SHORT COMMUNICATIONS

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Plague in a Colony of Gunnison's Prairie Dogs (*Cynomys gunnisoni*) Despite Three Years of Infusions of Burrows with 0.05% Deltamethrin to Kill Fleas

ABSTRACT: At Valles Caldera National Preserve in New Mexico, USA, infusing Gunnison's prairie dog (Cynomys gunnisoni) burrows with an insecticide dust containing 0.05% deltamethrin killed fleas which transmit bubonic plague. The reduction in the number of fleas per prairie dog was significant and dramatic immediately after infusions, with a suggestion that the reduction persisted for as long as 12 mo. Despite the lower flea counts, however, a plague epizootic killed >95% of prairie dogs after 3 yr of infusions (once per year). More research is necessary for a better understanding of the efficacy of insecticide dusts at lowering flea counts and protecting prairie dogs from plague.

Key words: Cynomys gunnisoni, DeltaDust®, deltamethrin, fleas, plague, prairie dog, pulicide, Yersinia pestis.

Prairie dogs (Cynomys spp.) are highly susceptible to plague, a bacterial disease caused by Yersinia pestis and transmitted by fleas (Siphonaptera), for two reasons: they live in colonies that foster the transmission of parasites and diseases (Hoogland 1979) and they have not evolved a good defense against an introduced disease that first appeared at prairie dog colonies in the 1930s (Cully and Williams 2001). Despite recent evidence for limited resistance in some species of *Cynomys* (Rocke et al. 2012), epizootic outbreaks of plague typically kill >95% of residents within infected colonies of all four species of prairie dogs that inhabit the western US (Cully and Williams 2001).

A population crash sometimes occurs following the invasion of a colony by *Y. pestis* via

plague-positive fleas carried by dispersing prairie dogs or hunting carnivores (Barnes 1993; Cully and Williams 2001). Recent evidence (Biggins et al. 2010) indicates that plague bacteria can persist within a colony over many years while causing chronic, low levels of mortality (enzootic phase) before sometimes causing a severe, sudden population decline (epizootic phase).

We infused Gunnison's prairie dog (Cynomys gunnisoni) burrows with a powdered insecticide that kills fleas. Researchers have obtained similar results with the same insecticide for three other species of prairie dogs (Biggins et al. 2010; Tripp et al. 2016). We wanted to provide protection from plague for the marked *C. gunnisoni* we were studying. Because the emphasis was protection of C. gunnisoni rather than a thorough investigation of the efficacy and longevity of the insecticide dust, we did not perform the proper controls to unequivocally demonstrate the latter. Our attempt to protect C. gunnisoni from plague via infusions failed in the fourth year of our research.

Gunnison's prairie dogs are medium-sized (300–1,000 g for adults at ≥ 4 mo after weaning), herbivorous, colonial, diurnal, burrowing rodents (Hoogland 1979). From September 2012–September 2016, we studied the behavioral ecology of C. gunnisoni at Valles Caldera National Preserve in northcentral New Mexico, USA. We studied a section of a 30-ha colony in Redondo Meadow (35°30′36′′N, 106°21′36′′E) at an elevation

Table 1. Long-term effects of infusions with deltamethrin powder on flea prevalence (mean and 95% confidence interval [CI]) and flea intensity (mean and 95% CI) on adult Gunnison's prairie dogs (*Cynomys gunnisoni*) of Redondo Meadow, Valles Caldera National Preserve, New Mexico, USA. Flea counts are from September. The counts from 2014 and 2015 were collected about 12 mo after infusions of the previous year but before infusions of the current year.

Year	Infusions in the prior September	Sample size	Flea prevalence (95% CI)	Flea intensity \log_{10} (95% CI)
2012	No	208	0.87 (0.82, 0.91)	$0.54\ (0.52,\ 0.57)$
2013	No	162	0.75 (0.67, 0.81)	$0.51\ (0.48,\ 0.55)$
2014	Yes	180	0.55 (0.48, 0.62)	0.32 (0.29, 0.36)
2015	Yes	155	$0.66\ (0.58,\ 0.73)$	$0.39\ (0.36,\ 0.42)$

of 2,500 m. Our study area occupied approximately 6 ha, and over 4 yr it contained a mean (SD) of 126 (67) adults in March and April, 197 (50) juveniles that weaned in late May and June, and 202 (39) adults in September.

We live-trapped, ear-tagged, and uniquely marked (with black fur dye) every C. gunnisoni living within our study area at least once per year. We combed the sides and back of each individual 10 times (30 total combings per C. gunnisoni) and we counted the number of ectoparasites that either fell to a wooden floor or that we removed with tweezers (Hoogland et al. 2004). Sucking lice (Anoplura) and ticks and mites (Acarina) were rare. Fleas were common, however, and the most likely species were Oropsylla hirsuta, Oropsylla labis, and Oropsylla tuberculata cynomuris (Pizzimenti 1975; Friggens et al. 2010). We studied C. gunnisoni every day from March-July of 2013-2016 and also for approximately 20 d/yr in September-October of 2012-2016.

In an attempt to reduce the number of fleas on *C. gunnisoni* and in their burrows, we used a DR5 air-powered duster (Birchmeier, Baden, Switzerland) to infuse burrows with about 4 g of DeltaDust® (Bayer Environmental Science, Research Triangle Park, North Carolina, USA), a powder that contains 0.05% of an insecticide called deltamethrin. Infusions occurred on 25 September 2013, 25 September 2014, and over 3 d in late September 2015.

Both before and after infusions with deltamethrin, most *C. gunnisoni* in our study area harbored <5 fleas and many harbored no

fleas. We investigated two aspects of our C. gunnisoni flea counts: 1) flea prevalence, which was the proportion of C. gunnisoni that harbored at least one flea, and 2) flea intensity, which included only flea counts of ≥ 1 , with the exclusion of counts of zero. Because flea prevalence was so low for the 10 mo following infusions, we only examined flea intensity in September (at 12 mo after the infusions of the previous September; Table 1).

Seasonal and annual effects on flea counts are pronounced for other species of prairie dogs (Hoogland 1979), as was true for the *C. gunnisoni* at our study colony (Friggins et al. 2010). We therefore have cautiously interpreted flea counts from >1 mo after infusions with deltamethrin because we do not have controls from colonies with no infusions. With this reservation, we analyzed the trends in flea prevalence immediately (within 1–2 wk) before and after infusions in 2013–2014 and at 6, 7, 8, 9, 10, and 12 mo after infusions in September of 2013–2015.

In the 1–2 wk before and after infusions of *C. gunnisoni* burrows, we detected no *C. gunnisoni* that appeared sick or disoriented (Lechleitner et al. 1962; Hoogland et al. 2004). Further, we observed no disappearances that could not be explained by the usual low frequency of undetected dispersals, undetected predations, or early immergences for hibernation—all of which occurred in September 2012 with no infusions.

Before any infusions of burrows with deltamethrin, the natural variation in the prevalence of fleas observed on *C. gunnisoni* during handling and marking in September—

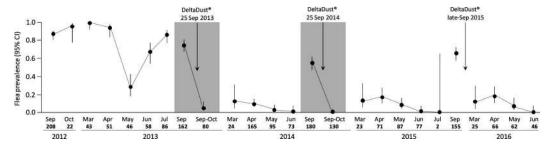


FIGURE 1. An overview of flea prevalence (mean and 95% confidence interval [CI]) on adult Gunnison's prairie dogs (*Cynomys gunnisoni*) during monthly intervals from 2012 into 2016 on a 6-ha study area of Redondo Meadow, Valles Caldera National Preserve, New Mexico, USA. Burrows in the study area were infused with DeltaDust, a powder containing 0.05% deltamethrin, on 25 September 2013, 25 September 2014, and over 3 days in late September 2015. The number below each month indicates the number of prairie dogs for which flea counts are available. Data are from adults only; see Fig. 2 for data from juveniles. The gray shading shows the flea counts in the 1–2 wk before and the 1–2 wk after infusions in September of 2013 and 2014; for September 2015 we only had flea counts before the infusions.

October 2012 and in March-July 2013 was substantial (Fig. 1). Infusions of deltamethrin had a dramatic effect on the prevalence of fleas over the short term in September-October of both 2013 and 2014 (shaded areas of Fig. 1). Our logistic regression revealed significant effects of both Year and Infusions (Year, Likelihood Ratio [LR] χ^2 =9.693, df=1, P=0.002; Infusions, LR $\chi^2=229.06$, df=1, P < 0.001). Over the short-term (mean=3.6 d after infusions), flea prevalence in September-October was reduced from 69% to 5% in 2013 and from 55% to 1% in 2014. The trend of low flea prevalence for up to 10 mo after application of deltamethrin (i.e., through July of following year; Fig. 1) is consistent with a lingering effect of the pulicide. Further, data on prevalence and intensity of flea parasitism from early and mid-September of 2014 and 2015 (before any infusions in those years), collected about 1 yr after infusions of the previous year, suggested deltamethrin had a residual effect for as long as 12 mo (Fig. 1, Table 1).

Finally, a long-term residual effect of deltamethrin is also suggested by the lower flea prevalence on *C. gunnisoni* juveniles in the spring of years (2014, 2015, and 2016) after infusions in the previous September than in the spring of the single year (2013) without any infusions in the previous September (Fig. 2). Flea intensity on juvenile *C. gunnisoni* at

12 mo postinfusions followed a pattern similar to that of flea prevalence at 12 mo postinfusions, but differences in flea intensity were less striking (Fig. 2). We detected >1 flea from an average of 60% of adult *C. gunnisoni* in September of the years following infusions (2014 and 2015) versus an average of 80% in September of the years with no previous infusions (2012 and 2013). Gunni-

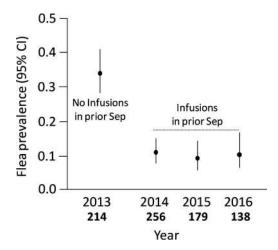


FIGURE 2. Flea prevalence (mean and 95% confidence interval [CI]) for juvenile Gunnison's prairie dogs (*Cynomys gunnisoni*) during May through July 2013–2016 at Redondo Meadow, Valles Caldera National Preserve, New Mexico, USA. Deltamethrin was infused into burrows during September of 2013, 2014, and 2015 but not in September 2012. The number below each month indicates the number of prairie dogs for which flea counts are available.

son's prairie dogs carrying at least one flea in September had an average of 3.34 fleas for the 2 yr following no infusions (2012 and 2013, before infusions on 25 September 2013) versus 2.27 fleas per *C. gunnisoni* at 12 mo after infusions. Some of these long-term differences in flea prevalence and flea intensity after infusions might have resulted from annual variation in flea counts. Our results underscore the importance of incorporating control areas with no infusions into future research that investigates the effectiveness of deltamethrin.

The number of adult *C. gunnisoni* in our 6-ha study area in early April was 222 in 2013, 113 in 2014, 102 in 2015, and 68 in 2016. This steady decline suggests that enzootic plague (Biggins et al. 2010) was causing low-level mortality from spring 2013 through spring 2016, but we have no information to confirm this possibility.

From daily counts of all *C. gunnisoni* at our study area, we detected no evidence of epizootic plague in September 2012 through July 2016. By September 2016, however, only two C. gunnisoni were still alive at our 6-ha study area. Visual counts throughout Redondo Meadow in September 2016 revealed an additional 11 C. gunnisoni in one small area of the 24 ha of suitable habitat that surrounded our study area. Fleas from this small area tested positive for Y. pestis by PCR in September 2016, thereby suggesting that plague was responsible for the death of >95% of the *C. gunnisoni* at our study area. Thus, despite the infusions of C. gunnisoni burrows with deltamethrin in September of three consecutive years, an epizootic of plague evidently occurred in our study area in August–September 2016 and killed almost all the residents there.

Previous investigators have demonstrated that infusions of burrows with deltamethrin reduce flea parasitism on three species of prairie dogs (Seery et al. 2003; Biggins et al. 2010; Tripp et al. 2016). In one study, deltamethrin continued to suppress flea counts for as long as 84 d after infusions (Seery et al. 2003). In another study, deltamethrin continued to reduce flea counts for

up to 10 mo after infusions (Biggins et al. 2010; Tripp et al. 2016). We documented that infusions with deltamethrin significantly and dramatically reduced flea counts for a fourth species of prairie dog immediately after infusions; less-dramatic reductions occurred for as long as 12 mo after infusions. In addition to coming from a new species, our results are unique because we have large sample sizes for flea counts from the same marked *C. gunnisoni* immediately before and up to 12 mo after infusions.

Our short-term results are especially persuasive because fleas almost completely disappeared from *C. gunnisoni* at our study area immediately after (≥2 wk) infusions in 2013 and 2014. Over longer intervals, our results are more difficult to interpret because seasonal changes in flea counts are common and might have confounded the apparent effectiveness of deltamethrin for as long as 12 mo after infusions.

Fleas are important in the transmission of plague within prairie dog colonies, but transmission also can occur via direct contact with infected individuals or carcasses or via aerosol transfer (Barnes 1993; Biggins et al. 2010). Perhaps these latter forms of transmission occurred at our study area and induced the epizootic of plague that occurred in August–September 2016.

With protracted, low-level mortality, enzootic plague can kill prairie dogs without an epizootic that typically, and suddenly, kills >95% of colony residents (Biggins et al. 2010). The steady decline in numbers of adult *C. gunnisoni* at our study site suggests, but does not confirm, that enzootic plague might have caused low-level mortality from spring 2013 through spring 2016.

Because fleas transmit Y. pestis to all four species of prairie dogs in western US, and because high flea counts probably enhance the transmission of plague (Lorange et al. 2005), many biologists have argued that reducing the number of fleas within colonies via infusions with deltamethrin should help to protect individuals from plague (Seery et al. 2003; Biggins et al. 2010; Tripp et al. 2016). Although our results suggest that deltamethrin

can be an effective pulicide for up to 12 mo, three consecutive years of infusions did not protect our marked *C. gunnisoni* from epizootic plague in August–September 2016. A detectable, significant effect of deltamethrin at killing fleas (Figs. 1 and 2, Table 1) evidently does not always translate into a biologic effect sufficient to suppress plague. Perhaps more-frequent infusions—as opposed to our single infusions in late September of each year—would have provided the protection necessary to deter the epizootic in our study area.

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