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FLEA ABUNDANCE, DIVERSITY, AND PLAGUE IN GUNNISON'S PRAIRIE DOGS (*CYNOMYS GUNNISONI*) AND THEIR BURROWS IN MONTANE GRASSLANDS IN NORTHERN NEW MEXICO

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ABSTRACT: Plague, a flea-transmitted infectious disease caused by the bacterium *Yersinia pestis*, is a primary threat to the persistence of prairie dog populations (*Cynomys* spp.). We conducted a 3-yr survey (2004–2006) of fleas from Gunnison's prairie dogs (*Cynomys gunnisoni*) and their burrows in montane grasslands in Valles Caldera National Preserve in New Mexico. Our objectives were to describe flea communities and identify flea and rodent species important to the maintenance of plague. We live-trapped prairie dogs and conducted burrow sweeps at three colonies in spring and summer of each year. One hundred thirty prairie dogs and 51 golden-mantled ground squirrels (*Spermophilus lateralis*) were captured over 3,640 trap nights and 320 burrows were swabbed for fleas. Five flea species were identified from prairie dogs and ground squirrels and four were identified from burrow samples. *Oropsylla hirsuta* was the most abundant species found on prairie dogs and in burrows. *Oropsylla idahoensis* was most common on ground squirrels. Two colonies experienced plague epizootics in fall 2004. Plague-positive fleas were recovered from burrows (*O. hirsuta* and *Oropsylla tuberculata tuberculata*) and a prairie dog (*O. hirsuta*) in spring 2005 and summer 2006. Three prairie dogs collected in summer 2005 and 2006 had plague antibody. We found a significant surge in flea abundance and prevalence, particularly within burrows, following plague exposure. We noted an increased tendency for flea exchange opportunities in the spring before *O. hirsuta* reached its peak population. We hypothesize that the role of burrows as a site of flea exchange, particularly between prairie dogs and ground squirrels, may be as important as summer conditions that lead to buildup in *O. hirsuta* populations for determining plague outbreaks.

Key words: Insect vectors, *Oropsylla tuberculata cynomuris*, reservoir hosts, small mammals.

INTRODUCTION

Plague is an infectious vector-borne disease caused by the bacterium *Yersinia pestis* and transmitted between mammals by fleas (Biggins and Kosoy, 2001). Since its introduction to the United States around 1899–1900, sylvatic plague has become established in native rodent species and contributed to the precipitous decline of endemic prairie dog (*Cynomys* spp.) populations (Cully and Williams, 2001; Gage and Kosoy, 2005). Prairie dogs are particularly susceptible to plague because they have no innate immunity and live in large colonies with elaborate burrow systems that favor reproduction and survival of the flea vector (Cully and Williams, 2001; Gage and Kosoy, 2005). Mortality rates in excess of 95% in exposed prairie dog colonies (Cully and Williams, 2001) affect not only prairie dogs, but also prairie-dog–dependent species including the black-footed ferret (*Mustela nigripes*) (Houston et al., 1986). Prairie dog epizootics can amplify plague by releasing large numbers of infected fleas into the environment. Predators that are attracted to the sick and dead prairie dogs can become infected by consuming

these animals or by being bitten by fleas. Predators can spread potentially infectious fleas to other sites, including previously unaffected prairie dog colonies, thereby contributing to the local spread of plague (Lechleitner et al., 1968). Although we have yet to identify the definitive reservoir host and flea vector species important to the maintenance and spread of plague within the United States, it is clear that prairie dog fleas are able to perpetuate plague among colony members over the course of the epizootic (Webb et al., 2006; Wilder et al., 2008). However, there is little evidence to suggest that these fleas, or the prairie dogs, can contribute to long-term plague maintenance cycles. Research focused on the factors that precede and lead to prairie dog plague epizootics are needed to identify other host and flea species that maintain plague in the ecosystem and transfer plague to prairie dog colonies.

Several rodent species have been proposed as enzootic or maintenance hosts, including grasshopper mice (*Onychomys* spp.) in black-tailed prairie dog towns (*Cynomys ludovicianus*) and *Peromyscus* spp. in white-tailed (*Cynomys leucurus*) and Gunnison's prairie dog towns (*Cynomys gunnisoni*) (Gage et al., 1995; Thiagarajan et al., 2008). Others have provided evidence for maintenance cycles that involve soil or flea stages (Lechleitner et al., 1968; Ayyadurai et al., 2008; Eisen et al., 2008). The most likely long-term scenario involves several factors and relies on a suite of animals, connected by flea vectors (Biggins and Kosoy, 2001). In general, flea species are more likely to transmit plague if they are highly susceptible to *Y. pestis* and have low host specificity (Gage and Kosoy, 2005). However, low-efficiency vectors may be important to the spread of plague if they are common in the environment (Kartman et al., 1962; Lechleitner et al., 1968; Eisen et al., 2006). Indeed, flea abundance is positively related to the vector potential for plague for a given flea species (Krasnov et al., 2006), and abundance and prevalence of flea species is an important determinant of *Y. pestis* transmission (Lorange, 2005; Eisen et al., 2006).

We report the results of a 3-yr survey of flea populations from Gunnison's prairie dogs and their burrows in montane grassland in New Mexico. We also analyzed flea and prairie dog blood samples for the presence of plague. Our objectives were to describe the flea communities within the Valles Caldera National Preserve (VCNP) prairie dog towns, compare animal and burrow flea loads across years and seasons in sites with and without plague, and identify flea species that may be important to the maintenance or transmission of plague within this population.

MATERIALS AND METHODS

Site descriptions

The study was conducted at the VCNP, Sandoval County, New Mexico. Study areas were in montane grassland habitat, with elevations of 2,460–2,640 m. Annual precipitation averages 638 mm, with approximately 45% falling during the summer monsoon season (July–September). Mean annual temperature is 4.5 C, with mean July temperatures of 15 C and mean January temperatures of −5.3 C. Three grassland habitats with prairie dog towns were selected for sampling (Fig. 1): Redondo Meadow (35°51′33″N, 106°36′15″W), El Cajete (35°50′18″N, 106°33′33″W), and Valle Grande (35°51′21″N, 106°29′29″W). The Redondo Meadow (elevation 2,459 m) vegetation was dominated by *Bouteloua gracilis*, *Potentilla hippiana*, *Erigeron flagellaris*, *Artemisia carruthii*, and *Polygonum douglasii*. Soils were classified as fine, smectic, superactive, frigid, Vertic Argialboll (Lyquilar series). The El Cajete (2,638 m) vegetation was dominated by *Bromus inermis*, *P. hippiana*, *Taraxacum officinale*, *E. flagellaris*, and *Achillea millefolium*. Soils were ashy, glassy, frigid, Vitrandic Argiustoll (Jaramillo series). Valle Grande (2,590 m) vegetation was dominated by *Festuca arizonica*, *Koelaria macrantha*, *Poa pratensis*, *Muhlenbergia montana*, *P. hippiana*, *Carex* spp., and *Antennaria rosea*. Soils were loamy over ashy-pumiceous, mixed over glassy, superactive, frigid Vitrandic Argisutoll (Vallande series).

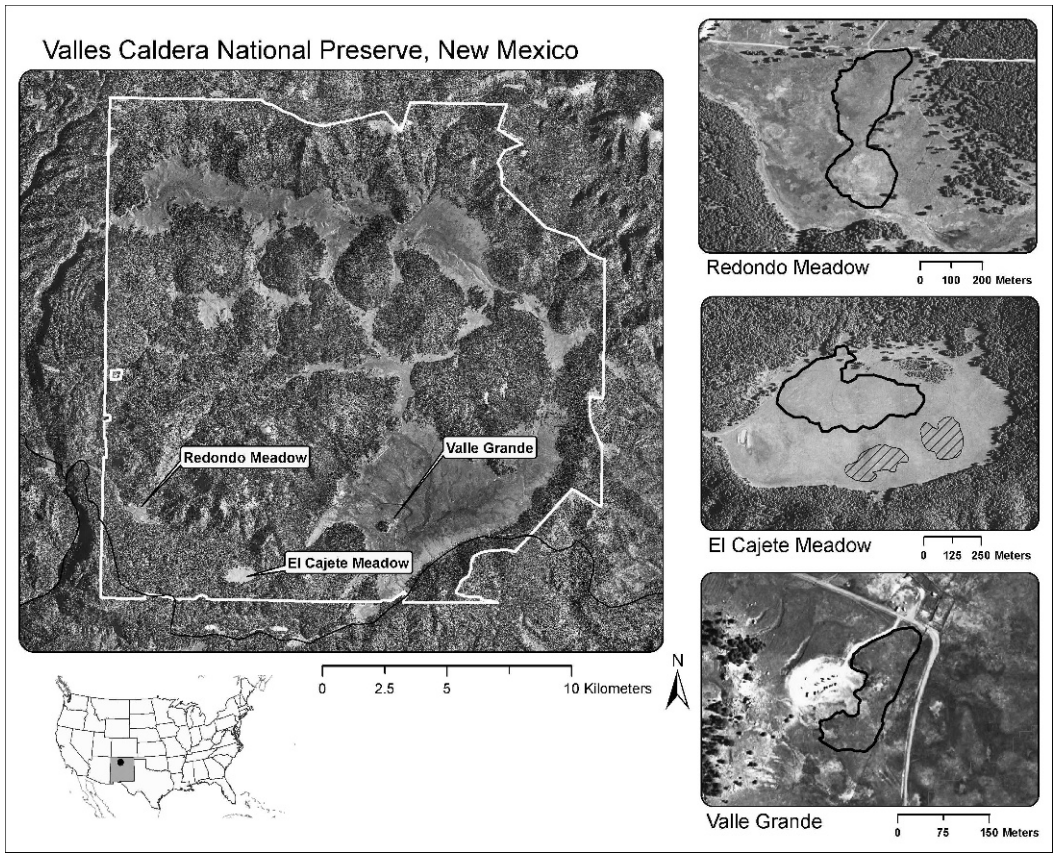

FIGURE 1. Location of three study sites in Valles Caldera National Preserve, northern New Mexico, USA. Outlines indicate perimeter of colony area that was the focus of trapping efforts and burrow sweeps, May 2004–September 2006. One colony, El Cajete, contained areas where prairie dog burrows were blocked at the time of study (hatched polygons).

Colony surveys

We live trapped prairie dogs and conducted burrow sweeps in spring (May–June) and summer (August–September) each year. El Cajete and Valle Grande were not sampled in 2004. Each colony lay in a valley bottom (Fig. 1) isolated from other colonies by mountain ranges, which prevented short-term movement of prairie dogs between sites. During the first trapping session at each site, we marked and took global positioning system (GPS) coordinates of active burrows. We determined activity by the presence of scat, scratching, or fly infestation at each burrow entrance. We updated these burrow characteristics at the start of each trapping period. If burrows appeared abandoned, we chose new burrows in the immediate area and processed them as described above. Two methods were used to estimate prairie dog densities: Active count (Severson and Plumb, 1998) and burrow survey transects (Biggins et al., 1993). Active count is an effective method of population estimation in both black- and white-tailed prairie dogs (Fagerstone and Biggins, 1986; Menkens et al., 1990; Severson and Plumb, 1998). In 2004, we attempted to quantify prairie dogs by counting the number of aboveground animals within a 300 × 300-m bounded area at 15-min intervals over a 3-hr period. However, after 2 days, we determined that accurate sightings were not possible because of vegetation and intermittent vehicle traffic. The latter negatively affected prairie dog activity patterns (prairie dogs retreated underground following a vehicle's passage). Therefore, we decided to approximate relative colony size by estimating burrow density with a belt-transect method. Though criticized (Powell et al., 1994; Hoogland, 1995; Severson and Plumb, 1998), this method has been used successfully to estimate relative differences

between towns and was found to correlate with prairie dog density in at least one study (Johnson and Collinge, 2004). To conduct burrow surveys, we used a GPS unit (Trimble Navigation, Sunnyvale, California, USA) to track our path as we walked the perimeter of each colony and to calculate area based on each perimeter. We marked the boundary with pin flags and walked a series of randomly placed 100 × 2-m transects until we covered 10% of the colony area. We counted and classified (active or inactive) burrows within 2 m of the transect with the use of a 2-m polyvinyl chloride pipe held perpendicular to the transect along a 100-m measuring tape.

Rodent trapping

Two to four Tomahawk® live traps (Size 70, Tomahawk Live Trap Company, Tomahawk, Wisconsin, USA) were set around each burrow for a total of 76–104 traps per colony. Traps were baited with a combination of rolled oats and sweet feed and wired open for at least 4 days prior to trapping to acclimatize prairie dogs to traps. Prairie dogs were trapped for 3 consecutive days following the prebaiting period. Traps were opened and baited before sunrise (approx. 5:30 AM) each morning and checked between 8:30 AM and 9:30 AM. Depending on capture success and weather conditions, traps were sometimes left open and checked every 45 min until noon. At a processing station distant from the trapping site, each prairie dog was weighed to the nearest gram with a spring balance (Pesola AG, Rebmattli, Baar, Switzerland), gender was recorded, and the animal was given a uniquely numbered ear tag (Gey Band and Tag Co., Norristown, Pennsylvania, USA). Animals were processed with the use of canvas cones described by Hoogland (2005). Blood samples were collected by clipping a toenail on a rear foot just distal to the quick and blotting the blood onto a Nobuto filter strip (Toyo Roshi Kaisha, Limited, Tokyo, Japan). Toenails were thoroughly cleaned with alcohol pads before being clipping and treated with a sulfur compound (styptic) after clipping to prevent infection. Nobuto strips were air dried and placed in an envelope for short-term storage.

Fleas were collected by holding each animal over a plastic basin containing a 20 × 20–cm felt cloth and thoroughly brushing the fur with a flea comb or toothbrush until all observed fleas had fallen into the basin. Fleas were then collected from the felt cloth, placed into a labeled cryovial, and flash frozen in liquid nitrogen (−70 C).

After processing, prairie dogs were returned to their traps and released at the site of capture. Prairie dogs were processed only during the first capture of each trapping period. Animal handling procedures were approved by the Animal Care and Use Committee of the University of New Mexico (04MCC002). Blood and flea samples were processed under the guidelines and standardized protocols for the safe handling of biohazard material where appropriate, including Mills et al. (1995), and written protocols from the laboratories of Paul Keim, Megan Friggens, and Kenneth Gage.

Burrow sampling

Fleas were collected from burrows immediately prior to, or following, prairie dog trapping. Twenty burrows were swabbed at each site each season by attaching a white flannel cloth (20 × 20 cm) to the end of a plumber's snake and extending the cloth into burrow to depth of at least 1 m. After 30 sec the cloth was removed and put into a 3.8-l plastic zipper-seal bag, which was sealed and placed in a cooler with dry ice. Burrow-sweep cloths were kept frozen until examination. Each cloth was examined for ectoparasites with the use of a dissecting microscope or magnifying glass. All ectoparasites were stored in labeled cryovials and frozen at −20 C until laboratory analysis.

Fleas were examined under a dissecting microscope and identified to genus, species, or subspecies according to Furman and Catts (1982), Hubbard (1947), and Lewis (2002). Voucher specimens of each flea species were deposited at the Division of Vector-borne Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado, USA.

Plague tests

Blood samples were analyzed for the presence of F1 plague antibodies with the use of a standard passive hema-agglutination (PHA) test (Williams et al., 1976; Chu, 2000). Briefly, blood samples were eluted overnight in a 1 M sodium borate solution and 25 µl of the eluent were used for PHA. Positive samples were confirmed with passive hemainhibition (PHI) tests. Antibody-positive results were recorded as reciprocal titers, denoting the concentrations as determined by titration. Titers <1:32 were considered nonspecific and not positive.

Fleas were examined for the presence of plague with the use of a multiplex polymerase chain reaction (PCR) as described by Stevenson et al. (2007). This analysis targets a region of the plasminogen (pla) activator gene of *Y.*

pestis (478 base pairs). Most fleas were analyzed individually for the presence of *Y. pestis* DNA. However, for nine hosts and two burrows that yielded >25 fleas, only the first 5 fleas were analyzed individually. The remaining fleas (104 fleas) were analyzed in pools of 2–5 fleas. DNA was obtained from the first 30 fleas by triturating individual fleas in 100 µl BHI (Becton Dickenson, Sparks, Maryland, USA). The remaining fleas were processed with a DNA extraction procedure described by Allender et al. (2004). Following processing, 2.5 µl of triturate or extracted DNA were used for PCR.

Statistical analysis

We did not compare flea abundance and prevalence across the sites because we surveyed colonies that were accessible by vehicle rather than randomly selecting among all available colonies in the VCNP. We assessed differences in flea abundance and flea prevalence between sampling periods, seasons (spring vs. summer), and years across all sites and within each site with the use of generalized linear model analysis (PROC GLIMMIX, Statistical Analysis Software, SAS 9.2, SAS Institute Inc., Cary, North Carolina, USA). For comparisons across all sites, we included colony as a random effect in our model. Prevalence data were analyzed with a binomial distribution and logit link, whereas abundance data were analyzed with a Gaussian distribution and log link. Statistical significance was set at $P<0.05$ and Tukey-adjusted P values were used to reduce the likelihood of type I errors.

RESULTS

The Redondo Meadow colony encompassed approximately 14 ha with an average of 273 active burrows/ha; the El Cajete colony was 15 ha with 60 active burrows/ha; the Valle Grande colony was 2.7 ha with 120 burrows/ha.

We captured 130 prairie dogs over 3,640 trap nights (including 22 recaptures). The majority (107, including 10 recaptures) were from Redondo meadow, 22 (including 1 recapture) were from Valle Grande, and none were caught in El Cajete. In addition, 51 golden-mantled ground squirrels (*Spermophilus lateralis*) were captured from El Cajete (40, including 5 recaptures) and Valle Grande (11, including 1 recapture). Voucher specimens of three *S. lateralis* were deposited in the Museum of Southwestern Biology, University of New Mexico, Albuquerque, New Mexico, USA (NK143015, NK 143434, and NK143450).

Prairie dogs were abundant in Redondo during 2004 (72 captures), but few animals emerged from burrows the following spring 2005. By summer 2005, many burrows showed signs of decline, and capture success was low (five animals). The population appeared to be in recovery in 2006 and we captured 10 and 15 prairie dogs in the spring and summer, respectively. El Cajete was reported to have a large and active population of prairie dogs in 2004 (Parmenter, pers. obs.). When trapping began at El Cajete in spring of 2005, >100 burrows were surveyed and, though open, very few appeared to be occupied by prairie dogs. Many additional burrows had blocked entrances. Trapping efforts yielded no prairie dogs, but 25 golden-mantled ground squirrels were caught in traps laid around prairie dog burrows. No prairie dogs and only 15 golden-mantled ground squirrels were captured from El Cajete in 2006. Valle Grande had small but stable populations of prairie dogs and golden-mantled ground squirrels over the course of this study, with 14 and 8 prairie dog captures, and six and five ground squirrel captures for 2005 and 2006, respectively.

We collected 633 fleas from prairie dogs, 167 fleas from prairie dog burrows and 66 from golden-mantled ground squirrels (Table 1). Golden-mantled ground squirrels were not sufficiently sampled for further analysis. Prevalence of infestation (percent infested with fleas) and abundance (number fleas/sample±SD) were positively correlated across sites and years for both prairie dogs ($r^2=0.61$, $P=0.007$, df=9) and burrows ($r^2=0.63$, $P=0.0007$, df=13). Trends in flea abundance and prevalence between prairie dogs and burrows were similar in Redondo Meadow prior to plague, but did not correspond

TABLE 1. Flea species and number collected from Gunnison's prairie dog burrows, Gunnison's prairie dogs, *Cynomys gunnisoni*, and golden-mantled ground squirrels, *Spermophilus lateralis*, caught in the Valles Caldera National Preserve in northern New Mexico, 2004–2006.

Source[a]	Flea species	Spring	Summer	Total
Burrow (n=280)	*Catallagia decipiens*	1	1	2
	Oropsylla hirsuta	85	47	132
	Oropsylla idahoensis	16	4	20
	Oropsylla tuberculata cynomuris	7	1	8
	Oropsylla tuberculata tuberculata	5	0	5
Total GPD (n=130)		114	53	167
	O. hirsuta	79	507	586
	O. idahoensis	5	23	28
	O. t. cynomuris	14	0	14
	O. t. tuberculata	3	2	5
Total GMGS (n=51)		101	532	633
	Eumolpianus eumolpianus cyrturus	3	2	5
	Opisodaysis enoplus	0	1	1
	O. hirsuta	0	9	9
	O. idahoensis	21	29	50
	O. t. tuberculata	1		1
Total		25	41	66
Grand total		240	626	866

[a] GPD = Gunnison's prairie dog; GMGS = golden-mantled ground squirrel.

after plague outbreaks. Trends in flea abundance and prevalence were not similar for prairie dogs and burrows at other sites.

Across all sites, mean abundance and prevalence of infestation were 4.89±8.31 and 65% for prairie dogs and 0.62±2.79 and 18% for burrow samples. Annual flea abundance on prairie dogs was significantly greater in 2006 (6.4±7.74) than 2005 (5.2±10.4) and 2004 (4.1±7.74; P=0.04, df=3). Annual abundance was greatest in 2005 (1.0±4.1) and lowest in 2006 (0.32 ±1.13) for burrows (P<0.05, df=5). Prevalence was highest in 2005 (76% and 23%) and lowest in 2004 (58% and 13%) for both prairie dogs and burrows, respectively (P<0.05 for both, df=3 or 5). Averaged across sites and years, abundance and prevalence of fleas in prairie dogs was more than two times greater in the summer than in the spring (5.9±8.1 vs. 2.52±8.35, and 78% vs. 35%, respectively). In burrows, summer prevalence and abundance of fleas were lower than spring prevalence and abundance (0.41± 2.1 vs. 0.83±3.34 and 13% vs. 24%, respectively).

Significant trends in flea abundance and prevalence for each sampling period within each site were found (Figs. 2, 3). Prevalence of fleas in burrows was significantly greater in 2005 than other years for both Redondo Meadow and El Cajete. Valle Grande had a significantly greater overall prevalence of fleas in spring than summer (Fig. 2B). Prior to the suspected plague outbreak (fall–winter of 2004), flea abundance and prevalence of infestation with fleas were lower in spring than summer for both prairie dogs and burrows in Redondo (Figs. 2A, B; 3A, B). However, flea abundance was significantly higher in burrows in the spring following plague exposure in Redondo Meadow (Fig. 2B). With the exception of 2005, when prevalence of flea infestation was 100% for both sampling periods, the prevalence of flea-infested prairie dogs increased significantly from spring to summer in Redondo Meadow (Fig. 2A).

Seven flea species were identified from the VCNP colonies (Table 1). *Oropsylla hirsuta* was the most abundant species found on prairie dogs and in burrows.

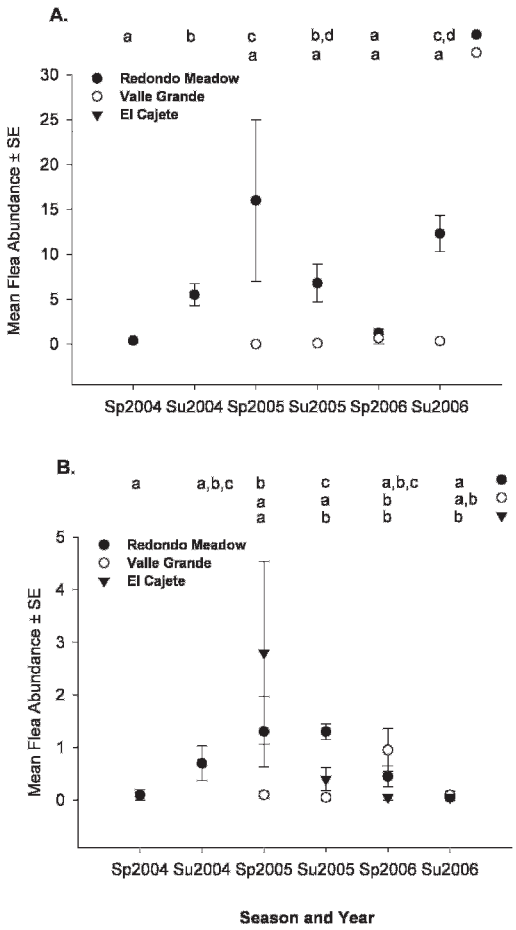

FIGURE 2. (A) Mean abundance (number of fleas/host±SE) of fleas from prairie dogs captured from two colonies in the Valles Caldera National Preserve during six collection periods 2004–2006. (B) Mean abundance of fleas collected from prairie dog burrows sampled from three colonies in the Valles Caldera National Preserve during six collection periods, 2004–2006. Letters signify significant differences ($P<0.05$) among sampling periods for each site, where those points which share a letter were not different across sampling periods.

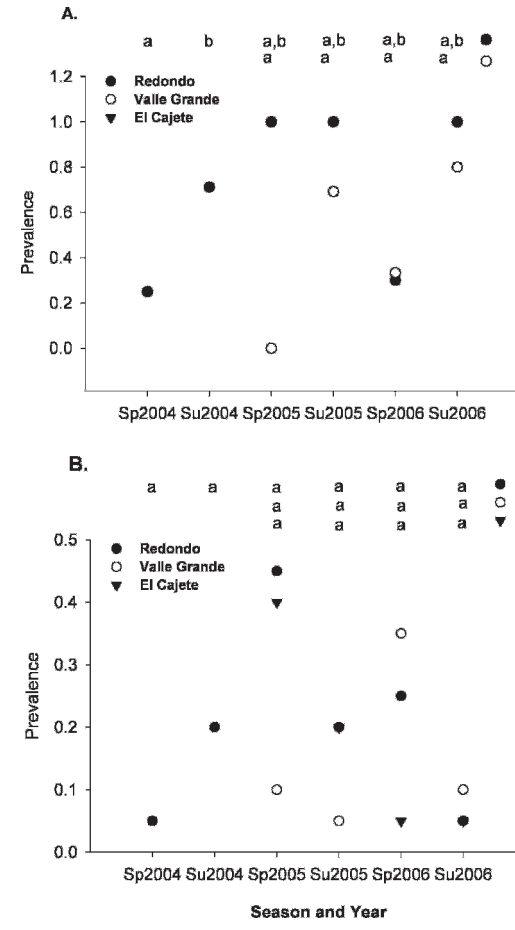

FIGURE 3. (A) Prevalence of flea infestation (number of infested animals/total animals) for prairie dogs captured from two colonies in the Valles Caldera National Preserve during six collection periods, 2004–2006. (B) Prevalence of flea infestation (number of infested burrow sweeps/total sweeps) from prairie dog burrows sampled from three colonies in the Valles Caldera National Preserve during six collection periods from 2004–2006. Letters signify significant differences ($P<0.05$) among sampling periods for each site, where those points which share a letter were not different across sampling periods.

Overall, flea diversity was higher during spring than summer sampling (3 vs. 2 and 3 vs. 2.3 species for prairie dogs and burrows, respectively). Species-specific trends in seasonality were apparent among the fleas, although patterns did not correspond between animals and burrows (Table 1). Fleas that were positive for plague were recovered from two burrows (two *O. hirsuta*, one *Oropsylla tuberculata* *tuberculata*) and one prairie dog (one *O. hirsuta*) from Redondo Meadow during the spring, 2005. Three prairie dogs collected in summer 2005 were antibody positive: one from Redondo Meadow with a titer of 1:256 and two from Valle Grande each with titers of 1:1024. Plague was detected again in the summer of 2006 in fleas recovered from a burrow in El Cajete

(1 *O. hirsuta*), and from a prairie dog captured in Redondo Meadow (14 *O. hirsuta*). Two prairie dogs from Redondo Meadow (one recapture) were antibody positive (1:512 and 1:2048) to plague in the spring of 2006.

DISCUSSION

During this study, plague epizootics occurred in two of three colonies. Although we found plague antibodies in two prairie dogs captured at Valle Grande, there were no other overt signs (e.g., infected fleas or prairie dog die-off) of a plague epizootic in this colony. In contrast, the prairie dogs inhabiting Redondo Meadow and El Cajete experienced severe population declines and both fleas and prairie dogs were found with recent exposure to plague. We were only able to detect plague immediately following the prairie dog population crash, an observation that is similar to those reported for other Gunnison's prairie dog colonies (Lechleitner et al., 1968), but stands in contrast to trends reported for white-tailed prairie dogs (Anderson and Williams, 1997).

We found that the number of fleas per host and per infested host and burrow were higher in plague-affected than nonaffected colonies. Anderson and Williams (1997) found significantly higher numbers of fleas in plague-affected than nonaffected white-tailed prairie dog colonies. We collected more fleas from a greater proportion of burrows and prairie dogs during the season of an epizootic and the season immediately following it than the years prior to or following an epizootic. In El Cajete, where prairie dogs were essentially eliminated, flea abundance declined following plague die-off (Fig. 2). In contrast, we did not detect a significant decline in the flea populations of Redondo Meadow where prairie dogs were in recovery. Indeed, by 2006, 2 yr after the epizootic, Redondo Meadow (recovering population) and Valle Grande (stable population), showed similar seasonal patterns in flea abundance and prevalence, as compared to El Cajete (Figs. 2, 3), a trend consistent with recovery at other prairie dog towns (Lechleitner et al., 1968). Significant declines in flea abundance following plague die-offs have been attributed to reduced host populations (Salkeld and Stapp, 2008) and subsequent low survival among off-host flea populations exposed to desiccation and possibly high temperatures (Gage and Kosoy, 2005). A similar density-dependent interaction may have fostered the increase in flea abundance in the summer of 2004, just prior to plague related die-offs (Figs. 2, 3). Most other studies have reported spring surges in flea populations prior to outbreaks, which corresponds more with the typical annual patterns in flea abundance seen in this study and elsewhere (Anderson and Williams, 1997; Cully et al., 1997; Stenseth et al., 2006). Whatever the cause, it seems likely that the increase in prevalence and abundance of fleas in summer 2004 was a key precursor to plague outbreak in this colony.

The seasonal trends in flea species composition at VCNP were very similar to those reported elsewhere: *Oropsylla tuberculata cynomuris* populations peaked in early spring (Cully et al., 1997; Salkeld and Stapp, 2008), *Oropsylla idahoensis* populations peaked in midsummer (Anderson and Williams, 1997), and *O. hirsuta* numbers were greatest during mid or late summer (Salkeld and Stapp, 2008). Again, the only exception was seen in 2004, just prior to the epizootic.

Oropsylla hirsuta and *O. t. tuberculata* were the primary fleas involved in prairie dog plague epizootics at VCNP. *Yersinia pestis*–infected *O. hirsuta* were collected from prairie dogs and burrows and plague-infected *O. t. tuberculata* were collected from burrows. These flea species were widespread at VCNP colonies and readily parasitize both golden-mantled ground squirrels and prairie dogs. *Oropsylla hirsuta* has been implicated in the spread

of plague in prairie dog towns (Cully and Williams, 2001) and appears to be the most important flea with respect to supporting fast-moving transmission during the epizootics commonly reported to occur in prairie dog colonies (Ubico et al., 1988; Cully et al., 1997). This is the first report of plague in *O. t. tuberculata* collected from prairie dog burrows, although its sister species, *O. t. cynomuris*, is commonly infected with plague (Ecke and Johnson, 1950; Lechleitner et al., 1968; Cully et al., 1997; Ubico et al., 1988; Anderson and Williams, 1997; Holmes et al., 2006). The separation of *O. t. tuberculata* and *O. t. cynomuris* into distinct taxa is not supported by all authorities, despite the general recognition that the latter come primarily from prairie dogs and former from ground squirrels (Lewis, 2002). Because most studies distinguish between *O. t. cynomuris* and *O. t. tuberculata*, it seems reasonable to preserve this level of classification in this study. We note, however, that *O. t. tuberculata* has been found on prairie dogs in the western United States, including northeastern Utah, on *C. leucurus* (Stark, 1958) and on *C. gunnisoni* in the relatively high elevation South Park region of central Colorado (Ecke and Johnson, 1950).

Ground squirrels may play a role in transferring infected fleas between reservoir host species (Lechleitner et al., 1968; Anderson and Williams, 1997). In addition, ground squirrels and prairie dogs often share flea species and flea exchange between these hosts is particularly evident during plague outbreaks (Ecke and Johnson 1950; Anderson and Williams, 1997; Cully and Williams, 2001). In the VCNP, golden-mantled ground squirrels were abundant in prairie dog towns, readily used prairie dog burrows, and ground squirrel-associated fleas found in prairie dog burrows had evidence of infection with *Y. pestis*. Thus, the presence of ground squirrels and the ready transfer of fleas between ground squirrels and prairie dogs provides an increased risk of plague exposure to prairie dogs.

Burrows are an important habitat for fleas and are a likely site of flea exchange and plague transmission among rodents in VCNP. In general, nonprairie dog fleas were more abundant and prevalent in burrows during spring in both this and other Gunnison's colonies in New Mexico (Cully et al., 1997). We saw clear shifts in the prevalence and abundance of *O. hirsuta* and *O. idahoensis* from burrow to prairie dogs as summer progressed (Table 1). In contrast, *O. idahoensis* were equally present on ground squirrel hosts during spring and summer (Table 1). Therefore, it appears that burrows provide favorable conditions for early season population increases in both prairie dog and non-prairie-dog–associated species. In addition, the capacity for *O. idahoensis* to utilize burrows successfully and parasitize prairie dogs readily is a strong indication that prairie dog burrows act not only as refugia to off-host populations of prairie dog fleas, but may foster flea exchange among hosts. This may also be a mechanism for flea exchange between prairie dogs and other species such as the American badger, black-footed ferret, and Burrowing Owl known to inhabit prairie dog burrows (Hoogland, 2005 and references therein).

Fleas are thought to play an important role in the maintenance of plague over time and are the primary mechanism by which plague is transmitted among hosts (Gage and Kosoy, 2005). At least two prairie dog flea species have been found in burrows and infected with plague up to a year after an epizootic (Lechleitner et al., 1968), implicating a significant role in plague maintenance cycles. However, the low resistance of prairie dogs to plague suggests that these fleas are unlikely to maintain plague in an enzootic cycle. On the other hand, burrows harbor plague-infected fleas of many species for many months following the occurrences of epizootics (Lechleitner et al., 1968; Ubico

et al., 1988; Anderson and Williams, 1997; Cully et al., 1997; Holmes et al., 2006). Also, the immigration of other rodent species into areas that have previously experienced plague epizootics (as reported by Ecke and Johnson, 1950 and Lechleitner et al., 1962) would allow fleas to continue to transmit plague to new animals. The findings of this study support the conclusion of Cully and Williams (2001) that prairie dog burrows provide ample opportunity for the interspecific spread of *Y. pestis* between prairie dogs and other animals. In addition, recent evidence for the persistence of plague in soil may point to a more direct role of burrows in providing refuge for plague pathogens (Ayyadurai et al., 2008; Eisen et al., 2008).

In conclusion, prairie dog burrows are an important component of plague cycles as a source for infectious off-host fleas, a site of flea exchange, and potentially by harboring the plague pathogen in soil. The ready exchange of fleas between ground squirrels and other species, in particular, prairie dogs, effectively increases prairie dog exposure to fleas and flea-borne pathogens and the likelihood of interspecific flea transfer in areas where *Spermophilus* and *Cynomys* coexist.

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