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Flea Abundance on Black-Tailed Prairie Dogs (*Cynomys ludovicianus*) Increases During Plague Epizootics

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Abstract

Black-tailed prairie dogs (*Cynomys ludovicianus*) on the Great Plains of the United States are highly susceptible to plague, caused by the bacterium *Yersinia pestis*, with mortality on towns during plague epizootics often approaching 100%. The ability of flea-borne transmission to sustain disease spread has been questioned because of inefficiency of flea vectors. However, even with low individual efficiency, overall transmission can be increased if flea abundance (the number of fleas on hosts) increases. Changes in flea abundance on hosts during plague outbreaks were recorded during a large-scale study of plague outbreaks in prairie dogs in north central Colorado during 3 years (2004–2007). Fleas were collected from live-trapped black-tailed prairie dogs before and during plague epizootics and tested by PCR for the presence of *Y. pestis*. The predominant fleas were two prairie dog specialists (*Oropsylla hirsuta* and *Oropsylla tuberculata cynomuris*), and a generalist flea species (*Pulex simulans*) was also recorded from numerous mammals in the area. The three species differ in seasonal abundance, with greatest abundance in spring (February and March) and fall (September and October). Flea abundance and infestation intensity increased during epizootics and were highest on prairie dogs with *Y. pestis*–infected fleas. Seasonal occurrence of epizootics among black-tailed prairie dogs was found to coincide with seasonal peaks in flea abundance. Concentration of infected fleas on surviving animals may account for rapid spread of plague during epizootics. In particular, the role of the generalist flea *P. simulans* was previously underappreciated.

Key Words: Black-tailed prairie dog—*Cynomys ludovicianus*—Plague—Yersinia.

Introduction

Plague, the disease caused by the bacterium *Yersinia pestis*, invaded western North America after 1899, at first in coastal cities but later as a flea-borne disease of wild rodents (Ecke and Johnson 1952, Gage and Kosoy 2005). Earliest reports of plague in black-tailed prairie dogs (*Cynomys ludovicianus* Ord, 1815) on the Great Plains are from the 1940s (Ecke and Johnson 1952), and prairie dogs experience rapid epizootics with mortality on towns approaching 100% (Cully and Williams 2001, Stapp et al. 2004, Pauli et al. 2006). Historically, black-tailed prairie dogs were abundant, but declines in their populations are attributed to habitat loss, eradication efforts, and plague (Antolin et al. 2002). Plague in prairie dogs has consequences for conservation of endangered species like black-footed ferrets (*Mustela nigripes* Audubon and Bachman, 1851) (Roelle et al. 2006) and represents health risks to humans: an estimated 14% of human cases in the United States are associated with plague in prairie dogs (Seery et al. 2003).

The primary fleas of prairie dogs are *Oropsylla hirsuta* (Baker, 1895) and *Oropsylla tuberculata cynomuris* Jellison, 1939. *Pulex simulans* Baker, 1895, which has a broad host range, has also been recovered from prairie dogs and their burrows in many western states (Hopla 1980, Cully et al. 2000, Holmes et al. 2006, Salkeld and Stapp 2008). Prairie dog fleas were considered inefficient vectors of *Y. pestis* because they rarely exhibit blockage (Eskey and Haas 1940, Burroughs 1947). Blockage in fleas' midguts occurs several days after an infectious blood meal as replicating bacteria form a biofilm, and in fleas like the Oriental rat flea *Xenopsylla cheopis* (Rothschild, 1903), blockage leads to efficient transmission as blocked fleas continually feed and regurgitate bacteria into the feeding site on mammalian hosts (Hinnebusch et al. 1996,

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Gage and Kosoy 2005, but see Eisen et al. 2007). An epidemiological model by Webb et al. (2006) showed that transmission by blocked fleas was unlikely to drive rapid epizootics among prairie dogs because of the time delay (>5 days) before blocked fleas transmit bacteria. On the other hand, fleas must play a role in epizootics, as application of insecticides to burrows during epizootics retards or prevents further plague spread (Seery et al. 2003, Hoogland et al. 2004). Further, seasonal plague occurrence and rapid spread of human plague epidemics after die-offs among commensal rats (*Rattus* spp.) provides additional evidence for the importance of flea-borne transmission. Epizootics and human epidemics are also associated with local climate and seasonal peaks in flea abundance on hosts (Indian Plague Commission 1908, Pollitzer 1954, Cavanaugh 1971, Enscore et al. 2002, Collinge et al. 2005, Savage 2007).

These observations suggest that alternative transmission routes have a role in rapid spread of plague among prairie dogs during epizootics, for instance by respiratory droplets or cannibalism of infected carcasses (Gage and Kosoy 2005, Webb et al. 2006). Within their extensive towns, prairie dogs live in coteries, social groups consisting of 2–4 adult females, 1–2 adult males, yearlings, and juvenile offspring. Coterie members defend territories surrounding the underground burrows they excavate and maintain (Hoogland 1995). Sociality within coteries could lead to direct transmission during daily interactions, and regular territorial disputes could lead to between-coterie transmission by biting and scratching. Prairie dogs are known cannibals, therefore infected carcasses could provide a short-term reservoir that facilitates within-colony spread of *Y. pestis* (Webb et al. 2006).

Recently, Wilder et al. (2008) demonstrated that efficient transmission by unblocked *O. hirsuta* and *O. t. cynomuris* occurs during the first 48 h after ingestion of infectious blood meals, well before blockage of the gut occurs. Although the mechanism responsible for this early phase transmission has not been identified, it is probable that early phase transmission is involved in epizootics among rodents (Eisen et al. 2006). Flea-borne transmission is related to flea abundance on hosts, with transmission more likely on hosts harboring large numbers of fleas (Pollitzer 1954, Krasnov et al. 2006). Burroughs (1947) reported that several flea species considered inefficient vectors because of poor blocking nonetheless transmitted *Y. pestis* when flea abundance was high.

The objective of this study was to describe seasonal patterns of flea abundance on hosts and flea species diversity infesting black-tailed prairie dogs in north-central Colorado, and to examine how flea abundance changes during plague epizootics. The three species commonly found on black-tailed prairie dogs display seasonal abundance patterns (Salkeld and Stapp 2008, Wilder et al. 2008), with the increased flea abundance on prairie dogs during epizootics pointing to additional mechanisms for rapid spread of *Y. pestis* within prairie dog towns.

Methods

Study area, trapping, and flea collection

This study was conducted in Weld County, Colorado, on the Pawnee National Grassland, the Central Plains Experimental Range, and privately owned land (Fig. 1). The Pawnee National Grassland consists of 78,129 hectares administered by the United States Department of Agriculture Forest Service. The Central Plains Experimental Range is 6280 hectares administered by the United States Department of Agriculture's Agricultural Research Service. The habitat is shortgrass steppe dominated by blue grama (*Bouteloua gracilis*) and buffalo grass (*Buchloe dactyloides*).

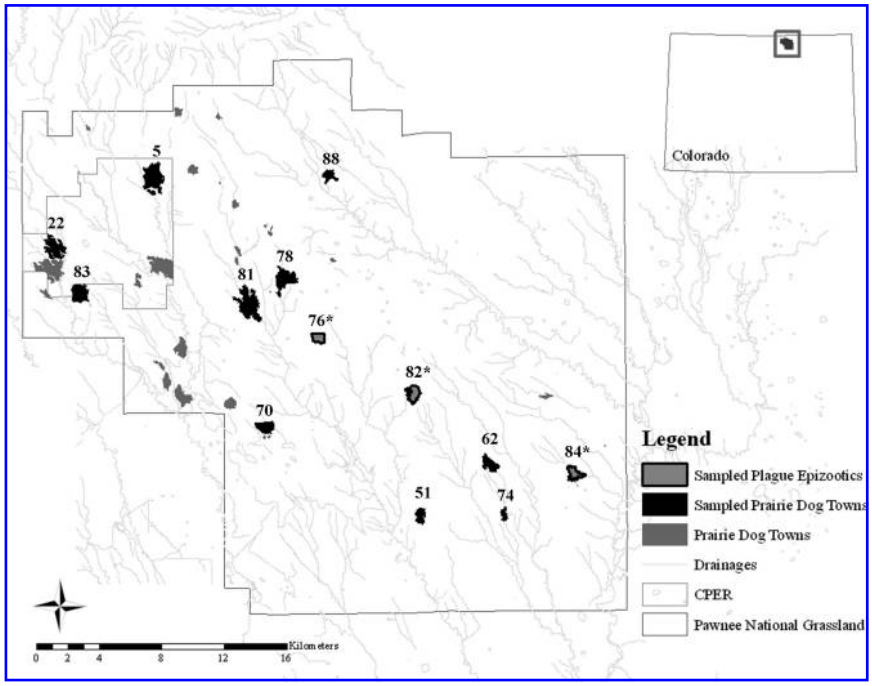

FIG. 1. Black-tailed prairie dog towns at their largest extent on the Pawnee National Grasslands, Weld County, Colorado, during the sampling periods in 2004–2007.

Black-tailed prairie dogs were captured from 13 towns between June 2004 and May 2007 (Fig. 1); three towns (76, 82, and 84) were sampled both before and during epizootics. No trapping occurred during December and January because of low prairie dog activity during adverse winter weather. Between 100 and 160 Tomahawk traps (Tomahawk Live Trap, Tomahawk, WI) were placed at active burrows in either a clustered (2004) or random (2005–2007) arrangement. For clustered arrangement, one area of active prairie dog habitat was selected within each quadrant of the town and 25 traps were placed at active burrows. For random arrangement, Universal Transverse Mercator coordinates within the towns were randomly selected using program Arc/INFO (Environmental Systems Research Institute, Redlands, CA). Traps were placed at active burrows within 15 m of each random point. Both trapping arrangements assured that sampling occurred across multiple coteries.

Before trapping, prairie dogs were acclimated to traps by leaving traps wired open and prebaited for 2–4 weeks, with 4–8 applications with 8% three-way sweet feed (Manna Pro, St. Louis, MO). During active trapping, traps were baited and set between 0600 and 0700 a.m. and checked for captures between 1000 and 1100 a.m.

Captured prairie dogs were anesthetized using isoflurane (Halocarbon Industries, River Edge, NJ) administered by an oxygen-driven vaporizer (Seven-Seven Anesthesia, Fort Collins, CO). A plastic tub was used to contain the anesthetized prairie dogs while they were brushed with a fine-tooth comb to remove fleas. Fleas were placed into vials containing 1.5% saline with 0.001% Tween 80 (ICN Biomedicals, Aurora, OH) and stored at -80°C until species identification. Prairie dogs were weighed; classified by sex, age (juvenile, yearling, or adult based on body condition and pelage), and reproductive status (reproductive, pigmented males, or pregnant or lactating females); and ear-tagged (National Band and Tag, Newport, KY). After prairie dogs fully recovered from anesthesia, they were released at the site of capture. Protocols were approved by the Institutional Animal Care and Use Committee at Colorado State University (03053A05).

Flea identification followed taxonomic keys of Stark (1958) and Hubbard (1968) was confirmed at the Centers for Disease Control and Prevention, and voucher specimens were deposited in the reference collection at the CSU Gillette Museum. Identification of *Pulex* species is problematic because female *P. simulans* and *Pulex irritans* L., 1758 cannot be distinguished by morphology, including seventh sternites and spermathecae (Hopla 1980). However, consistent with Hopla's (1980) descriptions for this area, all male *Pulex* were identified as *P. simulans* based upon the unique blade-like shape of the crochet and dorsal aedeagal sclerite. *Thrassis fotus* (Jordan, 1925), a flea of thirteen-lined ground squirrels (*Spermophilus tridecemlineatus* Mitchill, 1821) that co-occur in the study area, were occasionally collected.

The collection date of the first *Y. pestis*–positive flea or prairie dog carcass on a town was the earliest evidence of a plague epizootic; thus, all fleas collected before these dates were considered "before epizootic" samples, while fleas collected after these dates were "during epizootic" samples. The three towns that experienced epizootics (76, 82, and 84) were visited at least weekly for the majority of the sampling periods. Observations of the number of prairie dogs foraging above ground and the presence of recent digging at burrow entrances were conducted during these visits, and the towns were searched for dying animals and carcasses. Thus, it is likely that the discoveries of plague positive fleas and carcasses were made at or near the beginning of epizootics.

Laboratory diagnostic tests

To detect *Y. pestis* DNA in fleas, individual and pools of five or fewer fleas from the same host were tested, following the procedures described in Salkeld et al. (2007). This method reliably detects $\geq$10–100 colony forming units per flea (Stevenson et al. 2003). Liver and spleen tissue from prairie dogs suspected to have died from *Y. pestis* infection were tested at Centers for Disease Control and Prevention for presence of *Y. pestis* by direct immunofluorescent antibody tests to the F1 antigen, mouse inoculation, and culture (Chu 2000).

Data analyses

Flea abundance was defined as the number of fleas per host, prevalence as the number of infested hosts per total number of hosts examined, and infestation intensity was the number of fleas per infested host. Distributions of flea abundance were tested for fit to the negative binomial distribution using the R statistical package (R Development Core Team, Version 2.5.1, 2007). Aggregation of fleas on hosts is described by the negative binomial parameter k, defined as $X^2/(\sigma^2 - X)$, with average flea abundance X and variance σ^2. Low values of k, and certainly $k < 1$, indicate aggregation (Wilson et al. 2002). Values of k were estimated by maximum likelihood (Crawley 2007), with standard errors estimated by the THETA.ML procedure. Goodness of fit was tested using χ^2, with probabilities adjusted for multiple tests by the Dunn-Sidak procedure (Sokal and Rohlf 1995).

Factors for comparing flea abundance included prairie dog sex and age, sampling locations (towns), time of year in months and biweekly intervals, and plague status of towns.

Table 1. Summary of Fleas from Black-Tailed Prairie Dogs Captured on Towns with No Evidence of Plague

	Oropsylla hirsuta	Pulex simulans	Oropsylla tuberculata cynomuris	Thrassis fotus	*All fleas*
Total fleas (% of total fleas)	9867 (75.9)	2570 (19.8)	505 (3.9)	50 (0.4)	12,993
Prevalence % (no. of hosts infested)	80.9 (837)	31.3 (324)	5.4 (56)	4.5 (47)	87.6 (906)
Maximum flea abundance	149	54	79	2	149
Mean flea abundance (95% CI)	9.5 (8.6, 10.5)	2.5 (2.1, 2.9)	0.49 (0.2, 0.7)	0.05 (0.03, 0.06)	12.6 (11.5, 13.6)
Median flea abundance (95% CI)	4 (4, 5)	0 (0, 0)	0 (0, 0)	0 (0, 0)	7 (6, 8)
Infestation intensity (95% CI)	11.8 (10.7, 12.9)	7.9 (7.0, 8.9)	9.0 (4.9, 13.1)	1.06 (0.99, 1.14)	14.3 (13.2, 15.5)

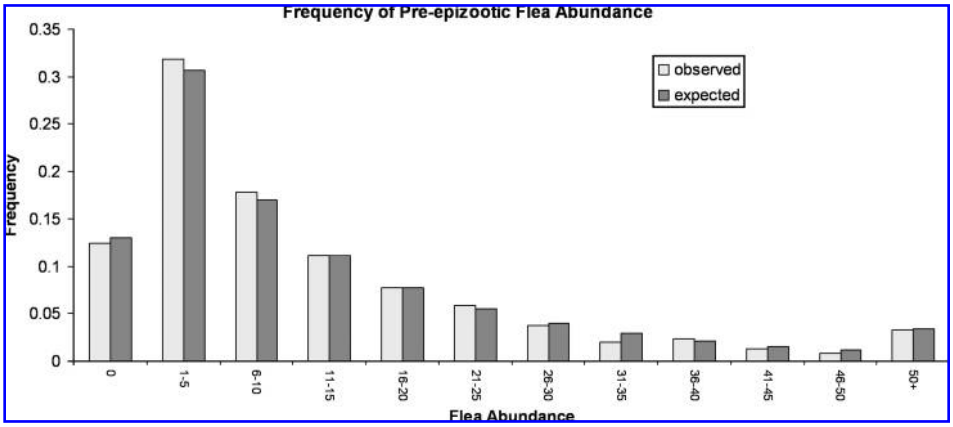

FIG. 2. Frequencies of observed flea abundance compared to expected frequencies from the negative binomial distribution, from towns with no evidence of plague. χ^2 goodness of fit to the negative binomial distribution $p = 0.6733$.

Generalized linear models (GLM) with negative binomial errors were fit using the GLMNB procedures in R. Measures of infestation intensity did not fit to the negative binomial distribution, and thus were analyzed using GLM with quasi-Poisson errors, with the mean:variance ratio as the dispersion parameter (Venables and Ripley 2002). Model fitting was carried out using stepwise procedures based on Akaike's Information Criterion and analysis of deviance via χ^2 tests implemented by the ANOVA procedure. Specific comparisons were tested by Tukey's honestly significant difference (HSD) procedure (Sokal and Rohlf 1995).

Results

A total of 1299 prairie dogs were captured and examined for fleas, 1034 on towns with no signs of plague and 265 during plague epizootics. The most common flea was *O. hirsuta*, with *P. simulans* the second most common and *O. t. cynomuris* accounting for a small fraction of the total (Table 1). The occurrence of *T. fotus* on prairie dogs was incidental, and too few were collected to include in further analyses. Flea prevalence was greater than 85%, with *O. hirsuta* having highest prevalence.

Distributions of each flea species on prairie dogs were skewed: average flea abundance on hosts was greater than the median, and maximum flea abundance was much greater than the averages (Table 1). Flea distributions fit the negative binomial distribution, with most prairie dogs harboring few or no fleas, and a few animals harboring many (Fig. 2). The negative binomial parameter k ($\pm$SE) for flea abundance from all prairie dogs captured through all seasons was 0.69 ($\pm$0.031) for all fleas and 0.54 ($\pm$0.025) for *O. hirsuta*. Values of k were 0.13 ($\pm$0.009) for *P. simulans* and 0.02 ($\pm$0.003) for *O. t. cynomuris*, reflecting both lower overall prevalence and seasonal occurrence of these species.

Flea abundance during plague epizootics

Fleas were abundant throughout the sampling periods, but average flea abundance seasonally fluctuated with peaks in spring (February and March) and late summer (September and October; Fig. 3). *O. hirsuta* were sampled in all months, with greatest abundance in late February (X = 16.3; 95% CI 11.0–21.6) and late October (X = 15.6; CI 10.7–20.6). *P. simulans* were found in all months sampled except February, with greatest abundance in late September (X = 24.1; CI 17.2–30.9).

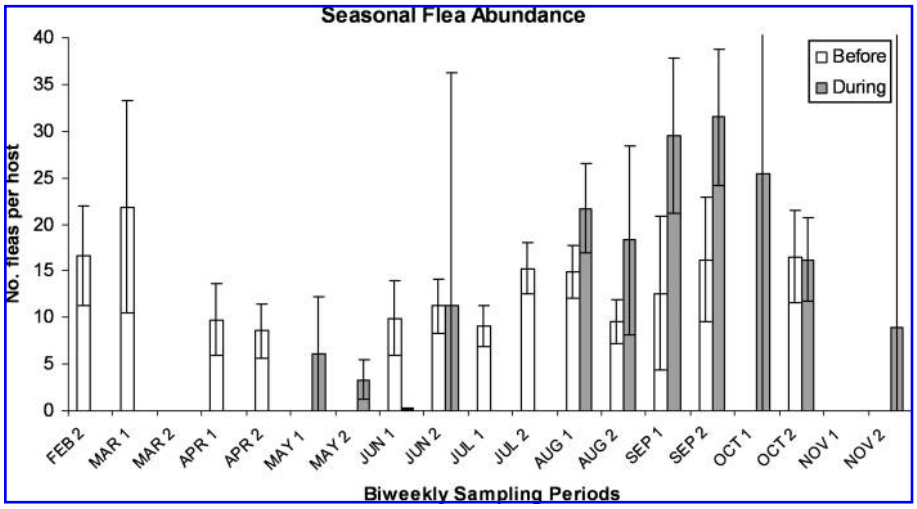

FIG. 3. Biweekly average flea abundance of fleas on black-tailed prairie dogs captured before and during plague epizootics. Error bars are 95% confidence intervals.

Table 2. Analysis of Deviance of Seasonal Effects (Monthly and Biweekly) and Plague Status on Flea Abundance (Data in Fig. 3), from a Nested GLM with Negative Binominal Errors (Only Significant χ^2 Shown)

Flea species	*Season and plague effect*	*df*	*Test of deviance* (χ^2)	p
O. hirsuta	Month	9	63.9	<0.0001
	Month/biweek	8	35.5	<0.0001
	Month/biweek/plague	10	87.5	<0.0001
P. simulans	Month	9	234.7	<0.0001
	Month/biweek	8	47.4	<0.0001
	Month/biweek/plague	10	75.4	<0.0001
O. t. cynomuris	Month	5	325.6	<0.0001
	Month/biweek	4	66.7	<0.0001

O. t. cynomuris were found only from February to July, with greatest abundance in early March (X = 18.7; CI 7.0–30.5); only 46 *O. t. cynomuris* were found in June and July.

Abundance of *O. hirsuta* and *P. simulans* varied seasonally and increased during epizootics (Table 2). Abundance of *O. t. cynomuris* varied seasonally, but we did not detect an increase during plague epizootics. Comparisons before and during epizootics depend on the timing of sampling: town 84 had an epizootic during the early spring period in 2006, when *O. t. cynomuris* would be abundant. The first *Y. pestis*–infected fleas (*P. simulans*) on town 84 were collected the previous October. When sampling resumed in May 2006, only 25% of the town was still active and the majority of the *O. t. cynomuris* season had passed.

Prairie dogs were captured at three towns (76, 82, and 84) that experienced plague epizootics and were extensively sampled both before and during the epizootics (Table 3). Abundance of all species differed between towns (Table 4), with fleas most abundant at town 82 followed by towns 84 and 76. No *O. t. cynomuris* were collected from town 82, but the trapping effort was during summer months when *O. t. cynomuris* adults were rarely found on prairie dogs.

Abundance of *O. hirsuta* and *P. simulans* increased during epizootics (Table 4), with overall flea abundance increasing from 10.4 (CI 9.1–11.8) before epizootics to 18.9 (CI 16.3–21.5) during epizootics. Abundance of *O. hirsuta* only increased at town 76 (Tukey's HSD, $p = 0.0191$), with no changes at towns 82 and 84 (town by plague status interaction in Table 4). As a further indication of the change in distribution during epizootics, *P. simulans* became more evenly distributed during epizootics, with the negative binomial parameter k increasing from 0.13 (±0.009) before epizootics to 0.35 (±0.037) during epizootics. *P. simulans* became both more abundant and less aggregated during epizootics. Aggregation of *O. hirsuta* did not change ($k = 0.7$ both before and during epizootics).

Y. pestis infected fleas

The increase in the number of fleas was even more pronounced on the 229 infested prairie dogs captured during epizootics (Fig. 4). The 31 prairie dogs with *Y. pestis*–infected fleas had infestation intensity twice that of the 198 prairie dogs with noninfected fleas (GLM analysis using quasi-Poisson errors: *O. hirsuta* $\chi^2 = 278.28$, df = 2, $p = 0.0014$; *P. simulans* $\chi^2 = 131.95$, df = 2, $p = 0.0012$). The greatest number of

Table 3. Summary of Flea Abundance from Black-Tailed Prairie Dogs Captured at Three Towns with Epizootics

	Town	*Plague status* (n)	O. hirsuta	P. simulans
Average flea abundance (95% CI)	76	Before (167)	6.1 (4.0, 8.2)	1.0 (0.6, 1.5)
		During (60)	11.3 (6.6, 16.0)	2.7 (1.7, 3.8)
	82	Before (167)	11.8 (8.7, 14.9)	6.3 (4.9, 7.7)
		During (120)	11.9 (9.0, 14.8)	12.8 (10.8, 14.8)
	84	Before (221)	8.5 (6.8, 10.2)	0.4 (0.2, 0.6)
		During (44)	8.5 (5.3, 11.7)	0.8 (0.2, 1.5)
	Total	Before (500)	8.4 (7.2, 9.7)	1.9 (1.5, 2.3)
		During (224)	11.1 (9.0, 13.2)	7.8 (6.5, 9.1)
Median flea abundance (95% CI)	76	Before (167)	2.0 (1.0, 3.0)	0.0 (0.0, 0.0)
		During (60)	6.5 (5.0, 10.0)	2.0 (1.0, 3.0)
	82	Before (167)	6.0 (4.0, 8.0)	4.0 (2.0, 5.0)
		During (120)	6.0 (4.0, 9.0)	10.0 (8.0, 12.0)
	84	Before (221)	4.0 (3.0, 5.0)	0.0 (0.0, 0.0)
		During (44)	4.0 (2.0, 8.0)	0.0 (0.0, 0.0)
	Total	Before (500)	3.0 (3.0, 4.0)	0.0 (0.0, 0.0)
		During (224)	6.0 (5.0, 8.0)	4.0 (3.0, 6.0)
Infestation intensity (95% CI)	76	Before (167)	9.2 (6.2, 12.2)	4.2 (2.7, 5.7)
		During (60)	11.7 (6.9, 16.6)	4.2 (2.7, 5.7)
	82	Before (167)	13.6 (10.1, 17.1)	7.1 (5.6, 8.6)
		During (120)	12.8 (9.7, 15.8)	13.5 (11.5, 15.5)
	84	Before (221)	11.0 (9.0, 13.1)	2.5 (1.4, 3.6)
		During (44)	9.8 (6.3, 13.3)	3.7 (1.4, 6.0)
	Total	Before (500)	11.2 (9.6, 12.7)	5.5 (4.5, 6.5)
		During (224)	11.9 (9.7, 14.1)	10.7 (9.1, 12.3)

Table 4. Analysis of Deviance of Differences in Flea Abundance Between Towns 76, 82, and 84, and Plague Status (Data in Table 3), from a GLM with Negative Binominal Errors (Only Significant χ^2 Shown)

Flea species	*Town and plague effects*	*df*	*Test of deviance* (χ^2)	p
O. hirsuta	Town	2	14.45	0.0007
	Plague	1	3.47	0.0626
	Town:plague	2	6.57	0.0374
P. simulans	Town	2	479.23	<0.0001
	Plague	1	41.52	<0.0001
O. t. cynomuris	Town	2	12.87	0.0016

Y. pestis–infected *O. hirsuta* on a single prairie dog was 26; the maximum number of plague-positive *P. simulans* was 12. Prevalence of *Y. pestis* infection among fleas was 12% for *O. hirsuta* ($n = 29$) and 8% for *P. simulans* ($n = 20$). We detected 56 positive *O. hirsuta* and 6 positive *P. simulans* from town 76; 16 positive *O. hirsuta* and 18 positive *P. simulans* from town 82; and 1 positive *P. simulans* and 1 positive *O. t. cynomuris* from town 84. Few positive fleas were found on town 84 because sampling occurred in late spring, toward the end of the epizootic when most prairie dogs had already died.

Host sex and age

Abundance of all three flea species was greatest on adult male prairie dogs (Table 5), and *P. simulans* abundance increased during epizootics on both males and females and for all age groups (Tukey's HSD, all $p = <0.02$) (Table 5). Sex had little influence on trap success with 685 male and 614 female prairie dogs captured during the study. The *Y. pestis*–infected carcasses of nine male and three female prairie dogs were collected during epizootics.

Discussion

Increases in flea abundance on hosts during plague outbreaks provide a mechanism for rapid spread of plague epizootics on prairie dog towns, especially when combined with early-phase transmission of *Y. pestis* by unblocked fleas within 48 h of ingesting infectious blood meals (Wilder et al. 2008). Flea abundance increases were greatest on towns during active epizootics, and infestation intensity was twice as high on prairie dogs with infected fleas than on prairie dogs with uninfected fleas (Fig. 4). Increased abundance on male prairie dogs may also account for between-coterie spread, as males are more likely to move between territories (Hoogland 1995) and either carry infected fleas with them or acquire infected fleas and return to their home territories.

Flea species diversity and seasonal abundance on black-tailed prairie dogs in northern Colorado were similar to descriptions from other localities (Maupin 1970, Brinkerhoff et al. 2006, Holmes et al. 2006), with the highest flea abundance in February and March and August through October. *O. hirsuta* abundance was relatively constant throughout the year with the addition of *O. t. cynomuris* in the spring and *P. simulans* during late summer. The absence of *O. t. cynomuris* on prairie dogs after July most likely reflects their life cycle, with active adults in spring, and teneral adults and/or pupae surviving within cocoons in prairie dog burrows during the remainder of the year (Reichardt and Galloway 1994). By excavating prairie dog burrows and examining nesting material to determine seasonal life cycles and occurrence of larvae, pupae in cocoons, and teneral adults, we could compare how these species compare to the annual cycle classification and specific flea species discussed by Krasnov et al. (2002). Regardless, *O. hirsuta* appears year round, *O. t. cynomuris* is active only in the winter and early spring, while *P. simulans* is active year round with peak abundance in the fall. Further study of *P. simulans* and whether this flea completes its life cycle on prairie dogs would be of interest. In particular, seasonal differences in reproductive activity of female fleas, which is related to blood feeding on prairie dogs, could also influence rates of flea-borne transmission (Krasnov et al. 2002).

The seasonal fluctuation of flea abundance coincided with the timing of 23 plague epizootics we observed on our study area from 2003 to 2007. Nine epizootics occurred during the spring peak in *O. hirsuta* and *O. t. cynomuris* abundance, and

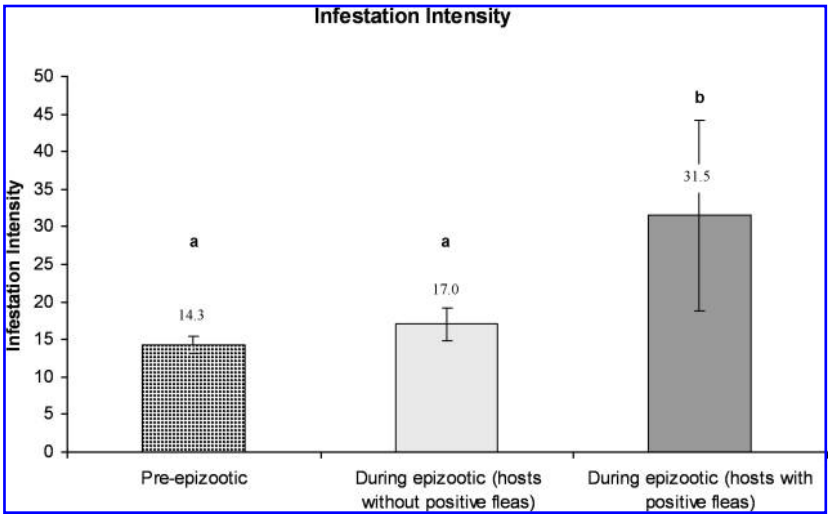

FIG. 4. Infestation intensity of fleas collected from infested prairie dogs captured before and during plague epizootics. Error bars are 95% confidence intervals; letters (a and b) represent Tukey's HSD differences.

Table 5. Analysis of Deviance of Flea Abundance Affected by Age and Sex of Prairie Dogs and Plague Status of Towns from a GLM with Negative Binominal Errors (Only Significant χ^2 Shown)

Flea species	*Age, sex, and plague effect*	*df*	*Test of deviance* (χ^2)	p
O. hirsuta	Age	2	87.65	<0.0001
	Sex	1	5.11	0.0239
P. simulans	Sex	1	5.69	0.017
	Plague	1	39.71	<0.0001
O. t. cynomuris	Age	2	71.65	<0.0001
	Sex	1	0.27	0.6045

14 occurred during the later summer and fall peak when *O. hirsuta* and *P. simulans* are most abundant. Although the start of these epizootics is not precisely known, the periods of rapid mortality were conspicuous and served as reliable indicators of epizootic activity. Human plague cases also occur seasonally and are influenced by local climate (Indian Plague Commission 1908, Pollitzer 1954, Cavanaugh 1971, Enscore et al. 2002). Sharp declines in human plague cases in India and Vietnam were observed when temperatures rose above 27°C (Indian Plague Commission 1908, Cavanaugh and Marshall 1972). Similarly, the epizootics at towns 76 and 84 began in October when the daily high temperature in the 28 days before the epizootics averaged only 20°C (data from National Climate Data Center 2007). Plague was first detected on town 82 on August 5, 2005; daily high temperature in the 28 days before the epizootic averaged 35°C but dropped to 31°C during the first 28 days of the epizootic. Observations of other epizootics in our study area also suggest lower prairie dog mortality during July and early August each year when temperatures are at their highest. Flea survival declines at high temperatures (Silverman et al. 1981, Krasnov et al. 2001), as does the ability of fleas to transmit plague (Kartman and Prince 1956, Cavanaugh 1971, Hinnebusch et al. 1998). The effects of high temperature on prairie dog fleas may be influenced by the ability of the fleas to seek refuge in the deeper reaches of burrows where temperature fluctuations should be buffered (Wilcomb 1954). Given that burrow system microclimates should vary much less than above-ground temperature, the effects of temperature on prairie dog mortality, flea questing and survival, and the ability of *Y. pestis* to persist and remain infectious in fleas deserve further study.

Little is known about the ability of *P. simulans* to transmit *Y. pestis*, but its direct role in plague epizootics in prairie dogs was underappreciated before this study. In our study area, *P. simulans* occurs on a wide range of species, including swift foxes (*Vulpes velox* Say, 1823) that tested positive for exposure to *Y. pestis* (Salkeld et al. 2007). The co-occurrence of *P. simulans* on prairie dogs and carnivores suggests that *P. simulans* may play a role in the transmission of plague between towns (Poland and Barnes 1979, Gage et al. 1994, Salkeld et al. 2007).

This study demonstrated that as host numbers decrease during epizootics, both average flea abundance and the frequency of prairie dogs with very high flea abundance increase. As prairie dogs die, the increasing pool of questing fleas concentrates onto the remaining hosts. More importantly, the greatest increase in flea infestation occurred on prairie dogs harboring *Y. pestis*–infected fleas. Increased flea abundance was also observed by Pauli et al. (2006) during epizootics in black-tailed prairie dogs, and by Anderson and Williams (1997) in white-tailed prairie dogs (*Cynomys leucurus*). The spatial and temporal spread of plague epizootics may be explained by increased flea abundance.

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