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## RESISTANCE TO DELTAMETHRIN IN PRAIRIE DOG (*CYNOMYS LUDOVICIANUS*) FLEAS IN THE FIELD AND IN THE LABORATORY

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**ABSTRACT:** Sylvatic plague poses a substantial risk to black-tailed prairie dogs (*Cynomys ludovicianus*) and their obligate predator, the black-footed ferret (*Mustela nigripes*). The effects of plague on prairie dogs and ferrets are mitigated using a deltamethrin pulicide dust that reduces the spread of plague by killing fleas, the vector for the plague bacterium. In portions of Conata Basin, Buffalo Gap National Grassland, and Badlands National Park, South Dakota, US, 0.05% deltamethrin has been infused into prairie dog burrows on an annual basis since 2005. We aimed to determine if fleas (*Oropsylla hirsuta*) in portions of the Conata Basin and Badlands National Park have evolved resistance to deltamethrin. We assessed flea prevalence, obtained by combing prairie dogs for fleas, as an indirect measure of resistance. Dusting was ineffective in two colonies treated with deltamethrin for >8 yr; flea prevalence rebounded within 1 mo of dusting. We used a bioassay that exposed fleas to deltamethrin to directly evaluate resistance. Fleas from colonies with >8 yr of exposure to deltamethrin exhibited survival rates that were 15% to 83% higher than fleas from sites that had never been dusted. All fleas were paralyzed or dead after 55 min. After removal from deltamethrin, 30% of fleas from the dusted colonies recovered, compared with 1% of fleas from the not-dusted sites. Thus, deltamethrin paralyzed fleas from colonies with long-term exposure to deltamethrin, but a substantial number of those fleas was resistant and recovered. Flea collections from live-trapped prairie dogs in Thunder Basin National Grassland, Wyoming, US, suggest that, in some cases, fleas might begin to develop a moderate level of resistance to deltamethrin after 5–6 yr of annual treatments. Restoration of black-footed ferrets and prairie dogs will rely on an adaptive, integrative approach to plague management, for instance involving the use of vaccines and rotating applications of insecticidal products with different active ingredients.

**Key words:** *Cynomys*, deltamethrin, insecticide resistance, *Mustela nigripes*, plague, pulicide, Siphonaptera, *Yersinia pestis*.

### INTRODUCTION

By killing hosts, introduced diseases can alter ecologic relationships and negatively affect a multitude of species (Jones et al. 2008). Consequently, conservation practitioners devote considerable attention toward introduced diseases and, when possible, the development and implementation of management strategies (Scott 1988; Daszak et al. 2000).

The conservation implications of introduced diseases are exemplified by sylvatic

plague, a flea-borne zoonosis caused by *Yersinia pestis*. Plague poses a risk to many mammals (especially rodents), including some endangered and threatened species (Gage and Kosoy 2006). Moreover, by killing mammals, plague alters trophic and competitive relationships (Biggins and Kosoy 2001). Thus, in addition to its implications for human health (Schmid et al. 2015), plague is important in conservation biology.

In North America, plague is especially devastating to populations of prairie dogs (*Cynomys* spp.), which are colonial, burrow-

ing rodents. Plague causes occasional epizootics in prairie dogs (90–100% mortality) and depresses their densities during the interceding enzootic periods (Biggins et al. 2010; Mize and Britten 2016; Salkeld et al. 2016). In doing so, plague inhibits prairie dogs from serving their historic functions as foundation and keystone species (e.g., by reducing their ability to function as a consistent prey base for predators, and their creation of burrows used by many species of wildlife; Antolin et al. 2002; Eads and Biggins 2015).

Also important, plague hampers efforts to recover populations of endangered black-footed ferrets (*Mustela nigripes*). Black-footed ferrets are limited to prairie dog habitats and experience prey limitations caused by plague, but they are also directly susceptible to plague infection (Williams et al. 1994). In most cases, ferret populations are unable to persist in the absence of plague management (Miller and Reading 2012). As a result, plague is widely regarded as an imminent biological threat to recovery of the species (US Fish and Wildlife Service 2013).

Plague might be transmitted by direct contact with infectious blood, inhalation of respiratory droplets, consumption of carcasses, and contact with contaminated soil or infectious protozoans (Richgels et al. 2016). However, adult fleas (Siphonaptera) are the primary agents of plague transmission under natural conditions (Gage and Kosoy 2006). The effects of plague on prairie dogs and black-footed ferrets are mitigated using a deltamethrin-containing pulicide that is infused into prairie dog burrows to kill the flea vectors. Upon initial use of deltamethrin, flea parasitism on prairie dogs is suppressed, in some cases by 45–85% for  $\geq 10$  mo (Biggins et al. 2010). During a study conducted in areas with enzootic, low-level plague, deltamethrin enhanced annual survival rates for adult prairie dogs by 31–45% (Biggins et al. 2010). In another study, also conducted in areas where epizootics were not observed, deltamethrin improved annual survival rates for adult ferrets by a factor of 2.4, an improvement also produced by vaccination of ferrets against plague (Matchett et al. 2010).

In portions of Buffalo Gap National Grassland (Conata Basin) and Badlands National Park, South Dakota (Conata–Badlands), one of the most successful reintroduction sites for black-footed ferrets, deltamethrin has been infused into black-tailed prairie dog (*Cynomys ludovicianus*) burrows on an annual basis since 2005 (Griebel 2009). Plague invaded the area in 2008 and extirpated prairie dogs on nearly all 5,665 ha of habitat that was not treated annually with deltamethrin (Griebel 2009). The continued persistence of dusted colonies on Conata–Badlands is evidence for the effectiveness of deltamethrin. However, fragmentation of colonies treated with deltamethrin for  $>8$  yr (Griebel 2014) and declines in numbers and survival of ferrets on those colonies (Livieri 2016) have led to questions about a possible decline in deltamethrin efficacy.

Repeated use of pulicides with the same active ingredient can result in changes within flea populations due to natural selection for individuals that are resistant to the active ingredient (Brogdon and McAllister 1998). Perhaps not surprisingly, many flea species throughout the world have developed resistance to insecticidal ingredients to which they have been repeatedly exposed, including deltamethrin (Rust 2016). Flea resistance to deltamethrin might help to explain why colonies of black-tailed prairie dogs in Conata–Badlands that have been treated with deltamethrin for  $>8$  yr are increasingly fragmented and support declining numbers of black-footed ferrets, presumably due to plague.

We evaluated the hypothesis that on colonies of black-tailed prairie dogs in Conata–Badlands, flea populations with  $>8$  yr of exposure to deltamethrin have developed resistance to deltamethrin. To evaluate the number of years needed for resistance to develop, we conducted an experiment at Thunder Basin National Grassland, Wyoming, where a colony of prairie dogs had been treated with deltamethrin for 5 to 6 yr. We hypothesized that fleas at Thunder Basin might exhibit low to moderate levels of resistance to deltamethrin.

## MATERIALS AND METHODS

### Study sites

All research was conducted under guidelines of the American Society of Mammalogists (Sikes et al. 2016) and Institutional Animal Care and Use Committee protocols (US Geological Survey, Fort Collins Science Center, Colorado, protocol 2015-07). Conata Basin (29,000 ha; 43°46'N, 102°18'W) is situated on Buffalo Gap National Grassland, South Dakota. Badlands National Park is about 99,000 ha in size, is adjacent to Conata Basin, and is administered by the National Park Service. Both areas are characterized by short-grass prairie, badland buttes, and drainages. Primary land uses are cattle grazing and recreation. Mean annual precipitation is 415 mm. During 2005–14 (Griebel 2015), each burrow in the dusted sites was treated annually with about 4–6 g of deltamethrin (DeltaDust®, 0.05% deltamethrin, Bayer Environmental Science, Research Triangle Park, North Carolina, USA). Thunder Basin National Grassland (230,000 ha; 43°45'N, 105°00'W) is characterized by mixed grass prairie and sagebrush steppe habitats. Land uses are cattle grazing and recreation. Mean annual precipitation is 370 mm. Black-tailed prairie dogs dominated our study areas. In a 1,620-ha colony, we sampled a 10-ha area treated with deltamethrin since 2011 and a nearby 12-ha not-dusted area. Each burrow in the dusted area was treated annually with about 4–6 g of deltamethrin dust during 2011–16.

### Indirect evaluation of deltamethrin resistance

As an indirect measure of resistance, we used a combing method to compare flea prevalence on prairie dogs from dusted and not-dusted colonies. The evaluation included data from Conata–Badlands and Thunder Basin. In some cases, a single flea can transmit sufficient plague bacilli to infect and kill a rodent (Eisen et al. 2009). Therefore, we analyzed data on flea prevalence, defined as the percentage of prairie dogs that carried at least one flea.

*Conata–Badlands, South Dakota:* The indirect evaluation of resistance in Conata–Badlands, South Dakota took place during July–October 2007–09 and 2013–15 and involved live-trapping prairie dogs and combing fleas from them. In trained crews, a false-negative rate of 0.7% is expected when a black-tailed prairie dog is combed for 30 s to assess flea prevalence (Eads et al. 2013).

During 2007–09, we sampled prairie dogs on the North Enclosure, a site treated annually with deltamethrin since 2005, and from three nearby not-dusted sites along Highway 44. During 2013–

14, we strategized our efforts further, and sampled prairie dogs on the North and South enclosures (each treated annually with deltamethrin since 2005) and on a not-dusted colony termed Cutbank. In 2014, we also collected data at Pinnacles, a not-dusted colony in Badlands. In 2015, we collected data at South Enclosure and Cutbank.

Trained personnel sampled black-tailed prairie dogs using Tomahawk live traps (Tomahawk Live Trap, Hazelhurst, Wisconsin, USA). We anesthetized each prairie dog and fleas on its body with isoflurane and combed the prairie dog as thoroughly as possible for 30 s to collect fleas (Biggins et al. 2010). One species of flea, *Oropsylla hirsuta*, comprised >99% of fleas collected; the species specializes on prairie dog hosts, transmits *Y. pestis* (Wilder et al. 2008), and is commonly found on black-footed ferrets in Conata Basin (Harris et al. 2014).

Subsets of data allowed for temporal consistency in comparisons of flea prevalence on dusted and not-dusted colonies, and comparisons of data from pre- and postdust periods on dusted colonies. Temporal consistency was important, because seasonal changes in flea populations are substantial and can confound comparisons of data from different sites (Wilder et al. 2008; Eads 2014). Intervals of interest are discussed in the results, with comparisons accomplished using chi-square tests of independence in R 2.13.2 (Prop.Test; R Development Core Team 2011). Effect sizes were calculated using Prop.Test. In the case of plague management for the purpose of conservation, one might argue that the costs of a “false negative” in our study (i.e., suggesting fleas do not exhibit resistance) are greater than the costs of a “false positive.” Thus, we present effect sizes as 90% confidence intervals.

In the Supplementary Material, we complement the indirect evaluation of flea resistance at Conata–Badlands with data on population attributes for prairie dogs and black-footed ferrets at the North and South enclosures, thereby summarizing the implications of deltamethrin resistance for their populations.

*Thunder Basin, Wyoming:* The evaluation of resistance in Wyoming took place during a before-and-after control-impact experiment in July and September 2015 and 2016, with sampling completed at the same times on dusted and not-dusted sites. Data were collected from a plot treated with deltamethrin during late July 2011–16 and a nearby not-dusted plot. Each year, prairie dogs were live trapped as above during a pretreatment period (14–16 July 2015, 11–12 July 2016) and a posttreatment period (14–15 September 2015, 18–21 September 2016). Using Prop.Test, we compared flea prevalence on the

dusted and not-dusted areas before and after dusting of burrows in 2015 and 2016, separately.

#### Direct evaluation of deltamethrin resistance

To directly evaluate resistance, we exposed fleas to technical-grade deltamethrin in Petri dishes (Ames 2011) and compared survival and recovery rates for fleas from dusted and not-dusted sites. The direct evaluation was accomplished at Conata–Badlands. During trapping in 2014, before deltamethrin was applied to the dusted sites, fleas from a subset of prairie dogs in the North and South exclosures (dusted) and Cutbank and Pinnacles (not dusted) were placed in 50-mL Corning® plastic conical centrifuge tubes (Corning Inc., Corning, New York, USA) using handmade aspirators. Fleas were combed into a white plastic tub and then aspirated into a conical tube. The lid of each tube was perforated with a 24-gauge needle to provide air for fleas. Strips of filter paper were placed in each tube so the fleas could attach to and climb on an object. During each day, conical tubes containing fleas were stored in coolers with ice packets surrounded by newspaper bedding. Immediately after all prairie dogs had been processed and released at their point of capture, the fleas were transported to a field station for testing.

Deltamethrin (Chem Service, West Chester, Pennsylvania, USA) was dissolved in acetone at a concentration of 0.0135 µg/µL in amber glass vials. Pilot trials with fleas from prairie dogs at our sites demonstrated that this concentration would kill 100% of *O. hirsuta* fleas after 50–60 min of exposure. Between uses, the amber vials were placed in a freezer.

Petri dishes were 100 mm in diameter and 20 mm deep. Before each assay, the bottom interior of each Petri dish was coated with 0.0135 µg/µL of deltamethrin and 1 mL of acetone and rocked evenly until complete evaporation was observed. The dishes were allowed to dry for 30 min in a dark location. After the waiting period, an aspirator was used to transfer groups of ≤20 fleas to new conical tubes; the number of fleas per tube depended on the number of fleas collected each day. Those groups of fleas were then tapped into separate Petri dishes (1 dish/conical tube), lids were placed on the dishes, and a timer was started when the last dish was covered. This transfer process involved about 4 min for all dishes combined/trial. The maximum number of fleas/dish was limited to 20 because it was difficult to monitor >20 fleas in the same dish.

Fleas that were alive at the start of an assay were assessed for mortality every 5 min and classified as paralyzed or dead if they were laterally recumbent and unable to right themselves when stimulated by tapping or lightly

shaking the Petri dish (Ames 2011). All fleas were paralyzed or dead after 50 min and many were stored in vials with ethanol for future identification. Petri dishes were washed with soap and water and allowed to dry between trials.

We compared survival rates for fleas from the dusted and not-dusted sites using the Kaplan–Meier model in MARK 8.1 (White et al. 2001). The model estimates survival probabilities when the probability of detection is 1.0; in our case, the defined fate of each flea was known (alive or paralyzed/dead). The sample was comprised of individual fleas with observations of survival or paralysis/mortality at 5-min intervals. Fixed effects included minute mark during trials (e.g., 0, 5, 10 min), presence or absence of dust at the collection sites, and observer identification ( $n=2$ ). We used a likelihood ratio test, with an approximate chi-square distribution, to determine if survival rates differed between dusted and not-dusted sites.

In 2014, the possibility of recovery was assessed for a subset of fleas in an exploratory manner. In these cases, immediately after the Petri dish trials, fleas from the Petri dishes were placed in centrifuge tubes each containing ≤14 fleas. The tubes were opened every 5–15 h to renew oxygen levels. The tubes were examined 40–46 h after initiation of the trials to determine if any of the fleas recovered. If a flea regained mobility, it was classified as recovered. Some fleas remained motionless, suggesting death. We compared recovery rates for fleas from dusted and not-dusted colonies using Prop.Test.

In 2015, we implemented more recovery trials for replication. We placed fleas in Petri dishes with deltamethrin (same concentration and methods mentioned earlier, ≤20 fleas/dish) for 55 min, at which time all fleas were paralyzed or dead. Fleas from each dish were then placed into separate, dry Petri dishes that had never been exposed to deltamethrin and assessed for recovery 40 h later. We compared recovery rates for fleas from dusted and not-dusted colonies using Prop.Test.

## RESULTS

#### Indirect evaluation of deltamethrin resistance

We combed prairie dogs on 1,945 occasions. Not all prairie dogs were individually marked; thus, we could not account for repeated measures from all prairie dogs. The primary influence of repeated measures probably is to cause underestimation of variances. If repeated measures of flea prevalence are often independent (Krasnov et al. 2006; Eads 2014), the amount of repeated sampling of

TABLE 1. Flea prevalence (90% confidence interval [CI]) on black-tailed prairie dogs (*Cynomys ludovicianus*) in Conata Basin, Buffalo Gap National Grassland and Badlands National Park, South Dakota, USA, 2007–09 and 2013–15. Prairie dogs were sampled (i.e., combed for fleas) on three colonies along Highway 44 that had never been treated with pulicides (not dusted); on the North and South enclosure colonies, which had been treated (dusted) with a commercial deltamethrin-containing pulicide annually since 2005; and on Cutbank and Pinnacles colonies, which had never been treated with pulicides. Flea prevalence was calculated as the percentage of combings with at least one flea detected.

Year(s)	Site	Treatment	Sampling period	No. of combings	Flea prevalence, % (90% CI)
2007–08	Highway 44	Not dusted	June–July	237	60 (54–65)
2007–09	North Enclosure	Dusted	June–July	225	3 (2–6)
2013	North Enclosure	Dusted	June–July	21	76 (56–90)
2013	North Enclosure	Dusted	August–September	51	47 (35–59)
2014	North Enclosure	Dusted	July	44	68 (55–79)
2014	North Enclosure	Dusted	August	52	71 (59–81)
2014	North Enclosure	Dusted	September	20	5 (0–23)
2014	North Enclosure	Dusted	October	34	53 (38–68)
2014	Cutbank	Not dusted	July	19	11 (2–31)
2014	Cutbank	Not dusted	August	59	76 (65–85)
2014	Cutbank	Not dusted	September	26	100 (87–100)
2014	Pinnacles	Not dusted	July	20	60 (40–78)
2014	Pinnacles	Not dusted	August	41	54 (40–67)
2015	South Enclosure	Dusted	July	121	42 (35–50)
2015	South Enclosure	Dusted	August	145	43 (36–50)
2015	Cutbank	Not dusted	July	81	51 (41–60)
2015	Cutbank	Not dusted	August	90	49 (40–58)

individual prairie dogs in our study was unlikely to have substantially altered variances of the data.

*Conata–Badlands, South Dakota:* In June–July 2013, about 1 yr after the North Enclosure was dusted in 2012 (year 8 of dusting), flea prevalence on the colony was 58–88% higher than it had been at that point during 2007–09 (years 3–5 of dusting;  $\chi^2_1=121.03$ ,  $P<0.001$ ). During June–July 2013, flea prevalence on the North Enclosure was higher than observed on the not-dusted Highway 44 plots during the same months in the years of 2007–08 ( $\chi^2_1=2.15$ ,  $P=0.142$ ). That is, there was a tendency for flea prevalence to be higher at a site dusted since 2005 than sites that had never been dusted (Table 1). Dusting in 2013 reduced flea prevalence in the North Enclosure by 10–48% into August–September, almost 2 mo postdusting (Table 1, June–July vs. August–September;  $\chi^2_1=5.11$ ,  $P=0.024$ ).

In early August 2014, before dusting that year, flea prevalence was similar in the North Enclosure and not-dusted Cutbank ( $\chi^2_1=0.38$ ,  $P=0.540$ ) and was 1–34% higher in the North Enclosure relative to not-dusted Pinnacles (Table 1;  $\chi^2_1=3.03$ ,  $P=0.082$ ). About 4–9 d after deltamethrin was applied to the North Enclosure in 2014, flea prevalence declined dramatically to 5%. Only 31–37 d later, flea prevalence rebounded to 53% (Table 1). During October at the South Enclosure, 28–36 d postdusting, 5 of 15 prairie dogs carried  $\geq 1$  flea.

In 2015, we sampled prairie dogs in the South Enclosure (no dusting in 2015) and Cutbank. During July, about 11 mo after the South Enclosure was dusted in 2014, flea prevalence was similar in the South Enclosure and not-dusted Cutbank (Table 1;  $\chi^2_1=1.40$ ,  $P=0.236$ ). In August, flea prevalence again was similar in the South Enclosure and Cutbank (Table 1;  $\chi^2_1=0.84$ ,  $P=0.359$ ).

TABLE 2. Flea prevalence (90% confidence interval [CI]) on black-tailed prairie dogs (*Cynomys ludovicianus*) at Thunder Basin National Grassland, Wyoming, USA, 2015 and 2016. Prairie dogs were sampled (i.e., combed for fleas) in an area of a colony treated (dusted) with a commercial deltamethrin-containing pulicide annually since 2011 and a nearby control area that had never been treated with pulicides (not dusted). The study was designed under a before-and-after control-impact design. Each year, prairie dogs were sampled for fleas during a pretreatment period (before: 14–16 July 2015, 11–12 July 2016) and a posttreatment period (after: 14–15 September 2015, 18–21 September 2016). Flea prevalence was calculated as the percentage of combings with at least one flea detected.

Year	Treatment	Sampling period	No. of combings	Flea prevalence, % (90% CI)
2015	Dusted	Before	139	43 (36–51)
2015	Not dusted	Before	100	49 (40–58)
2015	Dusted	After	90	21 (14–30)
2015	Not dusted	After	68	84 (74–90)
2016	Dusted	Before	101	35 (27–43)
2016	Not dusted	Before	101	38 (30–46)
2016	Dusted	After	100	24 (17–32)
2016	Not dusted	After	104	88 (82–93)

*Thunder Basin, Wyoming:* In early July 2015, about 10 mo after dusting in 2014 (year 4 of dusting), flea prevalence was similar in the dusted and not-dusted areas (Table 2;  $\chi^2_1=0.80$ ,  $P=0.372$ ). After dusting of burrows in 2015 (year 5 of dusting), flea prevalence declined to about 21% in the dusted area but increased in the not-dusted area (Table 2;  $\chi^2_1=61.02$ ,  $P<0.001$ ). In early July 2016, about 11 mo after dusting in 2015, flea prevalence was similar in dusted and not-dusted areas (Table 2;  $\chi^2_1=0.19$ ,  $P=0.660$ ). About 1 mo after dusting in 2016 (year 6 of dusting), flea prevalence declined to about 24% in the dusted area but increased in the not-dusted area (Table 2;  $\chi^2_1=86.36$ ,  $P<0.001$ ).

#### Direct evaluation of deltamethrin resistance

During the Petri dish assays in 2014, and considering all minute marks in the trials, fleas from the dusted North and South exclosures exhibited survival rates that were 15% to 83% higher than fleas from not-dusted Cutbank

and Pinnacles (Fig. 1; likelihood ratio  $\chi^2_1=52.74$ ,  $P<0.001$ ). The variable for observer identification was retained as a control ( $\chi^2_1=0.37$ ,  $P=0.539$ ). During the exploratory recovery trials in 2014, recovery rates were 14–28% higher for fleas from the dusted colonies than for fleas from not-dusted colonies (Fig. 1;  $\chi^2_1=45.63$ ,  $P<0.001$ ). During planned recovery trials in 2015, recovery rates were 19–30% higher for fleas from the South Exclosure relative to Cutbank (Fig. 1;  $\chi^2_1=32.66$ ,  $P<0.001$ ).

#### DISCUSSION

Results from South Dakota support the hypothesis that on two black-tailed prairie dog colonies treated annually with deltamethrin for more than 8 yr (North and South exclosures), *O. hirsuta* fleas developed some resistance to deltamethrin. Flea prevalence on prairie dogs rebounded within 1 mo of dusting and reached levels similar to or higher than levels observed at not-dusted areas. These results provide indirect evidence of deltamethrin resistance. We supplemented the indirect evaluation of resistance with a direct evaluation. When directly exposed to deltamethrin, fleas from the two dusted colonies exhibited higher rates of survival than fleas from the not-dusted sites. Moreover, and perhaps most important, after removal from deltamethrin, approximately 30% of fleas from the dusted colonies recovered, compared with 1% of fleas from the not-dusted sites. Put simply, deltamethrin paralyzed fleas from the two colonies with more than 8 yr of annual exposure to deltamethrin, but substantial numbers of those fleas recovered.

Although our data indicated that fleas from the North and South exclosures had developed some level of resistance to deltamethrin by 2013–15 (years 9–11 of dusting), we could not characterize the progression of that response over time; we collected reference data during 2007–09 but did not trap again until 2013 (when circumstantial evidence of resistance became compelling). Results from Thunder Basin, Wyoming suggested that, in



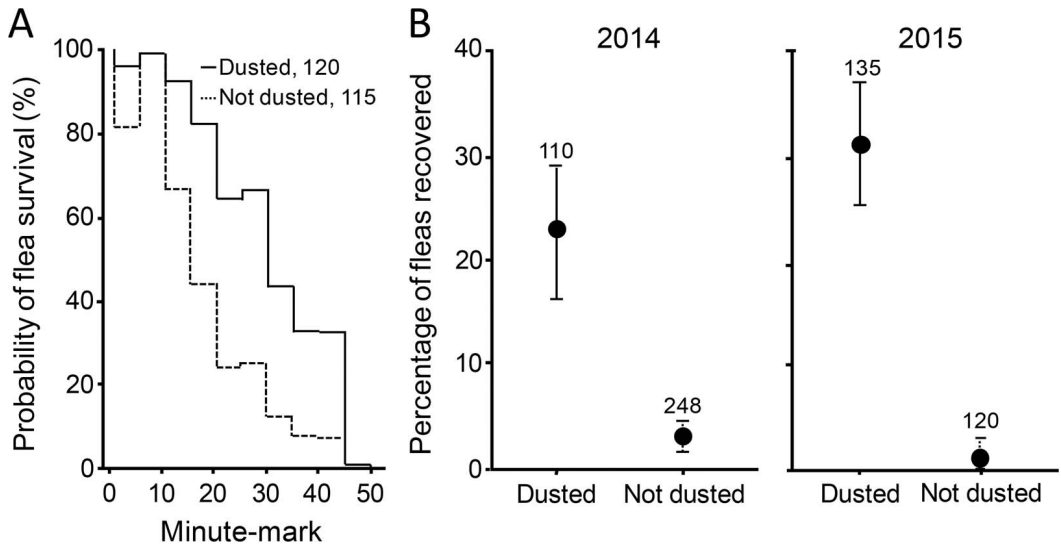


FIGURE 1. (A) Survival probabilities at 5-min marks for fleas subjected to 0.0135  $\mu\text{g}/\mu\text{L}$  deltamethrin in Petri dishes. Fleas were collected in South Dakota from colonies of black-tailed prairie dogs treated with a commercial deltamethrin-containing pulicide dust since 2005 (dusted) and colonies that had never been treated with pulicides (not dusted). Sample sizes are depicted next to the labels for dusted and not-dusted colonies. Survival probabilities are for fleas that were alive at the start of a particular 5-min mark (all fleas survived from the 5-min to the 10-min mark). (B) Recovery rates (90% confidence intervals) for fleas after 50 to 55 min of exposure to deltamethrin during 2014 and 2015; all fleas were paralyzed or dead after exposure to deltamethrin and were removed from deltamethrin and monitored for recovery about 40 h later. Sample sizes for the recovery trials are depicted above the recovery rates. In 2014, fleas were collected from two colonies with long-term exposure to deltamethrin, the North and South enclosures (dusted) and two colonies with no history of dusting, Cutbank and Pinnacles (not dusted). In 2015, fleas were collected from the South Enclosure (dusted) and Cutbank (not dusted).

some cases, fleas began to develop a moderate level of resistance to deltamethrin after 5–6 yr of treatment. During an experiment in Montana, prairie dogs carried 45% fewer fleas 10 mo after treatment (Biggins et al. 2010). In contrast, at about 10–11 mo postdusting at Thunder Basin, flea prevalence was similar in the dusted and not-dusted areas. At a site in Colorado, the prevalence of fleas on prairie dogs declined to 10% 1 month after burrows were infused with deltamethrin (Seery et al. 2003); at Thunder Basin, flea prevalence declined less so, to about 21–24% 1 mo posttreatment.

The results from Thunder Basin are perhaps suggestive of resistance, but logistical constraints inhibited an experimental design that would have allowed us to distinguish between an effect of flea resistance as opposed to other factors that might influence the efficacy of deltamethrin (e.g., attributes of

soils, local weather, etc.). The design would have benefited from additional replication and a treatment in which deltamethrin was infused into burrows at sites with no history of pulicide use (for comparison with data from sites with 5–6 yr of dusting). Continued research is needed to better evaluate the rate at which fleas develop resistance to deltamethrin.

The implications of our findings extend to all sites involved in efforts to conserve black-footed ferrets and prairie dogs. In prairie dog colonies where fleas have developed resistance to deltamethrin, the result could be catastrophic for ferrets. In many cases, prairie dogs and ferrets carry the same flea species (Harris et al. 2014; Mize et al. 2017). Fleas might, on occasion, transmit *Y. pestis* to ferrets (Matchett et al. 2010), as they do with prairie dogs and other rodents. However, the primary mode of plague exposure for ferrets is

thought to be via consumption of infectious rodent carcasses. In areas where fleas have developed resistance to deltamethrin, flea prevalence can rebound within 1 mo, perhaps allowing flea-borne plague to kill prairie dogs and other rodents. Subsequent to these rodent deaths, “the risk posed by even widely spaced carcasses could be serious for the relatively mobile foraging ferrets” (Godbey et al. 2006:236), which are likely attracted to rodent carcasses. This scenario might help to explain why numbers of ferrets on the enclosure colonies declined dramatically from 2007 to 2014, as fleas developed resistance to deltamethrin (Supplementary Material).

In addition to their direct susceptibility to plague, black-footed ferrets also experience prey limitations caused by plague (Williams et al. 1994; US Fish and Wildlife Service 2013). At the enclosure colonies, numbers of ferrets declined disproportionately faster than numbers of prairie dogs (the latter indexed using densities of burrow openings; Supplementary Material). This suggests that although plague and prey limitations might have collectively reduced survival rates for ferrets on the enclosure colonies, flea resistance to deltamethrin and plague-caused mortality in prairie dogs and other rodents (and resultant infection of predatory and scavenging ferrets) had a stronger effect than prey limitations. At conservation sites, black-footed ferrets should be vaccinated against plague as a proactive measure, even when deltamethrin is used for flea control.

It is important to note that shortly after the arrival of plague in Conata–Badlands during 2007–08, the disease extirpated nearly all prairie dogs on habitats that had not been treated annually with deltamethrin. It seems probable that if deltamethrin had not been used, very few prairie dogs would have survived the initial outbreak. If so, many or all of the black-footed ferrets would have been eliminated. Deltamethrin is an effective tool for plague management. We need to keep in mind that with repeated use of deltamethrin over time, fleas might develop resistance to the ingredient. With that said, fleas may not always develop resistance to deltamethrin

(e.g., resistance might be dampened if gene flow allows for the retention of susceptible genotypes in populations).

The development of deltamethrin resistance in flea populations can hamper but does not necessarily halt recovery efforts for black-footed ferrets. There is hope for the ferret’s recovery as new vector control tools are identified and added to deltamethrin-containing dusts as means for managing plague (e.g., other dust formulations and orally delivered systemic pulicides). The black-footed ferret recovery program ultimately seeks to develop an adaptive, integrative approach for plague management, for instance involving the use of vaccines for ferrets and rodents (Rocke et al. 2008, 2010) and rotating applications of pulicide products with different active ingredients.

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#### SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at <http://dx.doi.org/10.7589/2017-10-250>.

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