SEASON OF DELTAMETHRIN APPLICATION AFFECTS FLEA AND PLAGUE CONTROL IN WHITE-TAILED PRAIRIE DOG (Cynomys leucurus) Colonies, Colorado, USA

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ABSTRACT: In 2008 and 2009, we evaluated the duration of prophylactic deltamethrin treatments in white-tailed prairie dog (Cynomys leucurus) colonies and compared effects of autumn or spring dust application in suppressing flea numbers and plague. Plague occurred before and during our experiment. Overall, flea abundance tended to increase from May or June to September, but it was affected by deltamethrin treatment and plague dynamics. Success in trapping prairie dogs (animals caught/trap days) declined between June and September at all study sites. However, by September trap success on dusted sites (19%; 95% confidence interval [CI] 16–22%) was about 15-fold greater than on undusted control sites (1%; CI 0.3–4%; P < 0.0001). Applying deltamethrin dust as early as 12 mo prior seemed to afford some protection to prairie dogs. Our data showed that dusting even a portion of a prairie dog colony can prolong its persistence despite epizootic plague. Autumn dusting may offer advantages over spring in suppressing overwinter or early-spring flea activity, but timing should be adjusted to precede the annual decline in aboveground activity for hibernating prairie dog species. Large colony complexes or collections of occupied but fragmented habitat may benefit from dusting some sites in spring and others in autumn to maximize flea suppression in a portion of the complex or habitat year-round.

Key words: Black-footed ferret, Cynomys leucurus, deltamethrin, flea, Mustela nigripes, plague, white-tailed prairie dog, Yersinia pestis.

INTRODUCTION

Since its introduction into North America around 1900, plague—the disease caused by the bacterium Yersinia pestis—has caused local extirpation of prairie dog (Cynomys spp.) colonies, reduced colony sizes, increased variance in local population sizes, and increased distances between colonies (Lechleitner et al. 1962, 1968; Rayor 1985; Cully and Williams 2001). Mortality on individual colonies can be complete, but it is usually <85% in white-tailed prairie dog (Cynomys leucurus) complexes (Ubico et al. 1988; Cully and Williams 2001). In broader terms, plague can have catastrophic ecologic effects because impairing prairie dog abundance and persistence directly or indirectly impacts a multitude of other species that depend on prairie dogs for habitat and prey (Antolin et al. 2002; Augustine et al. 2008; Seglund and Schnurr 2010).

Plague is vectored and potentially harbored by fleas (Kartman et al. 1962; Gage and Kosoy 2005). Flea species vary in Y. pestis transmission efficiency (Eisen et al. 2009). Seasonal flea species diversity and peaks in abundance coincide with plague epizootics in black-tailed (Cynomys ludovicianus; Tripp et al. 2009) and white-tailed prairie dogs (Ubico et al. 1988). Insecticides (e.g., permethrin, deltamethrin) have been used to control flea abundance and suppress Y. pestis transmission in black-tailed, white-tailed, Utah (Cynomys parvidens), and Gunnison’s (Cynomys gunnisoni) prairie dog colonies (Seery et al. 2003; Hoogland et al. 2004; Biggins et al. 2010; Colorado Parks and Wildlife [CPW] unpubl. data).

As part of an ongoing species recovery program, black-footed ferrets (Mustela nig-
ripes) were reintroduced into the Bureau of Land Management (BLM) Wolf Creek Management Area (WCMA) in northwestern Colorado, US, between 2001 and 2008. The discovery of a plague-infected desert cottontail rabbit (Sylvilagus audubonii) carcass and scattered *Y. pestis*-positive prairie dog fleas in 2007 (Griffin et al. 2010) marked the beginning of a complex-wide decline in prairie dog abundance and eventual extirpation of black-footed ferrets at the WCMA (CPW unpubl. data). In September 2008, CPW and the BLM began emergency management to control fleas and reduce *Y. pestis* transmission in prairie dogs by preemptively dusting prairie dog burrows with the insecticide deltamethrin in the core black-footed ferret reintroduction site at WCMA (Fig. 1). Our study was conducted within the framework of that larger management effort in dusted areas and in adjacent, undusted (control) areas. Our objectives were to determine whether prophylactic deltamethrin treatments would protect prairie dogs from epizootic plague, to compare flea abundance and prevalence on white-tailed prairie dogs and in their burrows in dusted and undusted areas, and to evaluate the duration of flea suppression after autumn or spring deltamethrin application.

**MATERIALS AND METHODS**

**Study area**

The WCMA encompasses 21,000 ha of salt-desert shrub habitat managed by the BLM in Moffat and Rio Blanco counties in northwestern Colorado (Holmes 2008). Our study sites were located on 7,700 ha of active white-tailed prairie dog colonies (Fig 1) centered on 40°17′53″N, 108°26′48.2″W. The best prairie dog habitat within the WCMA recorded prairie dog density ranging from 6.6 to 9.1 prairie dogs per ha before the (re)emergence of plague (Holmes 2008). Based on prior surveillance (Griffin et al. 2010), we assumed *Y. pestis* transmission was occurring throughout the WCMA when we began our study.

**Study design**

To compare effects of insecticide application between seasons, we dusted one 369-ha plot (DA) within the WCMA 20/23 colony in September–October 2008 (autumn) and then dusted a separate 471-ha plot (DS) in April–May 2009 (spring) (Fig. 1). We established focal areas for prairie dog trapping and off-host flea collection within both dusted plots, and within two nearby undusted control colonies (WCMA 18 and 24, designated U1 and U2, respectively) (Fig. 1). We captured and sampled prairie dogs at all four sites on 9–18 June, 28 July–1 August, and 21–29 September 2009. We also collected fleas from prairie dog burrows in all four plots in May, July, and September 2009. The Colorado Division of (Parks and) Wildlife, Animal Care and Use Committee reviewed and approved protocols (file 05-2008).

**Insecticide application**

We applied deltamethrin per label instructions. Prairie dog burrows on dusted plots received 4–5 g of 0.05% deltamethrin powder (DeltaDust®, Bayer Crop Science, Research Triangle Park, North Carolina, USA; Seery et al. 2003; Biggins et al. 2010). To guide application, we created 40–100-m-wide transects across the plots by using ArcGIS (ESRI, Redlands, California, USA) and DNRGPS (Minnesota Department of Natural Resources, St. Paul, Minnesota, USA) softwares, and flagged boundaries. We applied dust into each burrow encountered using a specialized applicator (Technicide, San Clemente, California, USA) calibrated to deliver 4–5 g of deltamethrin. We recorded dusting with hand-held GPS equipment.

**Animal and burrow sampling**

Prairie dog capture, handling, and sampling followed established methods (Tripp et al. 2009). We placed about 50 traps (Tomahawk Live Trap Co., Tomahawk, Wisconsin, USA) at active burrows within focal areas (Fig. 1). We anesthetized prairie dogs by using isoflurane (Halocarbon Industries, River Edge, New Jersey, USA) vaporizers (Seven-Seven Anesthesia Inc., Fort Collins, Colorado, USA), and then we used a fine-tooth comb to remove fleas. We stored fleas in 1.5% saline frozen at −70 C until identified. We weighed and ear-tagged (Monel tag 1005-1, National Band and Tag Co., Newport, Kentucky, USA) prairie dogs, determined sex and age, and released each animal at its capture location upon recovery.

Within each plot, we randomly selected nine locations before each sampling occasion and swabbed 10 burrows within 50 m of each location by using methods of Ecke and Johnson (1952) as modified by Griffin et al. (2010). We keyed fleas to species (Stark 1958; Hubbard 1968) and pooled fleas of a single species from an individual prairie dog or burrow (<10 fleas/pool) for analysis.
Carcasses encountered in the course of dusting, swabbing, or capture were submitted for necropsy and *Y. pestis* screening.

**Laboratory analyses**

To detect *Y. pestis* DNA in carcasses or fleas, we tested tissue or flea pools by PCR assay (Griffin et al. 2010). This method reliably detected $\geq 100$ colony-forming units per flea pool (Griffin et al. 2010). For plague suspect carcasses, confirmation was provided by the Centers for Disease Control and Prevention (Fort Collins, Colorado, USA).

**Data analyses**

We compared prairie dog trap success, flea abundance (fleas/prairie dog), flea prevalence (infested/total examined), and flea infestation intensity (fleas/infested host) on prairie dogs captured on treatment sites to those captured at control sites. We used prairie dog trap success (total captures/total trap days) as a proxy for relative abundance. Because forage for prairie
dogs was abundant and similar across all study sites, which were separated by 3–4 km, we assumed that seasonal changes in forage abundance and quality impacted all study sites and trap successes equally during individual trapping occasions.

Comparisons of trap success and flea abundance on treatment and control plots, and between capture sessions, were made using χ² tests, with probabilities adjusted for multiple tests by the Dunn-Sidak procedure (Sokal and Rohlf 1995). We calculated confidence intervals for proportions by using the prop.test function in R 2.15.2 (R Development Core Team 2015).

Flea abundance distributions were tested for fit to the negative binomial distribution using R. On-host flea aggregation was described by the negative binomial parameter k; low values of k (especially k<1) indicate aggregation (Wilson et al. 2002). Values of k were estimated by maximum likelihood (Crawley 2007). We tested goodness of fit by using χ² tests.

Potential factors impacting flea abundance included prairie dog sex and age, sampling locations (sites), and month. Generalized linear models with negative binomial errors were fit using the GLMN B procedures in R. Model fitting was carried out using stepwise procedures based on Akaike’s Information Criterion and analysis of deviance via χ² tests implemented by analysis of variance procedure. Specific comparisons were tested by Tukey’s honestly significant difference procedure (Sokal and Rohlf 1995).

**RESULTS**

Plague continued spreading through the Wolf Creek prairie dog colony complex during our study, and its asynchrony and spatiotemporal variation influenced observable patterns. Moreover, the collapse of undusted colonies by autumn 2009 led to low prairie dog abundance and high flea abundance on control sites, thus rendering some a priori comparisons to dusted sites impractical.

Knowledge about plague activity on study sites was ample, but likely incomplete.

In 2008, before our study, burrows were swabbed in the WCMA. Flea abundance per burrow was similar across the complex in May (1.2), July (1.5), and August (1.0) (Griffin et al. 2010). In May 2009, 5.3 fleas/burrow were collected during opportunistic burrow sampling conducted just before dusting portions of the DS plot, but 70 d later (after dusting) only 0.22 fleas/burrow were collected.

**Prairie dog and plague trends**

Deltamethrin dust applied even 12 mo prior seemed to afford some protection to prairie dogs within dusted plots. The U1 plot yielded three *Y. pestis*-infected prairie dog carcasses in April and June 2009, and the U2 plot yielded four infected carcasses in May and July 2009. We found three infected prairie dog carcasses at DS in May 2009 before dusting, but we recovered no carcasses at DA. We attributed the general trend of declining trap success between June and September (Fig. 2) in part to waning above-ground prairie dog activity in autumn before hibernation (Bakko and Nahorniak 1986). However, undusted sites showed more precipitous declines than dusted (Fig. 2): by September 2009, trap success on dusted plots (19%; 95% confidence interval [CI] 16–22%) was about 15-fold greater than on paired undusted plots (1%; CI 0.3–4.0%) (P<0.0001; Fig. 2).
Flea sampling revealed a similar pattern: We detected *Y. pestis*-positive flea pools from prairie dogs and burrows on the undusted U1 plot in June and July, but none in September because no prairie dogs were captured (Table 1 and Fig. 2). Positive fleas were collected from U2 prairie dogs in July and from U2 burrows at all three samplings (Table 1 and Fig. 2). In contrast, the DA plot yielded relatively few *Y. pestis*-positive flea pools 9–12 mo after dusting (Table 1 and Fig. 2). Positive flea pools were not detected on prairie dogs at the DS plot until September, 4–5 mo after dusting (Table 1 and Fig. 2).

**Flea trends**

We collected 4,376 fleas from 341 prairie dogs (Table 1 and Fig. 2). *Oropsylla hirsuta* (72%) was most common, with fewer *Pulex simulans* (27%) and occasional *Oropsylla tuberculata cynomuris* (<1%). Flea distributions on prairie dogs fit a negative binomial distribution, with average flea abundance > median, maximum abundance >> mean, and low values of *k* (range 0.43–0.75).

Overall, flea abundance within plots tended to increase from May to September and was affected by deltamethrin treatment and plague dynamics (Fig. 2). Flea abundance on prairie dogs was heavily influenced by site (*P*<0.0001) and month (*P*<0.0001) of capture (Fig. 2; see Supplementary Material Table S1). Mean flea abundance was greater on undusted sites than on dusted sites in June and July, but by September plague and the resulting loss of prairie dogs had confounded flea dynamics at undusted sites (Table 1 and Fig. 2). At DS, flea abundance on prairie dogs did not differ between June and July (*P*=0.95), but it increased between July and September (*P*=0.0001). At DA, flea abundance on prairie dogs increased between June and July (*P*=0.02) and between July and September (*P*=0.0001). Similarly, flea abundance on prairie dogs from both undusted plots increased between June and July (*P*<0.0001). Flea abundance did not differ between July and September (*P*=0.11) at U2.

The effects of autumn and spring deltamethrin application diverged over the course of sampling. In June, flea abundance on prairie dogs did not differ between sites dusted 8–9 mo or 1–2 mo before sampling (*P*=0.89; Fig. 2). However, flea abundance was lower in July (*P*=0.02) and September (*P*=0.002) at DS. Prevalence of flea infestation on prairie dogs was largely unaffected across all study areas and treatments relative to flea abundance, with ≥50% of prairie dogs infested in all sampling occasions (Table 1). Flea infestation intensity on prairie dogs that harbored *Y. pestis*-positive fleas (*x*=35.9%, CI 25.8–46.1%) was 3-fold greater than on prairie dogs with no positive fleas (*x*=11.3%, CI 9.6–12.9%).

Trends in flea abundance from swabbed burrows approximated those observed on prairie dogs, with abundance generally greater on undusted than dusted plots (Table 1). Burrow flea abundance increased on both dusted plots from May to September as deltamethrin effects began to wane (Table 1). Spring dusting seemed to suppress flea abundance in burrows for at least 5 mo, whereas autumn dusting suppressed flea abundance in burrows for at least 8 mo compared to undusted plots (Table 1).

**DISCUSSION**

Our experiment was designed to measure and compare flea abundance and prevalence on prairie dogs and in their burrows between sites receiving deltamethrin in spring or autumn and undusted control sites. The plague-driven collapse of undusted colonies by September 2009 made those planned comparisons difficult, but it did afford the opportunity to compare dusting effects on prairie dog persistence and trap success. Despite the limitations imposed by plague dynamics and the opportunistic nature of our study, we observed trends in prairie dog and flea abundance that provided inference to the potential effectiveness of insecticides to conserve prairie dogs (and associated species) during plague outbreaks. Our data and
Table 1. Summary of white-tailed prairie dog (*Cynomys leucurus*) capture and flea collection data from the Wolf Creek Management Area in northwestern Colorado, USA. Prairie dog capture was conducted in June, July, and September 2009 in areas dusted with deltamethrin in autumn 2008 (DA) and spring 2009 (DS), and on adjacent undusted control colonies (U1, U2). Prairie dog burrows were swabbed in May, July, and September 2009 within these same dusted and undusted plots.

<table>
<thead>
<tr>
<th></th>
<th>Dusted plot</th>
<th>Undusted plot</th>
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<tr>
<td></td>
<td>DA</td>
<td>DS</td>
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<tr>
<td></td>
<td>May/June</td>
<td>July</td>
</tr>
<tr>
<td>Fleas from prairie dogs</td>
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</tr>
<tr>
<td>No. of prairie dogs sampled</td>
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<td>31</td>
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<tr>
<td>Flea prevalence</td>
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<td>No. of flea pools</td>
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<tr>
<td>Mean flea abundance/burrow</td>
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<td>No. of flea pools</td>
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<td>Positive flea poolsd</td>
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<tr>
<td>Proportion of pools positive</td>
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a Fleas collected from burrows in May or from prairie dogs in June.
b Proportion of sampled prairie dogs or burrows yielding at least one flea.
c No data because no prairie dogs captured.
d Positive for *Yersinia pestis* DNA by PCR assay.
observations also underscore the devastating impact of epizootic plague on white-tailed prairie dog colonies and, by extension, on dependent species such as black-footed ferrets, which had apparently disappeared from the WCMA by autumn 2010 (C.P.W. unpubl. data).

Dusting in either spring or autumn seemed sufficient to blunt the impacts of plague on treated plots by suppressing flea abundance. Although a single application to only a portion of a colony was insufficient to completely suppress plague activity, dusting even a portion of a prairie dog colony temporarily improved local persistence in the face of epizootic plague. Depending on the conservation goal, such an approach may be sufficient for protecting isolated colonies or ensuring prairie dog persistence at a given location. However, preventive management strategies intended to completely suppress plague across large colony complexes (e.g., black-footed ferret restoration sites) will likely involve regular (annual or semiannual) dust application at biologically relevant spatial scales (i.e., entire colonies or colony complexes, depending on prairie dog species and connectivity; Griebel 2012).

Dusting and epizootic plague had large effects on flea abundance (Fig. 2; see Supplementary Material Table S1). A strong site x month interaction demonstrated waning deltamethrin effects and large increases in flea abundance on prairie dogs from undusted plots succumbing to epizootic plague. The threefold higher flea infestation on prairie dogs with Y. pestis–positive fleas seemed consistent with observations of flea concentration during plague epizootics in black-tailed prairie dogs (Tripp et al. 2009). Although prophylactic dusting did not reduce flea prevalence or entirely eliminate plague in the treated colony and prairie dog abundance eventually declined, both dusted plots remained occupied longer than undusted plots and available for further conservation action (e.g., annual or semiannual dusting).

Dusting in spring seemed to suppress flea abundance for at least 3 mo. Positive fleas were absent from captured prairie dogs until 5 mo after the spring treatment, when 14% of the flea pools from prairie dogs were positive for plague and flea abundance on prairie dogs returned to relatively high levels. Reasons for this relatively quick flea rebound could include presence of plague in the area before the treatment was applied (positive fleas and carcasses found while dusting), attraction of badgers and other predators, dispersing prairie dogs, or alternate hosts (e.g., Salkeld et al. 2010) trafficking fleas back into dusted areas (refuge effect), and heavy precipitation in spring 2009 that could have hampered treatment. In contrast, dusting in autumn seemed to suppress flea abundance for at least 9 mo. Flea abundance on prairie dogs began to rebound at 10 mo after dusting and, by 12 mo flea abundance rose to relatively high levels (Table 1 and Fig. 2). Positive fleas were absent from captured prairie dogs until 10 mo after treatment.

Our observations of white-tailed prairie dogs are consistent with those of others, suggesting that annual dusting in Gunnison’s (D.W.T. et al. unpubl. data) and black-tailed prairie dogs (Griebel 2012) is effective in reducing fleas and dampening or preventing plague. Our data suggest that deltamethrin treatments may be more effective as a conservation tool when applied in advance of epizootic plague. Prairie dog social dynamics, burrow densities, precipitation frequency and intensity, and soil type likely have direct and indirect impacts on dusting efficacy. Autumn dusting may offer advantages over spring applications in suppressing overwinter or early spring flea activity that may facilitate epizootic plague (Fig. 2; Wilder et al. 2008). Timing of dusting should be adjusted in hibernating species such that application precedes the late autumn seasonal decline in aboveground activity to ensure sufficient carriage of deltamethrin deeper into burrow chambers by prairie dogs. Large colony complexes or collections of occupied but fragmented habitat may benefit from a dusting strategy that includes both spring and autumn dusting to provide maximum flea suppression for at least part of the complex year-round. Dusting combined with oral vaccination (e.g., Abbott

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SUPPLEMENTARY MATERIAL

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LITERATURE CITED


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