

THE ROLE OF FERAL MAMMALS ON WILDLIFE INFECTIOUS DISEASE PREVALENCE IN TWO NATURE RESERVES WITHIN MEXICO CITY LIMITS

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Abstract: Wild and feral medium-sized mammals were live trapped at two natural protected areas within the Mexico City limits to determine antibody prevalence for the most common infectious diseases (rabies, toxoplasmosis, and canine parvovirus) in dogs and cats. Mammals were trapped during the dry (March–April) and rainy seasons (July–August) of 1996 and 1997. A total of 68 individuals were captured, representing 8 species: opossums (*Didelphis virginiana*), ringtails (*Bassariscus astutus*), spotted skunks (*Spilogale gracilis*), weasels (*Mustela frenata*), rock squirrels (*Spermophilus variegatus*), Mexican gray squirrels (*Sciurus aureogaster*), feral cats (*Felis catus*), and feral dogs (*Canis familiaris*). There was marked seroprevalence for parvovirus (86.6%) and lower seroprevalences for both toxoplasma (23.9%) and rabies (17.9%). There were no significant prevalence differences among mammals in both protected areas, which were of contrasting size and isolation (i.e., small and isolated versus large and nonisolated). We suggest that high seroprevalence of these three infectious agents in wild mammals is a result of the high densities of feral dogs and cats in the two areas sampled. Feral dogs are able to maintain the infectious agents in these localities regardless of the protected area size and isolation. However, the native mammals of the small and isolated reserve are more vulnerable to infectious diseases because of small population size and genetic bottlenecks. Our results indicate that natural areas in and around Mexico City are a refugium for latent infectious agents, several of which are zoonotic. These findings suggest that conservation measures, such as eradication of feral mammals and vaccination programs, in the protected areas and surrounding areas could be beneficial.

Key words: Feral, mammals, Mexico, parvovirus, rabies, *Toxoplasma*.

INTRODUCTION

One of the most important causes of the decline of mammal populations is habitat fragmentation. There are a plethora of studies demonstrating both direct and indirect negative effects of habitat loss and fragmentation on mammal species in particular and wildlife in general.^{4,7,8,20} Current ecological and epidemiological theory predicts that native wild mammal species in smaller, fragmented, and isolated habitats are more likely to interact with invasive species and infectious diseases.^{8,9} Invasive species (e.g., feral mammals and exotic species) have been favored in shrinking ecosystems, their tolerance to different habitats and the absence of predators make them successful species to dominate the community. Due to their generalist nature, feral mammals are able to survive in an extremely wide array of relatively pristine and human-dominated ecosystems. They are able to restructure food webs and endanger endemic fauna. Cats, dogs, rats, and goats, among others, are carriers of multiple para-

sites and diseases and have contributed to the demise of native species throughout the world.^{3,4,15,16,21,28–30}

Along with invasive species, infectious diseases are becoming a major threat to wildlife in protected areas. One prime example of disease nearly decimating a wildlife population was canine distemper in the black-footed ferret (*Mustela nigripes*).^{27,32} Other examples include distemper in lions (*Panthera leo*) in the Serengeti¹⁴ and mycoplasma in the desert tortoise (*Gopherus agassizii*).¹¹ However, relatively few field studies have been undertaken to evaluate the relationship between shrinking ecosystems, invasive species, native species, and disease in Mexico.²⁶ Accordingly, the objective of this study was to evaluate seroprevalence of the three most common infectious diseases in stray dogs and cats in two Mexico City protected areas. We chose three infectious diseases (rabies, toxoplasmosis, and parvovirus) that have been shown to have a high degree of horizontal transmission, are prevalent in Mexico's stray dogs and cats, and are known to infect local wild mammals. These are endemic infectious diseases among domestic dogs and cats in Mexico City, although their prevalence in wild mammals in urban and suburban areas is unknown. Our objectives were to determine the seroprevalence for rabies, toxoplasmosis, and parvovirus in mammals in the two protected areas that differ in

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size and the degree of isolation and to provide disease management counseling aimed at maintaining the native mammals in both protected areas.

MATERIALS AND METHODS

The study sites were two reserves, El Pedregal Ecological Reserve (Pedregal) and the Desierto de los Leones National Park (Desierto), both found within the Mexico City limits, and differing in size and isolation. The Pedregal is located in the southern part of Mexico City and has been subject to long-term disturbance (fragmentation and isolation), which has had a decisive effect on the structure and composition of the endemic fauna. It is an isolated vegetation fragment of 160 ha with no natural corridors. In contrast, the Desierto (1867 ha), which encompasses a broad area of the Sierra de las Cruces, is connected to other protected areas by several corridors of natural vegetation. Both areas face serious conservation threats, including garbage pollution and introduced exotics (rats, mice, cats, and dogs), which can serve as vectors as well as reservoirs for diseases and parasites.

Mammals were captured using Tomahawk live traps (collapsible single door 20" × 7" × 7" Tomahawk Live Trap Co., Tomahawk, WI 54487, USA). This trap size is good for skunks, ringtails, and small feral dogs. Traps were baited with sardines and fruit, such as bananas and oranges, along two linear transects separated by 400 m. Fifteen traps were set on each transect, with 30 m between traps. All traps were baited just prior to sunset and checked early in the morning. Four trapping periods were established. There were 16 days in each trapping period, which were evenly divided between the Pedregal and Desierto. Two periods were in the dry season and two were in the rainy season for both 1996 and 1997.

All captured animals were immobilized with an intramuscular injection of a combination of a tranquilizer and a dissociative anesthetic (Xylazine 1 mg/kg Rompun, Bayer, Mexico City, Mexico; ketamine 10 mg/kg, Anesket, Pisa, Atitalaquia, Hgo., Mexico). A 5-ml blood sample was taken from the cephalic vein of each individual. Prior to release at each site of capture, the animals were ear tagged.

Blood samples were centrifuged within 24 hr of collection and serum was stored between -5°C and -20°C for further serum analyses in the Virology and Serology Laboratory, Veterinary Medicine School, National Autonomous University of Mexico (UNAM). Rapid fluorescent focus inhibition for rabies antibodies,²⁴ complement fixation for *Toxoplasma gondii* antibodies, and hemagglutination in-

hibition for canine parvovirus antibodies²² were performed.

The Shannon diversity index was used to compare the diversity between the two reserves, and the Sorensen index was used to compare composition and similarity between communities. Kruskal-Wallis and chi square (χ^2) statistical tests were used to compare seroprevalence between areas and seasonal prevalence. None of the 17 recaptures were included in the statistical analyses.

RESULTS

A total of 68 medium-sized mammals representing eight species, six families, and three orders were captured; these included 29 opossums (*Didelphis virginiana*), 20 ringtails (*Bassariscus astutus*), 6 six spotted skunks (*Spilogale gracilis*), one weasel (*Mustela frenata*), two rock squirrels (*Spermophilus variegatus*), one Mexican Gray squirrel (*Sciurus aureogaster*), six feral cats (*Felis catus*), and three feral dogs (*Canis familiaris*) (Table 1). Seventy-four percent of the animals were captured in the Pedregal, and the remaining 26% in the Desierto. Trapping success (total captured individuals/total of trap nights) was statistically greater in the Pedregal (6.7%) than in the Desierto (2%) ($\chi^2 = 24.5$, $P < 0.0001$).

Fifty animals representing six species were captured in Pedregal, while only 18 individuals representing six species were captured in Desierto. Using the Shannon diversity index, the Pedregal had higher mammalian diversity ($H' = 1.38$, $J = 0.77$) than Desierto ($H' = 0.834$, $J = 0.46$). There was no significant difference in species diversity between area size and isolation ($P = 0.22$). The two reserves had a 66% similarity in species composition (Sorensen index).

Sixty-seven blood samples were tested; one blood sample from *Sciurus aureogaster* was inadequate to be studied and it was not included in the prevalence analysis. Parvovirus was the most seroprevalent in both wild and feral mammals, with 86.6% of individuals antibody positive. The overall seroprevalence for toxoplasmosis was 23.9%, and rabies 17.9% (Fig. 1). Comparisons of seroprevalence between species were conducted. Feral cats and ringtails had the highest parvovirus seroprevalences. The highest rabies antibody response was detected in rock squirrels, followed by cats, dogs, and skunks, respectively (Fig. 2). Similar trends were observed for toxoplasmosis in which cats, dogs, and spotted skunks were the species with statistically significant highest seroprevalence (ANOVA $F = 4.77$, $P < 0.001$). All ringtails and feral cats showed parvovirus antibodies (Fig. 2). Long-

Table 1. Species and number captured (no. seropositive) of medium-sized mammals from two reserves in Mexico City, 1996 and 1997.^a

Species	Toxoplasma			Parvovirus			Rabies		
	Ped.	Des.	Total	Ped.	Des.	Total	Ped.	Des.	Total
<i>Didelphis virginiana</i>	25 (3)	4	29 (3)	25 (20)	4 (4)	29 (24)	25 (4)	4	29 (4)
<i>Spermophilus variegatus</i>	2		2	2 (1)		2 (1)	2 (1)		2 (1)
<i>Sciurus aureogaster</i> ^b									
<i>Bassariscus astutus</i>	11 (2)	9 (2)	20 (4)	11 (11)	9 (9)	20 (20)	11	9 (2)	20 (2)
<i>Spilogale gracilis</i>	6 (3)		6 (3)			6 (5)	6 (2)		6 (2)
<i>Mustela frenata</i>		1	1		1	1		1	1
<i>Felis catus</i>	4 (2)	2 (2)	6 (4)	4 (4)	2 (2)	6 (6)	4 (1)	2 (1)	6 (2)
<i>Canis familiaris</i>	2 (1)	1 (1)	3 (2)	2 (1)	1 (1)	3 (2)	2 (1)	1	3 (1)
Total	50 (11)	17 (5)	67 (16)	50 (42)	17 (16)	67 (58)	50 (9)	17 (3)	67 (12)

^a Pedregal (Ped), Desierto (Des). Values in parentheses represent the number of seropositive individuals.

^b Not done.

tailed weasel (*M. frenata*) did not show antibodies against any of the diseases (Fig. 2).

Feral dogs and cats presented high antibody titers for the three infectious agents combined; nevertheless, there were also differences among diseases and there were some differences between feral and wild mammals. Feral mammals had higher antibody titers for toxoplasmosis and rabies than wild mammals (Fig. 3); however, only toxoplasmosis seroprevalence was statistically higher ($P \leq 0.002$). Parvovirus was slightly higher in wild mammals but not significantly so ($P = 0.50$).

Interestingly, there was no significant difference in antibody response between the two reserves, despite their striking differences in size and isolation (toxoplasmosis $P = 0.535$, rabies $P = 0.97$, parvovirus $P < 0.25$). Contrary to what we expected, overall antibody prevalence in Desierto (94%) was higher than in Pedregal (84%).

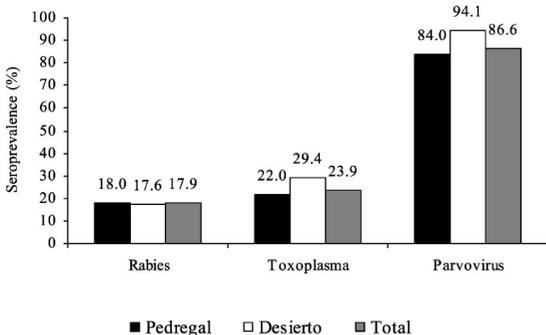


Figure 1. Overall seroprevalence.

DISCUSSION

Consequences of high antibody response to the three infectious agents on wild mammals are still unpredictable; however, we consider feral mammals the main disease reservoirs in both reserves. The high rabies antibody response found in this study may harbor important epidemiological concerns for either human or animal health. Rabies pathogenesis remains unclear in natural populations and the differences in virus properties among host species in wild populations may lead to different outcomes than those found in domestic animals. The results of this study demonstrate that a portion of unvaccinated wild and feral mammals had detectable levels of rabies antibodies. Some studies have shown similar seroprevalences. For instance, close to 22% of feral and wild mammals were seropositive based

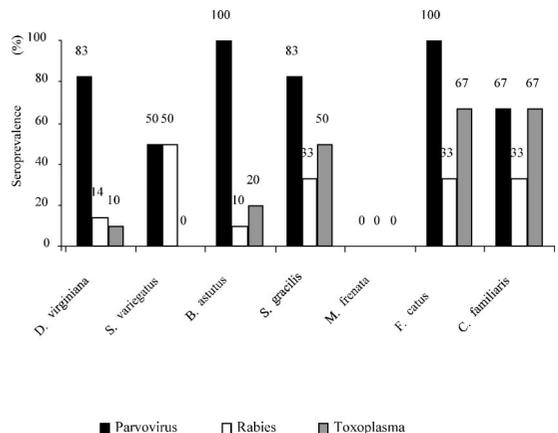


Figure 2. Seroprevalence among species.

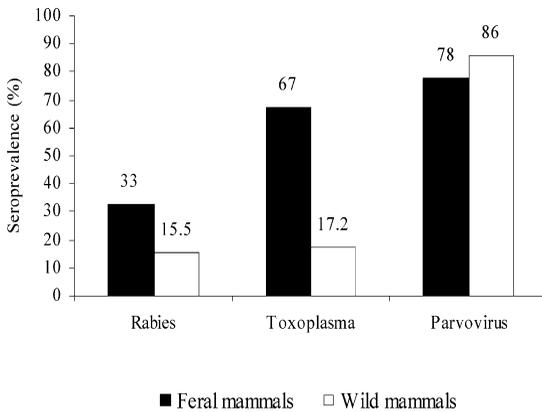


Figure 3. Seroprevalence in feral and wild mammals.

on a sample of 5,257 individuals in Canada.³¹ In a study conducted in Mexico between 1989 and 1994, 67,354 brains of different species (primarily stray dogs and cats) showed virus presence (Negri bodies) and rabies prevalence was 22%.²¹ Our study revealed a similar trend, although it was performed for antibodies, as close to 18% of captured mammals were seropositive for rabies.

Dogs have been the main source of human rabies in Mexico City; dog bites are responsible for 85.7% of human rabies cases.²¹ Rabies has a higher occurrence in urban areas with higher feral dog and cat densities.^{10,21} Rabies continues to be an animal- and public-health concern; estimations hover between 500,000 to a million feral dogs in Mexico City. The high rabies antibody response found in dogs, cats, and skunks for this study was not surprising because rabies is mostly associated with carnivores.² Some of the positive feral mammals could have been seropositive due to vaccination; however, most of the feral captures were young individuals that would not have been in contact with vaccination programs. Differences in rabies incubation periods among species may explain the lack of clinical signs for rabies in the seropositive individuals. No seasonal changes in seroprevalence of rabies were detected. At this time, we cannot determine if rabies seroprevalence is due to spill-over between species or if different rabies strains are endemic to the area. Long-term epidemiological research, including virus isolation, should be established to learn more about natural rabies ecology.

Feral cats commonly pass through or live in these natural protected areas. Cats are the main reservoir of *T. gondii*, and their feces contain the infectious agent.¹² Wild populations have a high risk of exposure to toxoplasmosis by ingesting the protozoan in raw meat or the oocysts in cat feces. Cats

are the only known definitive hosts for the sexual stages of *T. gondii*. Other carnivores do not play an important role in the epidemiology of toxoplasmosis, but do seem to acquire *T. gondii* infections. Toxoplasmosis is generally an asymptomatic infection, but could be virulent in immunosuppressed individuals or pregnant females and fetal death, abortions, and newborn mortality have been reported in several species.⁵ A toxoplasmosis epizootic was detected in *M. nigripes*, causing high mortality.³

Other serological carnivore studies for toxoplasmosis¹³ reported a seroprevalence of 10% of 62 individuals, and 24% of 273 individuals.²³ We observed similar results, with 23.9% seropositive carnivores (dogs, cats, and skunks) (Table 1). Martínez¹⁷ reported that 53% of feral dogs and cats tested in Mexico City had antibodies, while in our study, 67% of feral carnivores were seropositive. The high seroprevalence of toxoplasmosis in cats, dogs, and skunks justifies long-term epidemiological studies in wild populations.

Canine parvovirus is a recently evolved virus that mutated from feline panleukopenia virus and caused a global pandemic in the 1980s, dispersed primarily by canids.¹⁹ Various parvoviruses cause disease in wild mammals, but the most recent and well-established parvovirus is canine type 2 parvovirus, spread by several wild mammals species.^{1,25} A majority of the animals in this study were positive for parvovirus antibodies. Species with 100% seropositivity were feral cats and ringtails. The antibody titers were high in dry and rainy season, ranging from 1/32 to 1/4,096, suggesting that the virus is present throughout the year in both areas. No seropositive individuals showed signs of infection despite the high antibody titers.

The probability of becoming infected with parvovirus is higher than with the other diseases because parvovirus persists for a long time in the environment, primarily in feces.⁶ In this study, parvovirus antibodies were more prevalent compared with those of rabies and toxoplasmosis. Parvovirus consequences are unknown. Mortalities in wild mammals due to parvoviruses could reach 90% in some areas.²⁵ In the two reserves, the high prevalence of parvovirus antibodies in feral and wild carnivores justifies a long-term epidemiological study identifying endemic parvoviruses. Disadvantages to serology include the chance of cross-reaction of canine parvovirus with feline panleukopenia. There is a possibility that some seropositive cats may have feline panleukopenia, and further studies should be implemented to distinguish between the two diseases.

Another aim of our study was to determine which species act as disease reservoirs in these protected areas near Mexico City. The criteria we used to select the species that preserve infectious agents in the wild are: species distribution (well-distributed species are of greater importance in keeping and maintaining infectious agents); tolerance for different habitat types and environmental conditions, either in preserves or in perturbed areas; behavioral displays, e.g., tolerance for human presence, human settlements, and high contact rate with other species, including humans.¹⁹ Under these criteria, our study identifies feral carnivores as the species with major implications in disease dynamics in different habitats. Feral carnivores maintain disease outbreaks in the wild and affect human health.

High antibody response to the three infectious agents is related to the high degree of perturbation events (stressed areas) in both protected reserves and high densities of feral animals, which maintain these diseases and transmit them to wild-animal populations. The data obtained in this study will hopefully pave the way for further studies investigating fragment size and isolation and the detrimental effects of infectious diseases on wildlife populations. Linking epidemiology and conservation biology will identify preventive measures to maintain diversity as much as is feasible in an urban setting. The results of these types of studies may provide data to prevent possible outbreaks that could affect wildlife, domestic animals, and human health.

Conservation proposals

Virulence prediction of diseases is not easy because it involves a great number of factors, especially within natural communities. Wild populations in shrinking ecosystems are exposed to higher pressure from pathogens, which can become more virulent, due to higher rates of contact with exotic and invasive species in small patches. Antibody detection in wild populations can suggest the presence of an infectious agent and may play an important role as an indicator of diseases associated with fragmented ecosystems. Some measures that we propose as a result of this research are systematic monitoring programs to evaluate the impact of rabies, toxoplasmosis, and parvovirus in mammals from both areas; control and eradication of exotic and feral species; sterilization and vaccination campaigns in stray dogs and cats that live near the reserves; implementation of an information campaign inside the reserves advising visitors not to abandon pets at reserves and avoid feeding feral animals;

implementation of an information campaign emphasizing the importance of these reserves in conserving diversity in Mexico City and the detrimental effects that feral animals have on native fauna.

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