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# RISK FACTORS ASSOCIATED WITH PREVALENCE OF ANTIBODIES TO *LEPTOSPIRA INTERROGANS* IN A METAPOPOPULATION OF BLACK-TAILED PRAIRIE DOGS IN MEXICO

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**ABSTRACT:** Interest in the study of infectious diseases of wildlife has grown in recent decades and now focuses on understanding host-parasite dynamics and factors involved in disease occurrence. The black-tailed prairie dog (*Cynomys ludovicianus*) is a useful species for this type of investigation because it lives in heterogeneous landscapes where human activities take place, and its populations are structured as a metapopulation. Our goal was to determine if colony area, density, and proximity to human settlements are associated with prevalence of antibodies to *Leptospira interrogans* in black-tailed prairie dogs of northwestern Chihuahua State, Mexico. We captured 266 prairie dogs in 11 colonies in 2009 and analyzed 248 serum samples with the microscopic agglutination test (MAT) for antibody to any of the 12 pathogenic serovars of *L. interrogans*. Serologically positive test results for only serovars Bratislava, Canicola, Celledoni, and Tarassovi were considered for statistical analysis. Almost 80% of sera were positive for at least one pathogenic serovar (MAT titer  $\geq 1:80$ ). The highest recorded antibody prevalences were to serovars Bratislava and Canicola. Correlation analysis showed a negative relationship between *L. interrogans* antibody prevalence and colony area ( $r = -0.125$ ,  $P < 0.005$ ), suggesting that animals living in larger colonies were at a lower risk than those in smaller colonies. The correlation between the serovar Canicola and distance was negative ( $r = -0.171$ ,  $P < 0.007$ ), and this relationship may be explained by the presence of domestic dogs associated with human dwellings. This is the first study of *Leptospira* spp. antibody prevalence in prairie dogs, and it provides valuable insights into the dynamics of leptospirosis in threatened wildlife species. Further studies are needed to evaluate the impact of *Leptospira* serovars in metapopulations of prairie dogs and other domestic and wild mammals in grassland communities.

**Key words:** Chihuahua, Janos Biosphere Reserve, *Leptospira*, prairie dogs.

## INTRODUCTION

Leptospirosis is an important emerging infectious disease and zoonosis with wide distribution, caused by pathogenic spirochetes of the genus *Leptospira* (Leighton and Kuiken 2001; Higgins 2004). This multihost bacterium can be highly pathogenic and presents health implications for humans and domestic animals (Levett 2001). Leptospire are transmitted by direct contact with urine from animal reservoirs (studies have demonstrated leptospire in mammals, amphibians, and reptiles) or from contaminated water or soil. Symptoms of disease include fever, renal and hepatic insufficiency, pulmonary manifestations, and reproductive failure

(Adler and de la Peña 2010). Studies of wildlife have shown that some ecologic and environmental attributes can increase the prevalence of infection by *Leptospira* spp. (Derne et al. 2011), including spatial attributes such as area inhabited by reservoir hosts, and presence of human settlements, and ecologic attributes such as population density, sex, age, and weight of host (Wobeser 2006; Reis et al. 2008). However, few ecologic studies of *Leptospira* have focused on wildlife, and little is known about its effect on keystone species (Moinet et al. 2010).

Prairie dogs (*Cynomys* spp.) of North America are a keystone species of grasslands (Lomolino and Smith 2003); their populations have significant impact on

soil, vegetation structure, and predators (Miller et al. 1994). Some carnivores such as black-footed ferrets (*Mustela nigripes*) depend on prairie dog populations for their survival (Cully and Williams 2001). The area of distribution of the black-tailed prairie dog (*Cynomys ludovicianus*) in North America has been reduced to less than 2% of the historic range (Ceballos et al. 1993). One of the largest extant black-tailed prairie dog towns in North America is in the Janos Biosphere Reserve in the Janos-Casas Grandes complex of northwestern Mexico (Ceballos et al. 1993; List et al. 2010). Several factors have contributed to the decline of prairie dog populations, including human activities such as agriculture, overgrazing, and poisoning (Hoogland 1995). Diseases, especially bubonic plague, have caused local extinction of prairie dog colonies in the southern US (Reading et al. 1999; Keeling and Gilligan 2000). Little is known about *Leptospira*-related diseases of the black-tailed prairie dog in Mexico where the largest metapopulation occurs.

The study of the dynamics of pathogens in a metapopulation of a keystone species is critical to conservation programs, and *Leptospira* spp. offer an ideal model because of their recent worldwide increase in incidence and their association with public health issues. The impact of this bacterium on the metapopulation of the prairie dog is unknown. Our goal was to determine if the prevalence of antibodies to *Leptospira interrogans* in black-tailed prairie dogs is associated with population factors such as colony area, density, and proximity to human settlements or if individual attributes such as sex, age categories, and weight of prairie dogs are critical factors in the dynamics of *Leptospira*-related diseases.

#### MATERIALS AND METHODS

The study was conducted in the Janos-Casas Grandes complex in state of Chihuahua, northwestern Mexico, approximately 50 km south of the Mexico-US border (30°50'N,

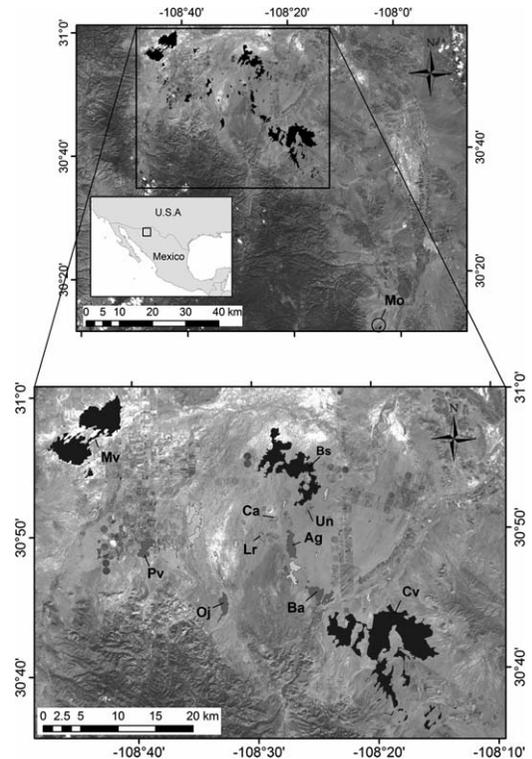


FIGURE 1. Map of black-tailed prairie dog (*Cynomys ludovicianus*) colonies in the northwestern corner of the state of Chihuahua, Mexico, sampled for this study. The large colonies (>1,000 ha) are La Báscula (Bs), El Cuervo (Cv), and Monte Verde (Mv); medium colonies (100–1,000 ha) are El Águila (Ag), Buenos Aires (Ba), Ojitos Sur (Oj), and Pancho Villa (Pv); small colonies (<100 ha) are La Cal (Ca), Loma Los Ratones (Lr), Mata Ortiz (Mo), and Uno Norte (Un). Modified from a map made by Laboratorio de Ecología y Conservación de Fauna Silvestre, Instituto de Ecología, Universidad Nacional Autónoma de México.

108°25'W; Pacheco et al. 2000). The study area is a mosaic of grassland, shrubs, and agriculture and constitutes part of the Janos Biosphere Reserve (List et al. 2010), a top conservation priority area in Mexico (Ceballos et al. 2005). The climate is temperate arid, with hot summers and cold winters (–15 to 50 C); the mean annual precipitation is 307 mm, with occasional snow in winter (García 1988). We selected samples from 11 colonies of black-tailed prairie dogs, with differences in colony area and distance to human settlements, which were safe for field work (Fig. 1).

We captured black-tailed prairie dogs between May and July 2009 with Tomahawk

traps (47.5×15×15 cm; model 204, Tomahawk Live Trap Co., Hazelhurst, Wisconsin, USA) baited with corn, oats, wheat, and molasses. Traps were placed in a 4×4 trap grid arrangement with 30 m between traps. Grids were separated by ≥500 m. Each trapping session lasted 4 days per colony. On the first day, we prebaited closed traps with the bait on one side of the traps. Over the next 3 d, traps were baited and opened for 8 h (4 h in the morning and 4 h in evening) and continuously monitored. Captured prairie dogs were handled in canvas bags (see Hoogland 1995), and individuals were weighed, and age (adult or juvenile) and sex were determined. All captured individuals were identified with uniquely numbered metal ear tags (model 1005-1, National Band & Tag Company, Newport, Kentucky, USA). A maximum volume of 1.5 mL of blood was collected by femoral venipuncture. Blood samples were centrifuged at 514 × G for 10 min to obtain serum and frozen at -20 C until laboratory analysis in the Veterinary School at Universidad Nacional Autónoma de México (UNAM). All individuals were released unharmed at the capture site, and all were handled humanely following prescribed procedures (Sikes et al. 2011).

Geographic coordinates for trapping sites were obtained with a global positioning system (model Garmin eTrex Legend, Garmin International, Inc., Olathe, Kansas, USA); colony areas were taken from previous studies (Marcé 2001; Avila-Flores 2009), and prairie dog density per hectare was estimated using the Schnabel method (Sutherland 1996). We defined human settlement as any area that included permanent human activity or occupancy, ranging from small, one-family farms to large towns consisting of >1,000 people. The distance between prairie dog colonies and human settlements was measured in a straight line using Arcview 3.2 (ESRI 1999). We selected the halfway grid between the closest grid and the farthest grid from the nearest human settlement to each prairie dog colony to obtain the distance. Capture success was defined as the percentage of traps with individuals captured (including recaptures) during 3 d of trapping (Romero et al. 2000).

A standard serologic microscopic agglutination test (MAT) was used to detect antibodies to *L. interrogans* in all serum samples (Faine et al. 1999). Testing of urine samples was not possible because of the difficulty of collecting urine in dry environments and because the prairie dogs are protected by laws that prohibit euthanization to obtain bladder and kidneys for leptospire identification.

We used 12 live strains of *L. interrogans* serovars (antigen): Autumnalis, Bataviae, Bratislava, Canicola, Celledoni, Grippotyphosa, Hardjo, Icterohaemorrhagiae, Pomona, Pyogenes, Tarassovi, and Wolffi. All serovars belong to the collection of the Department of Microbiology and Immunology, Veterinary School, UNAM, and Collaborating Centre for Reference and Research on Leptospirosis by agreement of the World Health Organization, Food and Agriculture Organization, and World Organization for Animal Health (WHO/FAO/OIE).

We prepared a 1:5 dilution of each serum sample collected in the field (25 µL of serum and 1 mL of saline solution). We placed 50 µL of 1:5 serum dilution in each well in flat, 96-well plastic microtiter plates (SARSTEDT AG & Co., Nümbrecht, Germany), organized in columns by serotype (one serotype in each of the 12 columns); 50 µL aliquots of the antigen were added for the screening test. The control (saline solution with the antigen) was placed in the first column. Plates were incubated at 30 C for 2 h, and the reactions of sera with antigens were observed in a dark field microscope (Myers 1985). Samples that reacted were retested in several twofold dilutions beginning at 1:10 to determine the final titer of serum for each serotype to which they reacted. The highest dilution with 50% agglutination of *Leptospira* was considered the sample titer (Cole et al. 1973).

Domestic animals with signs of infection and titer ≥1:100 are considered to have clinical leptospirosis (Faine et al. 1999). However, signs in wildlife are diverse and, for prairie dogs, unknown. We considered sera with titers ≥1:80 positive for *Leptospira*. Samples not meeting those criteria were considered nonspecific. For statistical tests, we only considered serovars with antibody prevalence of 20% or higher with MAT titers ≥1:80.

Statistical analyses were done using R package, Version 2.15.2 (R Development Core Team 2012). We performed correlation analysis to evaluate the relationship between the quantitative independent variables (colony area, population density, proximity to human settlements, and prairie dog weight) and prevalence of antibodies to *L. interrogans* serovars in black-tailed prairie dogs; sex and age variables were transformed (1=female, 2=male; 1=juvenile, 2=adult) before evaluation by correlation analysis;  $P < 0.05$  was considered statistically significant. Additionally, a multinomial logistic regression was performed using explanatory variables (quantitative variables were categorized), and odds ratios (OR) were estimated with 95% confidence intervals (CI).

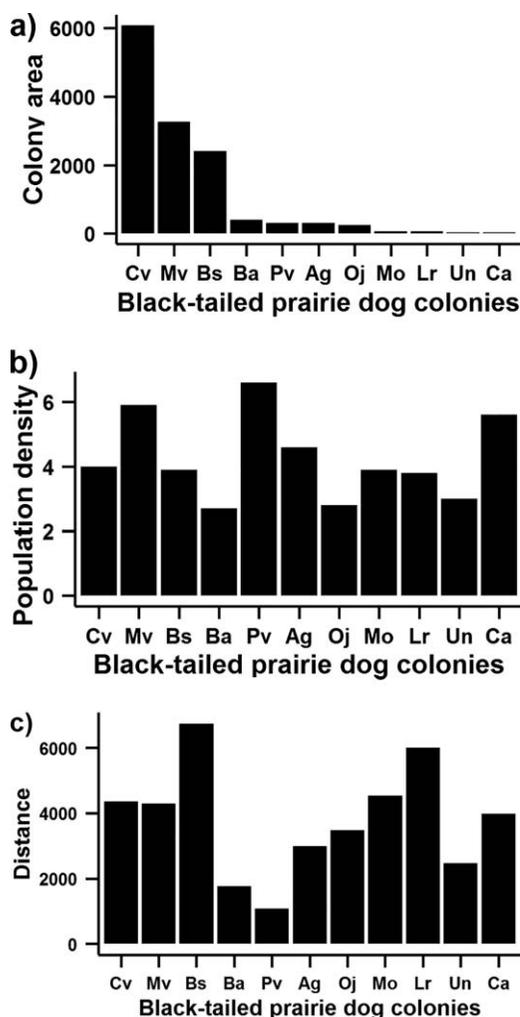


FIGURE 2. Environmental attributes of eleven black-tailed prairie dog (*Cynomys ludovicianus*) colonies: (a) colony area (ha), (b) population density (individuals/ha), (c) distance (m) to nearest human settlement. Ag=El Águila; Ba=Buenos Aires; Bs=La Báscula; Ca=La Cal; Cv=El Cuervo; Lr=Loma Los Ratones; Mo=Mata Ortiz; Mv=Monte Verde; Oj=Ojitos Sur; Pv=Pancho Villa; Un=Uno Norte.

## RESULTS

We captured 266 (90 male and 176 female) black-tailed prairie dogs from the 11 colonies sampled from May to July 2009. The area of prairie dogs colonies ranged 30 to 6,077 ha, population density was 2.7–6.6 individuals/ha, and distance to human settlements was 1,076–6,734 m (Fig. 2).

The average weight for males was 792 g (300–1,250 g), and for females, it was 743 g (360–1,205 g). We obtained 248 serum samples from the 266 captured individuals. Total *L. interrogans* antibody prevalence was 79%; 195 of 248 prairie dogs were positive for at least one serovar at the cutoff titer of  $\geq 1:80$  (Fig. 3).

### Correlation analysis

*Leptospira interrogans* antibody prevalence was negatively correlated with both colony area ( $r = -0.125$ ,  $P < 0.005$ ) and prairie dog population density ( $r = -0.191$ ,  $P < 0.005$ ). The serovars with the highest prevalence were Bratislava and Canicola ( $\geq 40\%$  with titers  $> 1:80$ ), followed by Celledoni (29%) and Tarassovi (25%). The highest titer was observed for serovar Bratislava (1:10,240), followed by Tarassovi (1:5,120), while a titer of 1:2,560 was recorded for Celledoni and 1:1,280 for Canicola.

**Serovar Bratislava:** Serovar Bratislava was distributed in all prairie dog colonies with high prevalence. Prevalence was not significantly associated with any variable we measured.

**Serovar Canicola:** Of the four serovars, only Canicola was significantly correlated with colony area ( $r = -0.26$ ,  $P < 0.005$ ), whereas prairie dog population density was negatively correlated ( $r = -0.31$ ,  $P < 0.005$ ). The distance from prairie dog colonies to human settlements was significant ( $r = -0.17$ ,  $P < 0.01$ ). Weight was significantly correlated with Canicola antibody prevalence ( $r = 0.14$ ,  $P = 0.027$ ), and variables sex and age were not significantly associated ( $r = 0.0169$ ,  $P = 0.791$ ;  $r = -0.0329$ ,  $P = 0.607$ , respectively).

**Serovar Celledoni:** Antibody prevalence of Celledoni was not significantly correlated with any independent variable.

**Serovar Tarassovi:** Prairie dog population density was negatively correlated with the

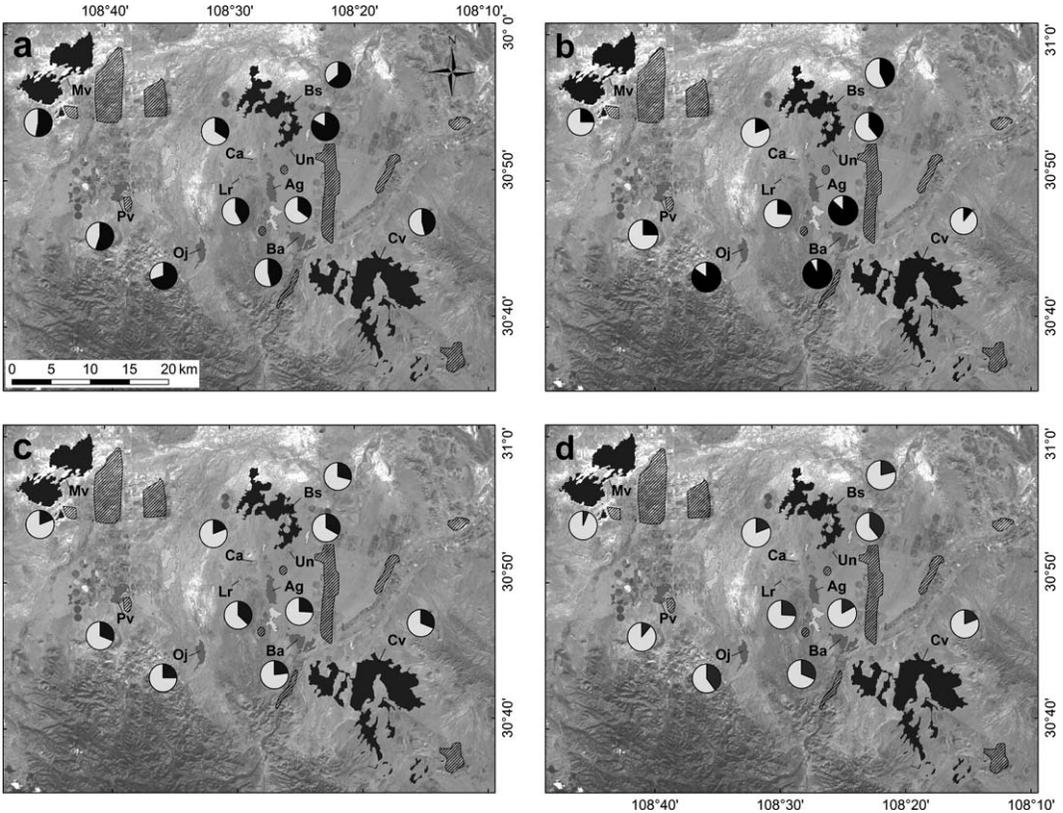


FIGURE 3. Distribution and prevalence of antibody to each of four *Leptospira* serovars in black-tailed prairie dogs (*Cynomys ludovicianus*): (a) Bratislava; (b) Canicola; (c) Celledoni; (d) Tarassovi. Dark part of pie charts represents proportion of prairie dog population positive by microscopic agglutination test; hatched polygons are areas of human settlements. Ag=El Águila; Ba=Buenos Aires; Bs=La Báscula; Ca=La Cal; Cv=El Cuervo; Lr=Loma Los Ratonés; Mv=Monte Verde; Oj=Ojitos Sur; Pv=Pancho Villa; Un=Uno Norte.

prevalence of antibody to serovar Tarassovi ( $r = -0.22$ ,  $P < 0.005$ ). Other variables tested were not related to *L. interrogans* antibody prevalence.

#### Multinomial logistic regression

The multinomial logistic regression showed that risk of infection (as determined by presence of antibody) with *L. interrogans* increases with low prairie dog population densities (OR=1.65, 95% CI=1.13–2.42). This relationship exists for the serovars Bratislava (OR=1.43, CI=1.09–1.88), Canicola (OR=1.88, CI=1.39–2.52), and Tarassovi (OR=1.41, CI=1.04–1.90). All the other variables tested were not related to *Leptospira* infection.

#### DISCUSSION

This is the first study in which antibodies against *Leptospira* spp. were detected in serum samples of black-tailed prairie dogs. In general, correlation coefficients were low but highly significant. We observed two important patterns of *L. interrogans* antibody prevalence in black-tailed prairie dogs. First, larger colonies had lower prevalences, suggesting that colony area may be protective against pathogenic leptospires. Second, prevalence was not positively correlated with prairie dog population density as expected. We found a negative relationship between prairie dog population density and the

occurrence of serovars *Canicola* and *Tarassovi*. We attribute this relationship to seasonal and interannual variation, as suggested by Davis et al. (2005). This observation contrasts with that of Redetzke and McCann (1980), who found a density-dependent relationship. *Leptospira* maintenance and transmission in Janos may depend on several factors, including temperature, frequency of droughts and rainy periods, and the accessibility of humid environments for *Leptospira* survival and reproduction (Derne et al. 2011). Simultaneously, *L. interrogans* maintenance and transmission depend on host population density and frequency of contact with infected hosts (Getz and Pickering 1983; Hartskeerl and Terpstra 1996; Ryder et al. 2007). We did not find significant relationships between distance to human settlements and *L. interrogans* antibody prevalence.

The infection dynamics of leptospirosis in prairie dogs are complex and may vary in space and time, and by serovar. Our results are consistent with previous studies on rodents from arid environments showing that serovars Bratislava and *Canicola* were common at titers  $\geq 1:160$  (Songer et al. 1983).

*Serovar Bratislava*: Similar to Goyal et al. (1992), we detected Bratislava antibody in nearly all individuals sampled, and there was no statistically significant relationship with the variables we assessed.

*Serovar Canicola*: *Canicola* is associated with dogs (*Canis lupus familiaris*) and other wild carnivores, including foxes (*Vulpes* spp.), coyotes (*Canis latrans*), and skunks (*Mephitis* spp.; Busch 1970; Faine et al. 1999; Kikuti et al. 2012), and it reportedly has a higher prevalence in urban than rural areas (Alton et al. 2009). Building on other research, our study raises the prospect that contact with domestic and feral dogs may be a constant source of pathogenic leptospires for black-tailed prairie dogs. We found *Canicola* to

be the second most frequent serovar in colonies near human settlements, and this observation is consistent with the higher prevalence of *L. interrogans* in wolves (*Canis lupus*) captured near farms compared with wolves sampled away from farms (Khan et al. 1991). These observations suggest that prairie dogs inhabiting small patches at lower population densities and near human settlements are at increased risk of *Canicola* serovar infection. We also noted a positive relationship between weight (a possible surrogate for adults or age) and prevalence of *Canicola* serovar infection. This relationship has been observed in other studies and suggests that adults have higher antibody prevalence because they have a higher cumulative risk of exposure to leptospires; this can be related to the positive relationship between *Canicola* infection prevalence and weight (Hathaway et al. 1981; Eymann et al. 2007).

*Serovar Celledoni*: There is little information on the relationship of serovar *Celledoni* with wildlife, but it has been reported in some cases of human leptospirosis (Faine et al. 1999). We found a few positive prairie dogs, including some with high antibody titers that could indicate recent infection.

*Serovar Tarassovi*: In North America, skunks are hosts for serovars Bratislava, Pomona, and *Tarassovi* (Bolin 2000). In Janos, skunks (*Mephitis macroura* and *Mephitis mephitis*) are often found in prairie dog colonies and could be involved in the infection by these serovars in black-tailed prairie dogs.

We found that prairie dog colonies closest to human settlements had higher population densities. This relationship may be due to anthropogenic effects that reduce the size of available prairie dog habitat patches and therefore generate crowding. An understanding of the transmission dynamics of *L. interrogans* in black-tailed prairie dog metapopulations requires

knowledge of the serovars and antibody titers in a range of domestic animals and wildlife. Our study demonstrated the value of MAT in the detection of *L. interrogans* despite its limitations. The inherent difficulty of obtaining urine samples in this dry prairie dog habitat and isolating strains of *Leptospira* spp. presented a unique challenge for molecular-based diagnostic procedures such as PCR and precluded their use.

On a larger scale, the study of pathogens in wildlife provides important tools for protection and management of natural populations, especially endangered and keystone species that have a crucial role in maintaining the ecosystem in which they live (Suzán et al. 2000). Safeguarding native grassland in Mexico and the wildlife associated with it depends on the successful protection and preservation of prairie dogs towns; the diagnosis and control of infectious diseases are essential to the conservation and management of this unique ecosystem. Our study is a step towards the understanding of patterns of leptospire infection in an endangered wild species—patterns that might be useful to guide management and conservation.

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