

## Disease Surveillance of California Ground Squirrels (*Spermophilus beecheyi*) in a Drive-through Zoo in Oregon, USA

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**ABSTRACT:** Rodents and other small wild mammals are often considered to be pests and vectors for disease in zoos that house small populations of valuable threatened and endangered animals. In 2005, three nonhuman primates at a drive-through zoo in Oregon, US, acquired tularemia from an unknown source. Due to an abundance of California ground squirrels (*Spermophilus beecheyi*) on zoo grounds, we instituted serosurveillance of this species from July through September 2008 to determine the prevalence of antibodies against pathogens considered to be potentially transmissible to collection animals. Serologic testing was performed for *Francisella tularensis*; *Leptospira interrogans* serovars Canicola, Grippotyphosa, Hardjo, Icterohemorrhagiae, and Pomona; *Toxoplasma gondii*; and *Yersinia pestis*. All squirrels were seronegative for *Yersinia pestis* (0%; 0/45) and *Toxoplasma gondii* (0%; 0/20); there was a prevalence of 2% (1/45) for *Francisella tularensis* antibodies and 57% (24/42) were positive for various *Leptospira* serovars. Although it remains unclear whether ground squirrels present a significant risk for transmission of disease to zoo animals, vaccination of high-risk zoo animals against leptospirosis warrants consideration. Beyond this, continued vigilance and persistence with various forms of pest control may reduce the likelihood of disease transmission from wildlife hosts to animals in human care.

**Key words:** Leptospirosis, plague, rodent, Sciuridae, serology, toxoplasmosis, tularemia.

Zoological gardens typically offer excellent habitat for squirrels (family Sciuridae) and other rodents by providing food and water resources, shelter, and some protection from natural predators. These animals, however, are often considered to be pests in zoos due to their destructive behaviors in exhibits and facilities, as well as their potential to act as vectors for disease transmission (Meerburg et al. 2009). Various forms of pest control are often incorporated into zoos' preventive med-

icine protocols to decrease the likelihood of disease transmission from wildlife to valuable collection animals; yet pest control is a challenge in zoos, as the use of some rodenticides is coupled with the risk of accidental toxicosis in nontarget species (Borst and Counotte 2002). Nonlethal forms of pest control have also been attempted, ranging from trapping and relocation of small mammals to surgical or chemical sterilization to control reproductive potential, but these strategies often require substantial time and resources (Nash et al. 2004). Despite efforts to eliminate the interface between free-ranging wildlife and animals in human care, some interactions inevitably still occur and numerous reports implicate wildlife as possible sources for diseases acquired by collections' animals (Neiffer et al. 2001; Ketz-Riley et al. 2009; Gyimesi et al. 2015).

Wildlife Safari is a 250-ha drive-through wild animal park located in Winston, Oregon, US (43°8'27"N, 123°25'39"W). The park is situated in the southwestern portion of the state, between the Umpqua National Forest to the east and the coast of Oregon to the west. Summers are hot and dry with daytime high temperatures averaging 29 C and overnight lows averaging 13 C, with little (<2.5 cm) monthly precipitation from June through September. Most of the region's annual precipitation (~80 cm) falls between October and May, when average high temperatures are approximately 10–20 C and lows are 0–10 C (US Climate Data 2017). The region's geography consists of valley lowlands with floodplains, terraces, and gently sloping hills supporting grasslands and mixed deciduous and coniferous forests (US Department of

Agriculture, Natural Resources Conservation Service 2003). Colonies of California ground squirrels (*Spermophilus beecheyi*) inhabit virtually all areas of the park, including expansive grazed pastures, building peripheries, and other areas of human disturbance, with nesting burrows distributed throughout the park.

In 2005, three nonhuman primates at Wildlife Safari were infected with tularemia; two died and one survived with treatment. The affected primates included a 15-yr-old male bushbaby (*Galago* sp.), a 13-yr-old male cottontop tamarin (*Saguinus oedipus*), and a 14-yr-old female cottontop tamarin. These animals were considered healthy prior to infection, although they may be classified as geriatric based on reported median life expectancies of 6 yr for bushbabies and 11.5 yr for cottontop tamarins in human care (Association of Zoos and Aquariums 2017). This outbreak prompted investigation into a potential source of disease. Due to the perceived overabundance of California ground squirrels observed within the park, a disease surveillance study was initiated in the summer of 2008 to determine the seroprevalence of antibodies against *Francisella tularensis*, as well as other potential pathogens, including *Leptospira interrogans* serovars Canicola, Grippotyphosa, Hardjo, Icterohemorrhagiae, and Pomona; *Toxoplasma gondii*; and *Yersinia pestis* in the local ground squirrel population. Serotests were chosen based on diseases that may occur in both rodent populations and various collection mammals (primates, carnivores, artiodactyls, perissodactyls, etc.). All wildlife capture, handling, and anesthesia protocols met with the approval of Wildlife Safari's animal health committee, as well as the Oregon Department of Fish and Wildlife.

Tomahawk live-traps (Tomahawk Live Trap Company, Hazelhurst, Wisconsin, USA) baited with various fruits, grains, and peanut butter were used to capture squirrels. Eleven traps were set at approximately 1700 hours for a total of 22 trap-nights between July and September 2008. Traps were placed non-randomly, at a minimum of 50 m and a

maximum of 500 m from adjacent traps, throughout the 250-ha park in locations where consistent and extensive squirrel activity was observed. Animals were collected from traps at approximately 0800 hours the following morning. A total of 45 adult squirrels were sampled. Four other mammals, including two Virginia opossums (*Didelphis virginiana*), one woodrat (*Neotoma* sp.), and one striped skunk (*Mephitis mephitis*), were inadvertently captured during the study period, and were promptly released.

Captured squirrels were transported to an on-site animal hospital for processing. Personal protective equipment (gloves and a facemask) was worn during procedures. Squirrels were induced with a combination of Telazol® (50 mg/mL tiletamine hydrochloride, 50 mg/mL zolazepam hydrochloride, Fort Dodge Animal Health, Fort Dodge, Iowa, USA), ketamine hydrochloride (100 mg/mL Ketaset®, Fort Dodge Animal Health), and xylazine hydrochloride (100 mg/mL AnaSed®, Lloyd Laboratories, Shenandoah, Iowa, USA). This Telazol®-ketamine-xylazine combination was mixed at a ratio of 2:1:1, and administered intramuscularly at a dosage of approximately 0.22 mL/kg. Up to 1 mL of blood was collected from a jugular or femoral vein from each squirrel. Squirrels were ear-punched using a Premier Uni-Punch (Delasco, Council Bluffs, Iowa, USA) biopsy punch to prevent resampling in the event of recapture.

Sera were submitted to Wyoming State Veterinary Laboratory (Laramie, Wyoming, USA) for *Francisella tularensis* microscopic agglutination testing (MAT) and *Yersinia pestis* competitive enzyme-linked immunosorbent assay testing, and to Oregon State University Veterinary Diagnostic Laboratory (Corvallis, Oregon, USA) for *Leptospira* spp. MAT and *Toxoplasma gondii* indirect hemagglutination testing.

There were no clinical signs of disease observed in any squirrel. Sera were negative for *Yersinia pestis* (0%; 0/45) and *Toxoplasma gondii* (0%; 0/20). Prevalence was 2% (1/45) for *Francisella tularensis*, which may reflect limited exposure, or alternatively, high cause-

TABLE 1. Microscopic agglutination test (MAT) results for 42 California ground squirrels (*Spermophilus beecheyi*) tested for *Leptospira interrogans* serovars Canicola, Grippotyphosa, Hardjo, Icterohemorrhagiae, and Pomona at Wildlife Safari between July and September 2008.

Titer	<i>Leptospira</i> serovars MAT titer prevalence (no. positive/no. tested)				
	% Canicola	% Grippotyphosa (n/N)	% Hardjo (n/N)	% Icterohemorrhagiae	% Pomona (n/N)
Negative	100	93 (39/42)	62 (26/42)	100	81 (34/42)
Positive					
1:100	0	0	5 (2/42)	0	7 (3/42)
1:200	0	5 (2/42) <sup>a,b</sup>	31 (13/42) <sup>a</sup>	0	10 (4/42)
1:400	0	0	2 (1/42) <sup>b</sup>	0	2 (1/42) <sup>c</sup>
1:6,400	0	2 (1/42) <sup>c</sup>	0	0	0

<sup>a</sup> One animal showed titers against serovars Grippo and Hardjo (both titers of 1:200).

<sup>b</sup> One animal showed titers against serovars Grippo (titer of 1:200) and Hardjo (titer of 1:400).

<sup>c</sup> One animal showed titers against serovars Grippo (titer of 1:6,400) and Pomona (titer of 1:400).

specific mortality. The source for the tularemia outbreak at this zoo remains undetermined. Though ground squirrels cannot be ruled out as a vector for the disease, other small mammals such as the house mouse (*Mus musculus*) may have also gained access to the primates' exhibits. Likewise, ectoparasites carried by small mammals may have been responsible for disease transmission.

Our most notable finding was the seroprevalence of *Leptospira* serovars in the local California ground squirrel population. Overall, 57% (24/42) of squirrels exhibited a titer for at least one *Leptospira* serovar, with 7% (3/42) showing titers against two serovars (Table 1). Positive titers were found to *L. interrogans* serovars Grippotyphosa (7%; 3/42), Hardjo (38%; 16/42), and Pomona (19%; 8/42) and sera were negative for serovars Canicola and Icterohemorrhagiae. In most squirrels, positive titers ranged from 1:100 to 1:400, with the exception of one animal with a titer of 1:6,400 against serovar Grippotyphosa. The MAT is considered the gold standard serotest for leptospirosis, with good sensitivity and specificity, although there is significant cross-reactivity among serovars and serogroups. The MAT is also limited in identifying chronic infection, as renal carriers of spirochetes may exhibit titers below the accepted minimum positive titer of 1:100 (World Organisation for Animal Health 2009).

Although leptospirosis has been well characterized in rodent and numerous other host populations worldwide, there are few studies of the bacterium in the family Sciuridae, and no known reports in California ground squirrels. In 2011, a study in Colorado, US, documented an antibody prevalence of 55% (12/22) to *L. interrogans* in fox squirrels (*Sciurus niger*), with the highest titers to serovars Grippotyphosa, Hardjo, and Icterohemorrhagiae (Dirsmith et al. 2013). One of 20 (5%) gray squirrels (*Sciurus carolinensis*) tested in Connecticut, US, in 2001 was seropositive for *L. interrogans*, with the highest titers to serovars Grippotyphosa and Canicola (Richardson and Gauthier 2003). Previous studies in the US documented leptospirosis in 11% (1/9) of fox squirrels in Iowa (Diesch et al. 1967), and 4% (1/27) of fox squirrels and 4% (1/26) of gray squirrels in southeastern states (Shotts 1975). Gozzi et al. (2013) summarized several studies documenting *Leptospira* serovars in South American and Asian squirrel populations.

Diseases carried by rodents and other wildlife may be devastating to small populations of threatened and endangered species maintained in zoos. Urinary PCR testing for leptospirosis may be useful in evaluating the risk of infected species by determining the occurrence of pathogen shedding. Olds et al. (2015) conducted serology and urinary PCR

testing for leptospirosis in captive black-tailed prairie dogs (*Cynomys ludovicianus*), also in the family Sciuridae. Serology showed 54.5% of study animals were exposed to spirochetes, exhibiting titers of at least 1:100 to *L. interrogans* serovar Bratislava; however, urinary PCR testing was negative for *Leptospira* serovars in all animals, so the authors concluded that the captive rodents were unlikely an important reservoir for the disease.

Our data provide a foundation for future regional studies to investigate the role of California ground squirrels as a host for leptospirosis, as well as to identify host-adapted serovars. Future studies should consider MAT testing for additional serovars, testing squirrels outside the park as study controls, and urinary PCR testing to determine the occurrence of pathogen shedding. Population densities of squirrels, environmental factors, disease status of collection animals, and spatial relationships between wild and collection animals may also influence the epidemiology of this pathogen. Based on our antibody survey results, vaccination of high-risk zoo animals against leptospirosis warrants consideration in areas where this pathogen is present. Beyond this, continued vigilance and persistence with various forms of pest control may reduce the likelihood of disease transmission between wildlife and animals in human care.

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