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Authors: Matchett, Marc R., Eads, David A., Cordova, Jennifer, Livieri, Travis M., Hicks, Holly, et al.

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FLEA CONTROL ON PRAIRIE DOGS (*CYNOMYS* SPP.) WITH FIPRONIL BAIT PELLETS: POTENTIAL PLAGUE MITIGATION TOOL FOR RAPID FIELD APPLICATION AND WILDLIFE CONSERVATION

Marc R. Matchett,[1,6] David A. Eads,[2] Jennifer Cordova,[3] Travis M. Livieri,[4] Holly Hicks,[5] and Dean E. Biggins[2]

[1] US Fish and Wildlife Service, Charles M. Russell National Wildlife Refuge, 333 Airport Road, Lewistown, Montana 59457, USA
[2] US Geological Survey, Fort Collins Science Center, 2150 Centre Avenue, Building C, Fort Collins, Colorado 80526, USA
[3] Arizona Game and Fish Department, PO Box 387, Seligman, Arizona 86337, USA
[4] Prairie Wildlife Research, PO Box 643, Wisconsin 54481, USA
[5] Arizona Game and Fish Department, 5000 West Carefree Highway, Phoenix, Arizona 85086 USA
[6] Corresponding author (email: randy_matchett@fws.gov)

ABSTRACT: Sylvatic plague is a widespread, primarily flea-vectored disease in western North America. Because plague is highly lethal to endangered black-footed ferrets (*Mustela nigripes*, BFFs) and the prairie dogs (*Cynomys* spp., PDs) on which BFFs depend for habitat and prey, minimizing the impacts of plague is a priority at BFF reintroduction sites. We developed a new, flour-based bait pellet containing 0.84 mg of fipronil and weighing ~1.25 g (FipBits). We measured the degree and duration of flea control on black-tailed PDs (*C. ludovicianus*) in Montana and on Gunnison's PDs (*C. gunnisoni*) in Arizona, USA from 2018–2020. FipBits were distributed on treated plots one time at a rate of 125/ha. Fleas were virtually eliminated in Montana from 1 mo posttreatment to 1 yr later and remained substantially depressed 2 yr posttreatment. With the split colony design, we probably underestimated the degree of flea control achieved with FipBits due to crossover edge effects along the arbitrary line dividing the plots. Flea control in Arizona was significant from 1 mo posttreatment to 1 yr later, but flea abundance had recovered by 2 yr posttreatment. Flea control was evaluated from 2020–2021 in South Dakota, USA on four plots treated with three concentrations of fipronil in FipBits (0.68, 0.71, and 0.83 mg/FipBit). Fleas were essentially eliminated for 10 mo on the 0.83-mg plot and were substantially reduced on the two 0.71-mg plots. Fleas were reduced on the 0.68-mg plot, but the degree of control was less than observed on other treated plots. Impacts of plague on PDs and BFFs would probably be greatly reduced by the levels of flea control observed with FipBits. Options for expanded FipBit evaluations are being pursued for what may become a highly practical, affordable, and effective plague mitigation tool.

Key words: Black-footed ferret, *Cynomys*, fipronil, flea control, *Mustela nigripes*, plague, *Yersinia pestis*.

INTRODUCTION

The primary biologic challenge to recovery of endangered black-footed ferrets (*Mustela nigripes*, BFFs) is sylvatic plague, caused by the bacterium *Yersinia pestis* and vectored primarily by fleas. Plague is highly lethal to both BFFs and the prairie dogs (*Cynomys* spp., PDs) on which BFFs are obligate dependents for habitat and prey. A priority action item in the current BFF Recovery Plan (US Fish and Wildlife Service 2013, p. 101) is to "Develop and implement as appropriate prophylactic methods for controlling sylvatic plague." A variety of insecticides has been used with varying degrees of cost and success to control fleas and minimize the effects of plague on PDs and other mammals (e.g., Hoogland et al. 2004, 2018; Biggins et al. 2010, 2021a, 2021b; Eads and Biggins 2019; Eads et al. 2019, 2021; Enscore et al. 2021; Goldberg et al. 2021). Given the need to minimize plague effects at BFF reintroduction sites, there have been intensive and extensive efforts to manage plague (e.g., Biggins et al. 2010, 2021a; Matchett et al. 2010, 2021; Rocke et al. 2017; Tripp et al. 2017). Such efforts continue with the development and testing of fipronil-laced bait pellets herein that have the potential to

FIGURE 1. Black-tailed prairie dog (*Cynomys ludovicianus*) with a fipronil bait pellet (FipBit) in its mouth, South Dakota, USA. Photo credit: D.A.E.

drastically reduce the cost of mitigating plague with flea control compared to other methods.

A progression of steps led to the development of fipronil bait pellets for flea control. Since 2016, grain (wheat) treated with 0.005% fipronil by weight has been effective in systemically controlling fleas on PDs (Poché et al. 2017, 2020; Eads et al. 2019, 2020). Fleas that have imbibed blood from a PD that consumed fipronil-treated grain are killed and larval fleas interacting with or feeding on feces from treated PDs may also be killed (Eads et al. 2019, 2023). During 2016–2019 in South Dakota, USA, flea control with fipronil grain was significant for at least 12 mo at 11 sites on three colonies of black-tailed PDs (*C. ludovicianus*, BTPDs; Eads et al. 2019, 2020).

Mass production of bait pellets to deliver an experimental oral plague vaccine to PDs was optimized by Corro et al. (2017). Kreiger and Matchett (2019) invented bait-dispensing units (triple-shooters, Model Avionics, Billings, Montana, USA) capable of rapidly distributing such bait pellets at 125 baits/ha across 20 ha/hr, or more, from an all-terrain vehicle. Author M.R.M., as a government employee, integrated the flea control success of efforts with fipronil-treated grain, bait manufacturing, and distribution capabilities to create a new fipronil bait pellet formulation that can be manufactured with methods similar to Corro et al. (2017) and rapidly distributed via triple-shooters. Specifically, he used inexpensive and readily available food-grade ingredients, simple formulation steps, and small amounts of fipronil to create "FipBits": blue, ~1.25 g, round (1.25-cm diameter) baits each containing 0.84 mg of fipronil (Fig. 1). Other ingredients include three types of flour, two thickeners, molasses, peanut butter, citric acid, two preservatives, and blue food dye. Production was completed at the Charles M. Russell National Wildlife Refuge headquarters located in Lewistown, Montana.

Eads et al. (2021) reported on experimental testing of FipBits with BTPDs from 2018–2020 in South Dakota, where FipBits suppressed fleas for 12 mo at three sites and up to 24 mo at two sites. Replication and additional experimentation are needed to further understand the degree and duration of flea control possible with FipBit treatments.

We investigated the degree and duration of flea control using FipBits on a colony of BTPDs in Montana and a colony of Gunnison's PDs (*C. gunnisoni*, GPDs) in Arizona from 2018–2020, plus two colonies of BTPDs in South Dakota from 2020–2021. Concentrations evaluated included 0.84 mg fipronil/FipBit in Montana and Arizona and 0.68, 0.71, or 0.83-mg fipronil/FipBit in South Dakota. A split-colony design (Biggins et al. 2010, 2021a) in Montana allowed us to investigate crossover edge effects on flea control along an arbitrary treatment plot boundary.

MATERIALS AND METHODS

FipBits used in Montana and Arizona were formulated with technical grade fipronil supplied by Chem Impex (935 Dillon Drive, Wood Dale, Illinois, USA); those used in South Dakota were formulated with a stock solution supplied by BASF Agricultural Products Group (BAS 350GA1, Batch 6771-34, Research Triangle Park, North Carolina, USA). Fipronil concentrations in FipBits (n=5 baits per lot) were measured and confirmed by extraction with acetonitrile, and the extract was analyzed by liquid chromatography with ultraviolet detection at the Analytical Toxicology Laboratory, Colorado State University, Fort Collins, Colorado, USA.

We implemented a before-after-control-impact (BA-CI) experimental design in Montana and Arizona evaluating flea control over 24 mo. Nontreated plots provided a baseline of flea parasitism before and after treatment. We implemented a before-after design from before treatment to 10 mo posttreatment in South Dakota. All experimental plots and adjacent habitat had no recent (≥2 yr) flea control treatments.

We established one pair of 4-ha test plots in Montana on the Charles M. Russell National Wildlife Refuge (47°37′59.9″N, 107°49′59.9″W) on an 8-ha BTPD colony and one pair (4 ha each) in Arizona on the Double O Ranch (35°34′59.9″N, 112°58′0.1″W) on an ~1,500-ha GPD colony. The paired plots in Montana were delineated by splitting a BTPD colony roughly in half by an arbitrary east-west line with no buffer zone between plots. The paired plots in Arizona were 2.9 km apart in areas with similar GPD burrow densities. We tested FipBits on four plots in South Dakota, each 1 ha, distributed between two BTPD colonies on the Buffalo Gap National Grassland; three plots were situated on the same ~75-ha colony and separated by >100 m, (43°32′4.9″N, 102°1′27.8″W), and the final plot (0.68 mg of fipronil/FipBit) was on a different colony (~500 ha), ~26 km southwest (43°26′44.5″N, 102°10′31.1″W) of the first colony.

We completed flea burden assessments on PDs by inspecting live-trapped, anesthetized PDs for fleas. Trap effort and sampling procedures were identical and simultaneous on paired treated and nontreated plots. Detailed PD handling methods can be found in Eads et al. (2019, 2020, 2021) and Matchett et al. (2021). Briefly, we live-trapped PDs using cage traps (Tomahawk Live Trap, Hazelhurst, Wisconsin, USA in South Dakota and Arizona; Tru-Catch Traps, Belle Fourche, South Dakota in Montana) baited with a rolled corn, oats, barley, and molasses livestock feed (laced with small amounts of peanut butter in South Dakota). We checked the traps by midmorning, anesthetized the PDs using isoflurane (Clipper Distributing Company, St. Joseph, Missouri, USA) on a cotton ball in an induction chamber in South Dakota and Arizona and in an induction chamber using isoflurane in oxygen through a vaporizer (Anaesthesia Equipment Service and Supply, Inc., Sanford, Florida, USA) in Montana. We recorded age (juvenile or adult), mass (g), and sex. The PDs in Montana and Arizona were batch-marked with a livestock crayon to facilitate immediate release if caught a subsequent time within a trapping session; BTPDs in South Dakota were ear tagged and immediately released on any subsequent recapture within a trapping session. We thoroughly combed each PD with a fine-toothed comb over a white plastic tub for 30 sec to dislodge and count fleas. We placed all fleas back on the PD to minimize any removal effect (Eads et al. 2021). Once fully recovered from anesthesia, we released PDs at the point of capture.

At all sites, resampling individuals in different trapping sessions could potentially influence our results. However, all fleas were placed back on each PD and no PDs were resampled within a trapping session. Any repeat samples from an individual would have been separated by a month to many months, providing time for PDs to acquire, or dispel, more fleas in burrows or from interacting with conspecifics.

Pretreatment flea assessments were made 5–6 August 2018 in Montana, 11–16 August 2018 in Arizona, and 12 July–2 September 2020 in South Dakota. FipBits were distributed on 7 August

2018 in Montana, 17 August 2018 in Arizona, and 3–4 September 2020 in South Dakota. We distributed FipBits uniformly at a rate of 125/ha by walking along transects (9×9-m grid) and dropping FipBits (Arizona and South Dakota) or from an all-terrain vehicle using a triple-shooter (Kreiger and Matchett, 2019) for application along transects (Montana). We made posttreatment flea assessments at approximately 1, 2–3, 9, 12, and 24 mo posttreatment in Montana and Arizona and 1 and 9–10 mo posttreatment in South Dakota.

Lorange et al. (2005) suggested that individual fleas are inefficient at plague transmission; thus, high flea burdens per host are oftentimes needed for successful transmission (with rat fleas *Xenopsylla cheopis*). Therefore, we focused our analyses on flea abundance, defined as counts of fleas per combed PD, including zeroes. We analyzed the Montana and Arizona data together and the South Dakota data separately. In all cases, we used negative binomial generalized linear models for analysis in R version 4.1.0 (glm.nb function in the *MASS* package; Ripley et al. 2021). All models included control variables for PD age and sex, given the importance of these variables in multiple studies of PDs and their fleas (e.g., Tripp et al. 2009; Eads and Hoogland 2016, 2017). We selected parsimonious models via Type III *F*-tests and backward elimination (α=0.050, anova function in *car* package; Fox et al. 2021). For interpretation, we extracted predictions of flea abundance and 95% confidence intervals (predictions function ggeffects package; Lüdecke et al. 2021) from the most parsimonious model. We present predictions for adult female PDs, specifically thereby controlling for PD age and sex, while evaluating changes in flea abundance from before to after FipBit treatments (the direction of the treatment effect was consistent for all sex and age classes).

Montana and Arizona

We investigated effects of the following factors on flea abundance: SITE (Montana or Arizona), TREATMENT (FipBit or nontreated), PERIOD (before or after treatment), and all possible two-way and three-way interactions (which helped account for SITE differences while assessing flea changes relative to TREATMENT and PERIOD; Eads et al. 2019). If FipBit treatment effectively controlled fleas, then flea abundance on treated habitat should have declined after treatment and remained low over time relative to nontreated habitat. We considered 3 posttreatment PERIODs to assess short-term, midterm, and long-term flea control (Montana: 1 to 3, 9 to 12, and 24 mo; Arizona: 1 to 2, 9 to 12, and 24 mo).

The split-colony design in Montana provided the opportunity to evaluate potential edge effects along the arbitrary treatment plot boundary. Capture location relative to the treatment dividing line determined whether a sample was tallied on the treated or nontreated plot. Those BTPDs living near the line probably spent time on both sides of the boundary. Thus, we considered a potential effect of DISTANCE to the treatment plot division line on flea abundance. We hypothesized that over the short- to midterm, FipBit treatment might have crossover effects on the nontreated side of the line by reducing flea abundance on BTPDs tallied as caught on the nontreated plot near the dividing line, yet may have consumed FipBit(s) because of their proximity to the treated plot. We hypothesized further that flea abundance levels might rebound on the FipBit-treated portion of the colony by 24 mo posttreatment (as suggested by trends of flea control with FipBits in Eads et al. 2021) by virtue of proximity to nontreated BTPDs. If so, we hypothesized that at 24 mo posttreatment, fleas on the FipBit side would be most abundant on BTPDs near the treatment line due to crossover from the nontreated plot. We evaluated these potential edge effects with a three-way interaction among DISTANCE, TREATMENT, and PERIOD.

South Dakota

We were primarily interested in differences in flea control at differing fipronil treatment rates and modeled effects for TREATMENT: 0.68 mg (1 plot), 0.71 mg (2 plots), or 0.83 mg fipronil/FipBit (1 plot); PERIOD: before treatment = 12 July–2 September 2020; 6 days to ~1 mo posttreatment = 9–23 September 2020, and ~9–10 mo posttreatment = 21 June–31 July 2021), and their interaction. Replicated research at the South Dakota study area had demonstrated that, in the absence of insecticide treatment or effective flea control, flea parasitism increases on BTPDs over summer-to-fall (Eads et al. 2018, 2019, 2020, 2021). We hypothesized that flea abundance would decline from before to 1 mo after FipBit treatments and fleas would remain suppressed 9–10 mo posttreatment.

Research in Montana was conducted under Smithsonian National Zoological Park IACUC no. 19-04, in South Dakota extended IACUC protocol 2015-07 (US Geological Survey, Fort Collins Science Center, Colorado), and in Arizona under Arizona Game and Fish Department (2016).

RESULTS

Montana and Arizona

In Montana, we combed BTPDs on 652 occasions and detected 2,082 fleas (Table 1). A total of eight fleas were combed from six

TABLE 1. Data from before-after-control-impact FipBit (0.84 mg fipronil/pellet) experiments with black-tailed prairie dogs (*Cynomys ludovicianus*) and Gunnison's prairie dogs (*C. gunnisoni*) sampled in Montana and Arizona, USA, respectively, including the state, sampling period, treatment, number of prairie dogs (PDs) combed for fleas, number of PDs with fleas, number of fleas, and flea parasitism indices; flea prevalence (hosts observed with flea(s)/hosts sampled), flea abundance (total fleas/hosts sampled), and flea intensity (total fleas/hosts with more than zero fleas) from 2018–2020. All PDs were combed once within a trapping session, but could be resampled between trapping sessions.

US State	Sampling period	Treatment	No. PDs combed	No. PDs with fleas	No. fleas	Flea parasitism index		
						Prevalence	Abundance	Intensity
Montana	Before treatment	Nontreated	59	56	529	0.95	8.97	9.45
		FipBits	63	63	560	1.00	8.89	8.89
	1–3 mo after	Nontreated	114	92	590	0.81	5.18	6.41
		FipBits	88	3	4	0.03	0.05	1.33
	9–12 mo after	Nontreated	100	44	151	0.44	1.51	3.43
		FipBits	96	3	4	0.03	0.04	1.33
	24 mo after	Nontreated	61	50	175	0.82	2.87	3.50
		FipBits	71	23	69	0.32	0.97	3.00
Arizona	Before treatment	Nontreated	9	8	19	0.89	2.11	2.38
		FipBits	14	12	56	0.86	4.00	4.67
	1–2 mo after	Nontreated	52	25	51	0.48	0.98	2.04
		FipBits	50	0	0	0.00	0.00	—
	9–12 mo after	Nontreated	76	33	111	0.43	1.46	3.36
		FipBits	42	7	12	0.17	0.29	1.71
	24 mo after	Nontreated	17	9	17	0.53	1.00	1.89
		FipBits	18	11	27	0.61	1.50	2.45

[a] — = data not calculable.

BTPDs among the 184 sampled from 1–12 mo posttreatment on the FipBit plot, compared to a total of 741 fleas from 136 BTPDs among the 214 sampled on the nontreated side of the colony. Flea species were not identified, but most were likely to be *Pulex simulans* (a generalist of mammal hosts) or *Oropsylla hirsuta* (a *Cynomys* specialist; Russell et al. 2018).

In Arizona, we combed GPDs on 278 occasions and detected 293 fleas (Table 1). A total of 12 fleas were combed from seven GPDs among the 92 sampled from 1–12 mo posttreatment on the FipBit plot, compared to a total of 162 fleas from 58 GPDs among the 128 sampled on the nontreated plot. Flea species were not identified, but most were likely to be *O. hirsuta* (Russell et al. 2018).

In the combined multivariate analysis, the PERIOD×TREATMENT×SITE interaction was eliminated (P=0.362). All two-way interactions were supported (P<0.001). Before treatment in Montana, fleas were similarly abundant on the FipBit and nontreated sides of the colony (Fig. 2, Table 1). Flea abundance declined on both sides of the colony from 1–3 mo and 9–12 mo posttreatment, but the decline was much greater on the FipBit side. At 24-mo posttreatment, flea abundance had started to rebound on the FipBit side, but fleas were still significantly less abundant on the FipBit side (approximately one flea per BTPD) compared to the nontreated side (almost three fleas per BTPD). Thus, in Montana, FipBits reduced fleas on BTPDs for at least 24 mo.

Before treatment in Arizona, fleas were more abundant on the plot selected for subsequent FipBit treatment than on the plot that would not be treated (Fig. 2; Table 1). Fleas remained similarly abundant on the nontreated plot from 1–24 mo posttreatment. In contrast, flea abundance was low on the FipBit plot at 1–2 mo posttreatment (no fleas

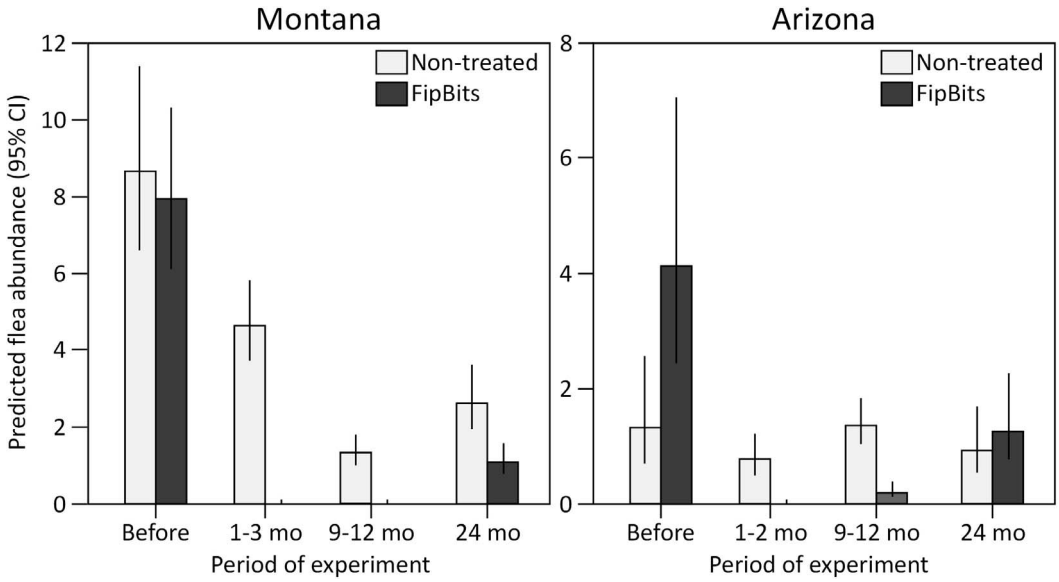

FIGURE 2. Predicted flea abundance (95% confidence intervals) on adult female black-tailed prairie dogs (*Cynomys ludovicianus*) in Montana, USA, and Gunnison's prairie dogs (*C. gunnisoni*) in Arizona, USA, during before-after-control-impact flea control experiments with FipBits (0.84 mg fipronil/pellet distributed at 125 pellets/ha; see also Table 1). Predictions are from a negative binomial generalized linear model with effects for prairie dog age and sex, and all possible two-way interactions between treatment (nontreated or FipBit), period of experiment (with three periods after treatment), and site (Montana or Arizona).

were combed from 50 GPDs sampled and just one flea was found in the induction chamber). Flea abundance remained low at 9–12 mo posttreatment. By 24 mo posttreatment, fleas were more abundant on the FipBit plot compared to the nontreated plot. Thus, in Arizona, FipBits reduced fleas on GPDs for at least 12 mo.

We probably underestimated the degree of flea control in Montana because of a crossover edge effect along the arbitrary line separating the plots. In the edge effect analysis, the DISTANCE×TREATMENT×PERIOD interaction was influential (P=0.025). Before treatment, there was no relationship between capture location DISTANCE to the plot division line and flea abundance on either plot (Fig. 3). In contrast, from 1–12 mo posttreatment, fleas were least abundant near the treatment division line, and most abundant far from the line, on the nontreated plot; that is, the suppressing effect of FipBits on fleas appeared to cross over from the treated plot into neighboring portions of the nontreated plot (Fig. 3). At 24 mo posttreatment, fleas were most abundant on BTPDs near the treatment line on the FipBit plot, and there remained a marked effect of DISTANCE on the nontreated side with fewer fleas on BTPDs near the treatment line, a probable continuing crossover effect from FipBit treatment on the other side of the line (Fig. 3). These edge effects, in both directions, would have caused us to underestimate the efficacy of flea control with FipBits. When excluding all posttreatment captures within 50 m of the dividing line, we detected just 55 fleas on 20 BTPDs among the 169 sampled on the FipBit plot over all posttreatment sampling occasions. In contrast, we detected 988 fleas on 155 BTPDs among the 190 sampled on the nontreated plot. The treated plot in Arizona was surrounded by nontreated GPDs, and this edge effect may have contributed to fleas rebounding there 24 mo posttreatment (Fig. 2).

South Dakota

We combed BTPDs on 543 occasions and detected 1,086 fleas (Table 2). Flea species

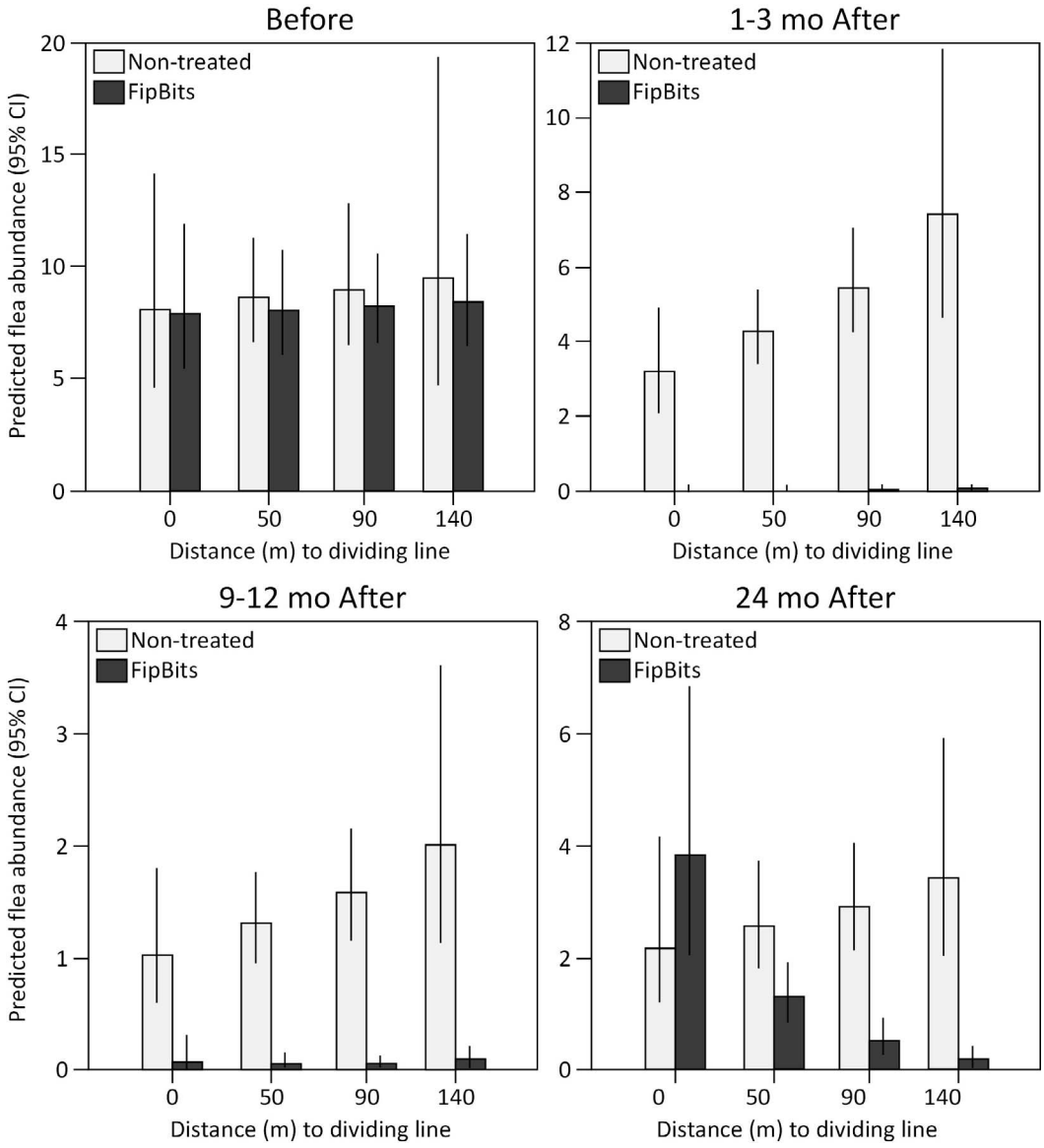

FIGURE 3. Predicted flea abundance (95% confidence intervals) on adult female black-tailed prairie dogs (*Cynomys ludovicianus*, BTPD), before and after treatment with FipBits (0.84 mg/pellet distributed at 125 pellets/ha), in Montana, USA, versus distance of capture locations to the treatment division line. Treated and nontreated portions of the same colony were separated by an arbitrary treatment division line (split-colony design with no buffer zone between plots). Predictions are from a negative binomial generalized linear model with effects for BTPD age and sex, and a three-way interaction among treatment (nontreated or FipBit), period of experiment (before treatment, 1–3 mo, 9–12 mo, and 24 mo posttreatment), and distance (m) to the treatment division line (presented here as four distance categories for ease of interpretation).

were not identified, but most were probably *O. hirsuta* (Eads et al. 2018). In the analysis of flea abundance, the TREATMENT×PERIOD interaction was supported (P=0.002). From before-to-after treatment, flea abundance declined on the plot treated with 0.68 mg FipBits, but more than two fleas were detected on many BTPDs up to 1 mo posttreatment, and flea numbers had increased considerably by 9–10 mo posttreatment (Fig. 4). Flea control was

TABLE 2. Data from before-after FipBit experiments with black-tailed prairie dogs (*Cynomys ludovicianus*, BTPDs) in South Dakota, USA, including average amount (mg) of fipronil per FipBit, sampling period, number of BTPDs combed for fleas, number of BTPDs with flea(s), number of fleas detected, and flea parasitism indices; flea prevalence (hosts observed with flea(s)/hosts sampled), flea abundance (total fleas/hosts sampled), and flea intensity (total fleas/hosts with more than zero fleas) from 2020–2021. All BTPDs were combed once within a trapping session, but could be resampled between trapping sessions.

Fipronil/ FipBit (mg)	Sampling period	No. BTPD combings	No. BTPDs with fleas	No. fleas	Flea parasitism index		
					Prevalence	Abundance	Intensity
0.68	Before treatment	52	34	359	0.65	6.90	10.56
	1 mo after	95	41	261	0.43	2.75	6.37
	9–10 mo after	18	14	84	0.78	4.67	6.00
0.71	Before treatment	115	39	185	0.34	1.61	4.74
	1 mo after	94	21	41	0.22	0.44	1.95
	9–10 mo after	73	6	14	0.08	0.19	2.33
0.83	Before treatment	31	23	141	0.74	4.55	6.13
	1 mo after	50	1	1	0.02	0.02	1.00
	9–10 mo after	15	0	0	0.00	0.00	—

[a] — = data not calculable.

relatively strong on the two plots treated with 0.71 mg FipBits, and even stronger on the plot treated with 0.83 mg FipBits, where only one flea was found among the 65 BTPDs combed during all posttreatment sampling. Efficacy of FipBit flea control appeared to increase with fipronil concentration per bait (Fig. 4).

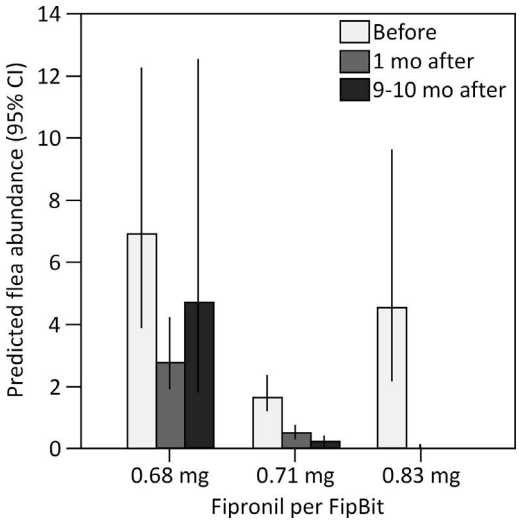

FIGURE 4. Predicted flea abundance (95% confidence intervals) on adult female black-tailed prairie dogs (*Cynomys ludovicianus*) during before-after flea control experiments with FipBits in South Dakota, USA (see also Table 2). Predictions are from a negative binomial generalized linear model with effects for prairie dog age and sex, and a two-way interaction between treatment (nontreated or FipBits containing 0.68, 0.71, or 0.83 mg of fipronil/pellet), and period of experiment (before treatment, 1 mo after, and 9–10 mo after).

DISCUSSION

Our results and those from Eads et al. (2021) are similar to successful flea control achieved with deltamethrin dust infused into PD burrows (e.g., Seery et al. 2003; Eads and Biggins 2019) and with fipronil grain treatments (Poché et al. 2017, 2020; Eads et al. 2019). Such flea control has effectively minimized the impacts of plague in many cases (Biggins et al. 2010, 2021a; Matchett et al. 2010; Tripp et al. 2017). We expect FipBit treatments would provide similar plague reduction effects, but continued study is needed for confirmation.

In addition to near-immediate adult flea control with FipBit treatments, we hypothesize sustained flea control over 1–2 yr also results from effects on flea larvae, which can die after interacting with or consuming fipronil residues in PD feces in burrow systems where flea larvae develop (Eads et al. 2019, 2023; see also Tsurim et al. 2021a, 2021b). Sunlight causes photodecomposition of fipronil to fipronil desulfinyl on the soil surface; this degrades fairly rapidly with a

TABLE 3. Estimated costs (US$/ha) for product, treatment rates (ha/h), and expected cost savings (%) using FipBits (0.84 mg fipronil/pellet distributed at 125 pellets/ha) for flea control, compared to infusion of deltamethrin dust into black-tailed prairie dog (*Cynomys ludovicianus*) burrows and depositing 95 g (1/2 cup) of fipronil-treated grain per burrow (per label directions).

Cost, treatment rate, savings	Flea control method		
	Deltamethrin dust[a]	Fipronil grain[a]	FipBits
Product (US$/ha)	18.50	40.71	3.40[b]
Treatment rate (ha/h)	5.7	20.8	125.0
FipBit product savings (%)	82	92	—
FipBit labor savings (%)[c]	95	83	—
FipBit total savings (%)	89	88	—

[a] Based on 2021 US Forest Service, Buffalo Gap National Grassland contracts.

[b] Ingredients cost US$1.15/ha, manufacturing labor estimated.

[c] Assumes applicator salary the same for all three methods.

[d] — = data not applicable.

half-life of 41–55 days (Ying and Kookana 2002). Dark PD burrow systems (Wilcomb 1954) may facilitate persistence of fipronil residues underground (Eads et al. 2021). Ying and Kookana (2002, p. 1095) found "The half-life of the 'total toxic component' (fipronil and its metabolites) in field soil was 188 days on average," but may range from 3–7.3 mo depending on soil type, UV radiation, pH, humidity, temperature, and microorganisms (US Environmental Protection Agency 1996; Bonmatin et al. 2015).

Eads et al. (2021) described that nearly 80% less fipronil is distributed with FipBits (0.84 mg/FipBit, 125 baits/ha, 0.105 g of fipronil/ha) compared to treating an average of 99 burrows/ha with fipronil-treated grain: per label instructions, 95 g/burrow (1/2 cup), giving 0.470 g of fipronil/ha (Scimetrics Limited Corp, Wellington Colorado, USA), which may reduce concerns about FipBit effects on nontarget species. Burrow densities may vary greatly within and between PD colonies (Jachowski et al. 2008), which affects product and labor costs for products deposited at, or into, each burrow. Material handling and logistics are another consideration in that 3,800 kg of fipronil grain would be needed to treat a modest 400 ha (99 burrows/ha) compared to 62 kg of FipBits. Table 3 illustrates the expected relative cost savings using FipBits for flea control compared to other methods. If biennial FipBit treatments prove satisfactory, the cost savings could be greater.

Fipronil grain safety feeding trials with captive BTPDs were begun in 2016 before field trials commenced (Eads et al. 2019). We chose an initial fipronil concentration of 0.84 mg/FipBit to deliver a dose of ~1 mg/kg for an average sized adult BTPD (~1 kg; Koford 1961). This is approximately 1% of the acute lethal dose (LD_{50}) in laboratory rats (*Rattus norvegicus*; 97 mg/kg; Tingle et al. 2003). The concentration was informed by knowledge that no ill effects had been observed with captive BTPDs fed grain with 0.005% fipronil by weight; that grain treatments had proved effective in controlling fleas on wild BTPDs (Poché et al. 2017, 2020; Eads et al. 2019); and that the delivered dose to a wild PD will vary considerably based on body mass and the number of baits found and consumed. For further safety assessments, authors D.A.E. and M.R.M. initiated in 2020 (still ongoing), with many partners, feeding trials with baits containing fipronil to captive BTPDs, deer mice (*Peromyscus maniculatus*), and BFFs. Because we used small amounts of fipronil, we did not expect safety concerns. No ill effects were observed feeding two to five FipBits to BTPDs; similarly, no ill effects were expected or observed (including posttrial histologic and

pathologic examinations) feeding those FipBit-fed BTPD carcasses to six, 7-yr old BFFs in captivity for 30 days.

In addition to PDs, we expect that other mammals will also consume FipBits. A 20-g mouse would ingest 42 mg/kg if consuming one FipBit (they could consume more), which might be lethal given that the LD_{50} in laboratory mice (*Mus musculus domesticus*) is 91 mg/kg (Tingle et al. 2003). Even within a taxonomic group, there is wide variation in acute toxicity of fipronil (Tingle et al. 2003; Gibbons et al. 2015), and for many species the LD_{50} has not been determined. Ongoing and future studies may clarify nontarget effects on birds, mammals, amphibians, and arthropods that would be helpful for managers to evaluate tradeoffs. It is hoped FipBit treatments, specific to the ectoparasites on target hosts (Eads et al. 2020), will be more precise and have fewer side effects on nontarget species than does insect control with broad spectrum and long-lasting insecticides such as deltamethrin (e.g., Goldberg et al. 2022).

Fipronil dosing rates are expected to influence efficacy of flea control, perhaps up to some upper threshold at which efficacy approaches 100% (dos Santos et al. 2021). Our study suggests that fipronil concentrations of $\leq$0.68 mg/FipBit distributed uniformly at a rate of 125/ha may provide less than desired levels of flea control, although we only treated one plot at this rate and more replication is needed. More experimentation is needed to evaluate varied fipronil concentrations in baits, and application rates, to optimize flea control and to minimize treatment costs and any negative effects on nontarget species.

We recommend fipronil bait treatments in mid- to late summer for BTPDs because Abbott et al. (2018) and Matchett et al. (2021) showed high levels of bait uptake under those conditions, while Eads et al. (2019, 2020, 2021) showed effective flea control on BTPDs with fipronil bait treatments during the same time periods. We recommend similar treatment timing for GPDs, Utah PDs (*Cynomys parvidens*) and white-tailed PDs (*Cynomys leucurus*), considering that treatments need to be completed before the onset of hibernation. Applications early in the morning with favorable weather conditions for high PD activity (e.g., avoiding treatments when excessive heat, rain, cloudy, cool, or windy conditions are forecast) should maximize bait consumption by diurnal PDs versus consumption by small mammals that are generally most active at night.

In summary, FipBits, with 0.71, 0.83, or 0.84 mg of fipronil/pellet, distributed uniformly at a rate of 125/ha, have been effective in flea control on PDs for 1–2 yr (results herein; Eads et al. 2021). FipBits might become a practical and affordable tool to mitigate the impacts of plague at BFF reintroduction areas. The US Fish and Wildlife Service is pursuing options to enable expanded FipBit evaluations and assessments of effects on target and nontarget organisms. Similar to other flea control tools (Eads et al. 2022), FipBits could potentially be used to minimize plague risk to humans, where fleas are currently controlled on rodents with insecticides. It may also be possible, but we need additional developmental effort, to rotate different active ingredients (fluralaner, afoxolaner, lotilaner, spinosad, etc.) for control of various disease-vectoring ectoparasites in an integrated pest management strategy using the FipBit manufacturing and distribution model. Fipronil treatment effects (positive or negative) on PDs, BFFs, and other species continue to be investigated with ongoing field and captive animal studies. It could be argued that any negative effects need to be evaluated in the context of how plague continues to disrupt ecosystem functions and limits endangered BFF conservation (Biggins and Kosoy 2001; Eads and Biggins 2015).

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