, yearling, adult). See Table 2 for further descriptions.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Factor</th>
<th>(w_i)</th>
<th>Best model</th>
<th>(\Delta_i) next</th>
<th>Exposure probability (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FPV</td>
<td>Age</td>
<td>1.00</td>
<td>Age(3)</td>
<td>4.56</td>
<td>Kit 0.42 (0.34–0.57), Yrl 0.43 (0.28–0.58), Ad 0.88 (0.79–0.93)</td>
</tr>
<tr>
<td>FCoV</td>
<td>Age</td>
<td>0.74</td>
<td>Age(3) + Pop(4)</td>
<td>0.35</td>
<td>GR: Kit 0.04 (0.01–0.16), Yrl 0.05 (0.01–0.19), Ad 0.08 (0.02–0.27)</td>
</tr>
<tr>
<td></td>
<td>Population</td>
<td>0.61</td>
<td></td>
<td></td>
<td>YE: Kit 0.11 (0.04–0.23), Yrl 0.12 (0.05–0.26), Ad 0.20 (0.09–0.37)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TR: Kit 0.08 (0.02–0.25), Yrl 0.09 (0.02–0.32), Ad 0.15 (0.04–0.41)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SR: Kit 0.15 (0.03–0.46), Yrl 0.17 (0.04–0.48), Ad 0.27 (0.10–0.54)</td>
</tr>
</tbody>
</table>

* GR = Garnet Range, YE = Yellowstone, TR = Teton Range, SR = Snowy Range.
Consistent with our predictions for a solitary carnivore, we identified only two pathogens, FIV and FPV, as endemic in cougars. In both cases, high antibody prevalence and continuous presence are probably related to prolonged opportunities for transmission. One of few studies to date that has examined long-term dynamics of infectious diseases in a large carnivore was done in a gregarious species, the African lion (Packer et al., 1999). Discrete epidemics for FPV, FCoV, CDV, and FCV at intervals of 4–12 yr characterized virus dynamics in Serengeti lions, in contrast to the shorter or nonexistent interepidemic intervals our data showed for these pathogens. Higher host contact rates in the group-living lion could be responsible for the more pronounced epidemics, but more comparative studies are needed to separate effects of social organization from other factors, including variability among pathogen strains and different ecological and climatic conditions.

Antibody prevalence was surprisingly similar between cougar populations. This lack of spatial heterogeneity was remarkable not only because it occurred over distances of several hundred kilometers but also because the sampled populations differed in significant parameters such as presence/absence of cougar hunting. It suggests that pathogen presence and transmission in these cougar populations might be driven by certain unifying ecological factors. One such factor could be a comparable suite of mammalian prey and sympatric carnivore species present at each of our four sampling sites. For pathogens shared among multiple mammalian host species, such as CDV and Y. pestis, this could translate into similar levels of exposure in cougars. Considering their low density relative to other potential hosts, cougar numbers probably have little effect on the dynamics of these pathogens which can cause significant mortality in wild carnivore populations (Williams et al., 1994; Roelke Parker et al., 1996).

Aside from FIV, for which there is compelling evidence of a cougar-specific strain (Troyer et al., 2005), it is unclear whether the remaining feline viruses found in Rocky Mountain cougars (FPV,

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**Figure 4.** Estimated FIV exposure probability in relation to age for male and female cougars in four populations: (a) Garnet Range, (b) Yellowstone, (c) Teton Range, and (d) Snowy Range. See Table 2 for parameter estimates and measures of uncertainty.
Infectious diseases of wild mammals include FCoV, FCV, and FPV. These viruses are known to be present in bobcats and Canada lynxes. FCoV and FCV are shared with other species, while FPV is often associated with considerable mortality. The latter virus is currently endemic in cougars. The authors discuss the potential risk of FPV transmission to other species, which could conceivably act as a source of transmission to other species. This could be relevant for species of conservation concern, such as the Canada lynx, for which recovery efforts in the Rocky Mountain region are currently underway.

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