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Possible Vector Dissemination by Swift Foxes following a Plague Epizootic in Black-tailed Prairie Dogs in Northwestern Texas

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ABSTRACT: To determine whether swift foxes (*Vulpes velox*) could facilitate transmission of *Yersinia pestis* to uninfected black-tailed prairie dog (*Cynomys ludovicianus*) colonies by acquiring infected fleas, ectoparasite and serologic samples were collected from swift foxes living adjacent to prairie dog towns during a 2004 plague epizootic in northwestern Texas, USA. A previous study (1999–2001) indicated that these swift foxes were infested almost exclusively with the flea *Pulex irritans*. Black-tailed prairie dogs examined from the study area harbored only *Pulex simulans* and *Oropsylla hirsuta.* Although *P. irritans* was most common, *P. simulans* and *O. hirsuta* were collected from six swift foxes and a single coyote (*Canis latrans*) following the plague epizootic. Thus, both of these canids could act as transport hosts (at least temporarily) of prairie dog fleas following the loss of their normal hosts during a plague die-off. All six adult swift foxes tested positive for antibodies to *Y. pestis*. All 107 fleas from swift foxes tested negative for *Y. pestis* by mouse inoculation. Although swift foxes could potentially carry *Y. pestis* to uninfected prairie dog colonies, we believe they play only a minor role in plague epidemiology, considering that they harbored just a few uninfected prairie dog fleas (*P. simulans* and *O. hirsuta*).

Key words: Black-tailed prairie dogs, *Cynomys ludovicianus*, fleas, *Oropsylla hirsute*, plague, *Pulex irritans*, *Pulex simulans*, swift fox, *Vulpes velox*, *Yersinia pestis*.

Sylvatic plague is a flea–transmitted bacterial disease caused by *Yersinia pestis* that frequently causes epizootics resulting in 90–100% mortality rates in prairie dog (*Cynomys* spp.) colonies in western North America (Olson, 1981; Barko, 1997; Cully and Williams, 2001; Gasper and Watson, 2001). During a plague epizootic in a prairie dog population, the number of *Y. pestis*–infected free-living fleas may increase as host mortality increases (Ubico et al., 1988). Some fleas will use alternative mammalian hosts if their primary host is not available (Gasper and Watson, 2001). Sick and dead prairie dogs attract carnivores and raptors that could potentially disseminate fleas, possibly spreading *Y. pestis* to other areas (Ubico et al., 1988). Cully and Williams (2001) suggested that fleas infected with *Y. pestis* were carried long distances by coyotes (*Canis latrans*) or raptors. Thus, fleas switching from their natural rodent hosts to other animals could contribute to the geographic spread of *Y. pestis* during plague epizootics (Gasper and Watson, 2001). The swift fox (*Vulpes velox*) is a small endemic carnivore that inhabits the plains grasslands of western North America. We hypothesized that it could also act as an alternative flea host and disseminate *Y. pestis*–infected rodent fleas, especially during and following plague epizootics in prairie dog colonies. In order to better understand their importance as disseminators of *Y. pestis* vectors, we collected ectoparasite and serologic samples from swift foxes in northwestern Texas during and immediately after a plague epizootic in black-tailed prairie dogs. Our objectives for this study were to determine 1) species composition and richness of swift fox flea populations during plague epizootic versus interepizootic periods; 2) whether fleas from swift foxes were infected with *Y. pestis* during an epizootic; and 3) whether swift fox blood samples had antibodies to *Y. pestis* indicating exposure. Research protocols for this study were approved by

the Animal Care and Use Committee at Texas Tech University (Lubbock, Texas).

Die-offs in black-tailed prairie dog (*Cynomys ludovicianus*) towns on the Rita Blanca National Grassland (RBNG) in northwestern Texas, USA, were first observed in February 2004 (Nicholson, 2004). In April 2004, fleas collected from one prairie dog colony in the RBNG (index site) by Kansas State University (KSU; Manhattan, Kansas, USA) researchers tested positive for *Y. pestis*. By May 2004, fleas collected from all the prairie dog colonies in our swift fox study area and from 21 other sites on the RBNG as well as on adjacent private property in Dallam and Sherman Counties (Texas, USA) tested positive for *Y. pestis*. Fleas were collected by the flagging technique. A 30×36-cm white flannel (flag) cloth was attached to a plumber's snake. The flag was pushed down a prairie dog hole to a maximum depth of 4 m. If present, at least some fleas became entangled in the flannel fibers. The extracted flag was placed in a sealed plastic bag. Fleas (n=408) were killed by halothane (Fluothane®, Wyeth-Ayerst, Philadelphia, Pennsylvania, USA) or by freezing and stored in saline.

The US Centers for Disease Control and Prevention (CDC; Fort Collins, Colorado, USA) tentatively identified fleas from flagged prairie dog dens under a dissecting microscope, then ground them up with a mortar and pestle in pools of ≤25 fleas each and suspended the residue in approximately 2 ml of 0.85% physiologic saline. Subsequently, 0.5 ml of the suspension was inoculated subcutaneously into four specific pathogen-free 4- to 6-wk-old Swiss-Webster white laboratory mice (CDC). Mice were checked twice daily for signs of illness over a 21-day period. Those that died were necropsied to obtain liver, spleen, and lymph node tissues for direct immunofluorescent antibody staining (Jackson ImmunoResearch Laboratories, Inc., West Grove, Pennsylvania, USA) and bacterial isolation in sheep blood agar at 28 C for 48 hr (CDC). Surviving mice were killed on day 21 postinoculation and tested in the same manner.

Sixty-five sites within the 2,048-km^2 area between 102.1°W to 102.9°W and 36.2° to 36.5°N were subjectively evaluated for evidence of a plague epizootic based on previous experience following visual assessment for prairie dog activity by Department of State Health Service (DSHS) staff (61 sites) and a KSU researcher (4 sites). The swift fox study site is on a 100-km^2 area that includes parts of the RBNG and private lands in Dallam County (36.2°N, 102.4°W). Pence et al. (2004) described the study site and documented the swift fox ectoparasite fauna during an interepizootic period (1999–2001) for plague in the endemic prairie dog colonies.

During June 2004, 12 swift foxes were captured and handled using methods described by Kamler et al. (2002). Ectoparasites were collected according to the methods of Pence et al. (2004) and placed in labeled vials that contained saline solution. Collected samples were stored at 4 C and transported to the CDC for *Y. pestis* testing and flea species identification as previously described. Blood samples were collected from foxes on Nobuto® filter strips (Advantec Manufacturing, Inc., Dublin, California, USA) via toenail clippings. Nobuto® strips were placed in labeled envelopes and transported to DSHS Molecular and Serological Analysis Branch (Austin, Texas, USA) where they were examined for antibodies against *Y. pestis* in a passive hemagglutination assay. The narrow portion of Nobuto® strips were cut into equal halves, placed in a covered serum vial containing 0.4 ml of sodium borate buffer, and passively eluted overnight at 4 C. The filter strips were then compressed with a plastic (nonabsorbent) rod and heat-inactivated at 56 C for 30 min. Next, 50–100 ml of sheep red blood cells (SRBC; MD Anderson Cancer Center, Bastrop, Texas, USA) were added

to cooled specimens and allowed to adsorb for 30–60 min at ambient room temperature or overnight at 4 C. Samples were centrifuged for 10 min (3,000 rpm) to sediment the SRBC. Supernatant (containing antibodies) was transferred to fresh tubes and used to run the agglutination test. Positive titers for dilutions of $\geq 1:10$ were considered positive for *Y. pestis*.

Fleas tested for *Y. pestis* by mouse inoculation were tentatively identified under a dissecting microscope at CDC. All specimens were subsequently destroyed during the process of preparing mouse inoculums. Voucher specimens were not retained for proper flea species determination by mounting on glass slides and examination by light microscopy. Subsequently, in August 2004, we made a second collection of ectoparasites at the study site by the previously outlined methods from 10 swift foxes, one coyote, and eight black-tailed prairie dogs. Fleas were preserved in vials of 70% ethanol. These specimens were transported to Texas Tech University Health Sciences Center (Lubbock, Texas), where they were processed and identified according to the methods outlined in Pence et al. (2004). Males of the genus *Pulex* were identified as either *P. irritans* or *P. simulans* using reference specimens (R. E. Lewis, Ames, Iowa, USA). Voucher specimens are deposited in the US National Parasite Collection (Beltsville, Maryland, USA: accession numbers 095662.00 to 095664.00 and 095667.00 from swift foxes, 095659.00 to 095661.00 from the coyote, 095665 to 095666.00 from black-tailed prairie dogs).

Capture locations were recorded for swift foxes using a handheld Magellan GPS 315 (Magellan Corporation, San Dimas, California, USA). Minimum distance to the nearest prairie dog town was calculated in ArcGIS 9.0 (Environmental Systems Research Institute, Redlands, California).

Fleas from eight of 21 (38%) DSHS-flagged sites were infected with *Y. pestis*. Because flea collections from flagged sites were treated as pooled specimens for *Y. pestis* testing, exact numbers of infected fleas were not determined. Twenty-one pools consisting of a total of 312 fleas tentatively identified as *Oropsylla hirsuta* were tested. Of these, six pools consisting of a total of 131 fleas tested positive for *Y. pestis*. Twelve pools consisting of a total of 60 fleas tentatively identified as *P. simulans* were tested, with one pool containing 11 fleas testing positive for *Y. pestis*. One *Pulex* spp. tested negative.

Of the 65 sites evaluated for prairie dog activity, 16 (25%) had very recently (during 2004) become inactive, seven (11%) exhibited low activity, four (6%) had long been inactive (probably ≥ 2 yr), 10 (15%) had apparently been poisoned, 27 (42%) exhibited normal activity, and one (2%) was of undetermined status. Plague activity in prairie dogs was localized between 102.6° to 102.9°W and 36.3° to 36.4°N. Prairie dogs in towns on the southwestern periphery of the RBNG (west of 102.8°W, south of 36.4°N, and east of 102.6°W) appeared to have escaped the plague epizootic.

Ectoparasites and blood samples were collected from 12 swift foxes (10 adults and two juveniles) captured during 15–18 June 2004. The minimum distance from swift fox capture locations to the nearest prairie dog town was $\bar{X} \pm SE = 8.8 \pm 1.2$ km. The DSHS staff surveillance of all prairie dog towns on our study site indicated that they either had animals that tested positive for *Y. pestis*, or they exhibited decreased or no activity, probably because of the presence of plague. One hundred seven fleas (40 males and 67 females) from swift foxes collected on the study site were forwarded to CDC. Following cursory examination of the male genitalia under a dissecting microscope, technical personnel tentatively identified all these fleas as *P. simulans* prior to their preparation for mouse inoculation. All of the inoculated mice survived the incubation period for developing symptoms of

plague. Thus, all fleas removed from swift foxes in June 2004 tested negative for *Y. pestis*. Because of inadequate blood samples, only six of the 12 captured swift foxes were tested for antibodies to *Y. pestis*. All six were adults that tested positive.

An additional 10 swift foxes (four adults and six juveniles) were captured from 26 August 2004 to 28 August 2004. Three of the adults were recaptures from the June trapping effort. We identified three flea species (*P. irritans*, *P. simulans*, and *O. hirsuta*) from 567 ectoparasite specimens (Table 1). Also, one female tick identified as *Ixodes sculptus* was found. *Pulex irritans* was the most common ectoparasite, occurring on all 10 foxes examined and representing 97% of the male *Pulex* spp. collected, but seven male specimens of the prairie dog flea *P. simulans* were found on six of the 10 swift foxes examined. Also, two specimens of another common prairie dog flea, *O. hirsuta*, were collected from one adult swift fox. Although it has been found in swift fox dens (Kilgore, 1969), *O. hirsuta* has not been found previously on this host. So, herein we record a new host record for this flea. Additionally, all three of these flea species were found in small numbers on a single juvenile coyote collected in the study site. Only *P. simulans* and *O. hirsuta* were found on eight prairie dogs we collected during this period from an active town just at the periphery of our study site.

More than 20 species of fleas have been collected from prairie dogs or their burrows (Cully and Williams, 2001). The five most commonly reported species on this host that are implicated in the transmission of *Y. pestis* are *O. hirsuta*, *Oropsylla tuberculatus cynomuri*, *Opisocrostis labis*, *Neopsylla inopina*, and *Pulex* spp. (Olson, 1981; Cully and Williams, 2001; Pybus and Williams, 2003). However, *Pulex* spp. was reported to be a poor vector for *Y. pestis* (Burroughs, 1947; Cully and Williams, 2001).

Pulex irritans is the most prevalent and abundant ectoparasite on swift foxes (Kilgore, 1969; Miller et al., 1998; Pybus and Williams, 2003; Pence et al., 2004). Kilgore (1969) collected 76 male *P. irritans*, five male *P. simulans*, and 133 females identified only as *Pulex* spp. from 18 swift foxes or at their den entrances in the Oklahoma Panhandle. Miller et al. (1998) found only *P. irritans* on 22 swift foxes in southeastern Colorado. Pence et al. (2004) found three species of fleas (*P. irritans*, *Euhoplopsyllus affinis*, *Dactylopsylla percernis*) and one species of tick (*I. sculptus*) on swift foxes in northwestern Texas; *P. irritans* was the most common ectoparasite on 57 swift foxes examined from 1999 to 2001 during an interepizootic period for plague in prairie dogs. Likewise, we found that the prevalence and abundance of *P. irritans* remained high (Table 1) in swift foxes examined from the same area in 2004 immediately following a prairie dog plague epizootic. Additionally, the two prairie dog fleas *P. simulans* and *O. hirsuta* had become part of the swift fox ectoparasite fauna. We surmise that these fleas transferred to swift foxes from their dead rodent hosts as the foxes came in contact with prairie dog towns where plague was present. However, none of the fleas we tested were positive for *Y. pestis* even though their fox hosts were seropositive.

Evidence that swift foxes feed in prairie dog towns was supported by the fact that all of the foxes we tested were positive for antibodies to *Y. pestis*. The presence of antibodies to *Y. pestis* indicates exposure to the bacteria and existence of plague at some point in time within the swift foxes' home ranges (Gasper and Watson, 2001). Antibodies to *Y. pestis* were not found in serum from 26 swift foxes in Colorado (Miller et al., 2000). Prior to our study, only Harrison (2003) had demonstrated antibodies to *Y. pestis* in one of 16 swift foxes tested in New Mexico.

Based upon the close proximity of most swift fox dens to prairie dog towns in our study site, we speculate that many adult swift foxes could have direct contact with

TABLE 1. Estimates of prevalence, intensity, and abundance of ectoparasites on swift foxes during a plague epizootic in black-tailed prairie dogs on Rita Blanca National Grassland, Texas, USA, August 2004.

Species	Prevalence[a]		Abundance[b]	Intensity[c]		
	NI/NE	%	$\bar{X}\pm$SE	$\bar{X}\pm$SE	Range	Total[d]
Pulex irritans	10/10	100	21.20±4.86	21.20±4.86	4–56	216
Pulex simulans	6/10	60	0.70±0.21	1.17±0.17	1–2	7
Pulex spp.[e]	9/10	90	35.80±7.74	39.78±7.42	6–74	341
Orospsylla hirsuta	1/10	10	0.20±0.20	1.00±0.00	0	2
Ixodes sculptus	1/10	10	0.10±0.10	1.00±0.00	0	1

[a] Number of hosts infected/number of hosts examined.

[b] Total number of specimens collected/number of hosts examined.

[c] Total number of specimens collected/number of hosts infected.

[d] Total number of specimens collected.

[e] Females could not be identified to species.

Y. pestis–infected prairie dogs or fleas. The minimum distance to a prairie dog town from swift fox capture locations was 8.80±1.22 km. This is within swift foxes' reported average nightly travel distances of 5.7–18.5 km (Hines and Case, 1991; Covell et al., 1996).

The immune response to *Y. pestis* mounted by most rodent-consuming carnivores is usually sufficient to prevent development of clinical symptoms of the disease (Barnes, 1982; Gage et al., 1995; Gasper and Watson, 2001). Domestic dogs (*Canis lupus familiaris*), coyotes, red foxes (*Vulpes vulpes*), and gray foxes (*Urocyon cinereoargenteus*) typically seroconvert when exposed to *Y. pestis* and rarely die from the infection (Gage et al., 1995). Williams et al. (1991) found high prevalences of antibodies against *Y. pestis* but with no gross or microscopic evidence of plague in badgers (*Taxidea taxus*), coyotes, and striped skunks (*Mephitis mephitis*). In our study, we believe that swift foxes probably fed on infected prairie dogs and/or other rodents, acquiring antibodies to this bacterium (Pybus and Williams, 2003) without developing signs of clinical illness.

Herein, we have shown that swift foxes can at least temporarily acquire fleas from their plague-diseased prey species and that they could potentially transmit *Y. pestis* to rodent hosts in uninfected areas. Serologic surveillance of free-ranging swift foxes has shown that they are capable of acquiring the infection without developing overt clinical signs of disease. However, none of the fleas we tested from swift foxes were positive for *Y. pestis*. Thus, we question how effective this host and its flea vectors are in the transmission of this pathogen.

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