Investigating Pseudorabies Virus as a Mortality Factor for California Mountain Lions

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ABSTRACT: Wild pigs in North America carry multiple pathogens capable of causing diseases in wildlife, people, and domestic animals. Wild pigs are the reservoir host of pseudorabies virus (PrV) which is a fatal infection in wild carnivores. We previously conducted surveillance for PrV in wild pigs from Kern County, California where the distribution of wild pigs overlaps with native mountain lion. We found that 100% of wild pigs were exposed to PrV, and 6% were shedding the virus. Black bears and pumas have been observed preying on wild pigs in the region suggesting that they are vulnerable to exposure to PrV from pigs. We conducted retrospective, post-mortem surveillance for PrV on 16 pumas from five counties in south central California. None of the pumas tested positive for PrV. In Florida, PrV is attributed to one-third of the deaths in Florida panthers. Wild pigs are a large portion of the diet of panthers in Florida, but studies in California suggest pigs may not be utilized as frequently. This difference in diet could account for the lack of evidence that PrV causes measurable mortality in California pumas. Nonetheless, in management areas with carnivores, wild pig management should be carefully considered.

KEY WORDS: Aujeszky's disease, cougar, invasive species, mountain lion, pathogen pollution, *Puma concolor*, *Sus scrofa*

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INTRODUCTION

Invasive species disrupt ecosystems and threaten species with extinction via multiple ecological mechanisms such as predation, competition, alteration of nutrient flow, and resource degradation (Parker et al. 1999). Invasive species can also alter ecosystems via pathogen pollution, whereby they transmit pathogens to naïve faunal communities (Daszak et al. 2000). The introduction and rapid spread of *Yersinia pestis*, the causative agent of plague, by black rats (*Rattus rattus*) brought into the port of San Francisco in 1900 (Biggins and Kosoy 2001), illustrates this process. It then spread through native rodent communities from the west coast to the Great Plains where it has altered population processes of prairie dogs (*Cynomys* spp.) and the black-footed ferret (*Mustela nigripes*). Pathogens caused the extinction of some native Hawaiian birds from avian malaria (*Plasmodium relictum*) (Warner 1968); amphibians from ranavirus (Picco and Collins 2008); and has permanently altered the agro-ecological landscape for livestock and food plant production (Anderson et al. 2004).

Wild pigs (*Sus scrofa*) are not native to North America and can carry more than 30 pathogens known to cause clinical or subclinical illness in other species including livestock, wildlife, companion animals, and humans (Miller et al. 2017). One of these pathogens, pseudorabies virus (PrV), is an easily transmitted alphaherpesvirus that causes little to no clinical illness in adult pigs, but is deadly to unweaned piglets and carnivores (Müller et al. 2011). The virus establishes a lifelong infection in adult pigs (Mettenleiter 2000), with periodic reactivation and shedding of the virus (Capua et al. 1997) that renders the animal infectious to conspecifics and carnivores (King et al. 2012). Thus, wild pig populations have the capacity to negatively impact native carnivores in North America.

PrV is found in pigs throughout North America and, nationwide, the abundance and distribution of wild pigs has increased in recent years and (Wood and Barret 1979). The average prevalence of antibodies to PrV (seroprevalence) in wild pigs is 25% in the US, but seroprevalence is variable among regions and locales (Pedersen et al. 2013). In Florida, the state-wide average seroprevalence was 40% from 2011-2014, but ranged from 0 to 67% among managed populations (Wisely, unpublished data). Pseudorabies virus has been shown to impact the Florida panther (*P. concolor coryi*), a federally endangered species: the third leading cause of panther deaths in Florida was attributed to acute neuropathy associated with PrV (M. Cunningham, pers. comm.).

In California, pseudorabies has been detected in feral pigs in 13 counties (McDougle 2017). A serological study of PrV in a wild pig population in Kern County, California, found that 100% of wild pigs were exposed to PrV and six percent were actively shedding the virus (Wisely et al. 2017). The high prevalence of the virus and sympatric distribution of wild pigs and mountain lions in California suggest that PrV is a potential risk to mountain lion health.

Prior to this investigation, PrV was not considered a possible cause of mortality for California mountain lions (*P. concolor*). Given the high prevalence of PrV in wild pigs in California, and that wild pigs are a potential prey base for mountain lions, we undertook a retrospective study of the seroprevalence of PrV in mountain lion carcasses collected by the California Department of Fish and Wildlife (CDFW) to determine whether PrV is a significant mortality factor for this species in California.

METHODS

Sample Collection

Tissues used in this study were acquired from the CDFW Wildlife Investigations Laboratory, which recently initiated a state-wide mountain lion health surveillance program to assess the mortality and health status of mountain lions throughout California. Lion carcasses were collected by CDFW and included lions taken on depredation permits or other mortalities such as vehicle-strikes, poaching, humane euthanasia due to illness, or those found dead. Carcasses were frozen in -28°C freezers until they could be necropsied to determine the cause of death, nutritional status, sex, and age. Tissue samples were archived in -80°C freezers.

We used lung and salivary gland tissue from 16 California mountain lions collected in four counties (Monterey, Santa Barbara, Madera, and Kern Counties) from November 2015 to February 2017. These counties were chosen because seropositive wild pigs had been previously discovered by the United States Department of Agriculture Wildlife Services.

Laboratory Analysis

We extracted viral DNA from lung and salivary gland tissue using the QIAGEN DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) following the manufacturer's instructions modified to maximize the concentration of recovered DNA. Modifications to the protocol included vigorous mixing of the samples prior to extraction and a 15 min incubation period during final DNA elution. The concentration and purity of recovered DNA was quantified using the Epoch Microplate Spectrophotometer running the Gen5 software, version 2.09 (BioTek Instruments, Winooski, VT). Purity was assessed as the ratio of absorbance at 260 and 280 nm. A ratio of 1.8 is considered to have low concentrations of protein which can inhibit PCR. Recovered DNA was stored at -20°C until further processing.

We used primers and a probe targeting the 5' coding region of the PrV glycoprotein B (gB) gene (also known as UL27) in order to detect PrV DNA (Sayler et al. 2017). Assays were run with negative controls (molecular grade water) and extraction controls (i.e., no template controls) to eliminate detection of false positives due to contamination. The PrV-gB qPCR assays were performed on the ABI 7500 fast thermocycler by using Brilliant III Ultra-Fast qPCR Master Mix (Agilent, Santa Clara, CA), with 2 µL of template DNA, 0.4 µL of PrV-gB forward primer at 20 µM, 0.4 µL of PrV-gB reverse primer at 20 µM, and 0.4 µL of PrV-probe at 10 µM. Cycling conditions were set as follows: 95°C for 3 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 30 sec. The cutoff cycle value for this qPCR assay was 35 quantification cycles (Cq), which corresponded to the average number of Cq for the detection of 10 copies of PrV DNA and represented the lower limit of detection of the assay (Sayler et al. 2017). Any Cq values >35 were considered a negative result. Positive qPCR samples were confirmed by triplicating the assay and considered positive if at least two-thirds of the replicates had Cq values ≤35.

RESULTS

The 16 mountain lions were representative of multiple age classes and included two kittens, two yearlings, four subadults, and eight adults. Four animals were females and 12 were males. We successfully extracted DNA from 16 lung and 15 salivary gland samples. DNA concentration averaged 265 ± 57 ng/ul (mean ± SE) for lung and 322 ± 62 ng/ul for salivary gland. The ratio of absorbance ranged from 1.83-1.90 for lung tissue and 1.87-2.01 for salivary gland indicating highly pure DNA.

Of the 31 DNA samples from 16 individuals, two animals tested positive at the threshold of detection for the real time assay (Cq = 35 for both samples) during initial screening. Each positive sample came from a different tissue type (one positive lung sample and one positive salivary gland sample). Upon screening each sample in triplicate, no positive samples were detected. Based on our criteria, we concluded that no animals were positive for PrV DNA in either lung or salivary gland tissue.

DISCUSSION

None of the 16 mountain lions tested were positive for PrV DNA. Although we initially found two samples which were positive at threshold of detection, we could not replicate those results. We thus deemed those two animals as negative for viral DNA in either tissue.

Although PrV is broadly distributed in wild pigs in California in general and in Kern County in particular, we were not able to ascribe PrV as a mortality factor for mountain lions. Several explanations for the negative findings are possible, including inadequate sample size and sample selection (e.g., disease spatial clustering was not considered; some samples came from lethally-controlled animals or degraded carcasses). If the prevalence of disease was low in mountain lions, the probability of detection would also have been low. Spatial heterogeneity in PrV prevalence in pigs, pig density, and mountain lion behavior could all influence the likelihood of a mountain lion exposure to PrV.

Regardless of sampling design, mountain lions may not be exposed to PrV in wild pigs as frequently in California as they are in Florida. Wild pigs comprise an estimated 23-59% of Florida panther diet (Maehr et al. 1990). While diet is likely variable across California ecosystems, in Mendocino County, Allen et al. (2015) showed that mountain lions primarily consume black-tailed deer with no evidence of wild pig in their diet. In Kern County, however, evidence of mountain lion predation of wild pigs has been observed (Eric Covington, pers. comm.). While the present study showed no evidence of PrV in mountain lions, the risk of exposure to the virus is clearly present. For isolated populations of mountain lions that are vulnerable to local extinction, wild pigs in the area should be considered.

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