Current Research in Parasitology & Vector-Borne Diseases 3 (2023) 100124

Contents lists available at ScienceDirect

Current Research in Parasitology & Vector-Borne Diseases

journal homepage: www.editorialmanager.com/crpvbd/default.aspx

Plague mitigation for prairie dog and black-footed ferret conservation: Degree and duration of flea control with 0.005% fipronil grain bait

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ARTICLE INFO

Keywords:
Cynomys
Fipronil
Mustela nigripes
Siphonaptera
Yersinia pestis

ABSTRACT

Sylvatic plague, a primarily flea-borne zoonosis, is a significant threat to prairie dogs (*Cynomys* spp., PDs) and their specialized predators, endangered black-footed ferrets (*Mustela nigripes*, BFFs). Host-fed fipronil baits have proven effective in controlling fleas on PDs for the purposes of plague mitigation and BFF conservation. Currently, annual treatments are the norm. We tested the long-term efficacy of fipronil bait treatments with black-tailed PDs (*C. ludovicianus*, BTPDs) and BFFs in South Dakota, USA. During 2018–2020, we provided BTPDs on 21 sites with grain bait formula, laced with 0.005% fipronil (50 mg/kg); 18 non-treated sites functioned as baselines. In 2020–2022, we live-trapped, anesthetized, and combed BTPDs for fleas. Flea control was significant for at least 639–885 days. Flea abundance on the treated sites was < 0.5 fleas/BTPD for ~750 days. During 2020–2022, we sampled BFFs for fleas on 4 BTPD colonies treated with fipronil grain bait and 8 non-treated colonies. Flea control was significant with BFFs, but flea abundance began to rebound within ~240 days post-treatment. When feasible, the combination of insecticide treatments, such as fipronil baits, and BFF vaccination against plague provide a “two-pronged” protection approach for these endangered carnivores. If fipronil bait treatments are less effective with predatory BFFs than PDs, as found herein, the “two-pronged” approach might be used to protect BFFs and biennial fipronil bait treatments might be used to protect PDs. If BFF vaccination is not possible, or few BFFs can be vaccinated, annual fipronil bait treatments might be used as a precaution to protect BFFs. Flea densities might be surveyed to determine when/where more frequent treatments seem useful.

1. Background

Sylvatic plague, a primarily flea-borne zoonosis, poses a significant threat to colonial prairie dogs (*Cynomys* spp., PDs) and their specialized predators, endangered black-footed ferrets (*Mustela nigripes*, BFFs). The causative agent, *Yersinia pestis*, was introduced to California in the early 1900s and invaded interior grasslands and PD colonies by 1932, or earlier (Eskey & Haas, 1939). Catastrophic PD die offs were noticed (Barnes, 1993) and BFFs were thought to be susceptible (Forrest et al., 1988). That suspicion was confirmed when fatal plague was documented in a captive BFF (Williams et al., 1994). Later, 30 captive BFFs were accidently exposed to *Y. pestis*-infected PD meat; 27 of the BFFs died (Godbey et al., 2006). In a subsequent study, a BFF died after licking, sniffing, and/or biting, but not eating, a *Y. pestis*-infected mouse (Rocke et al., 2006). Thus, BFFs are considered highly susceptible to plague (Livieri et al., 2022).

BFFs are conserved *via* captive breeding, reintroduction to the wild, and management of free-ranging populations. Many studies indicate plague bacteria persist on, or near, PD colonies (review in Eads et al., 2022a). Biologists use insecticides for flea control and plague mitigation at PD complexes where BFFs have been reintroduced (~7070 ha) in five U.S. States and one Canadian province. Most commonly, field personnel infuse deltamethrin dust into PD burrows, a practice accomplished for more than 20 years (Matchett et al., 2010; Biggins et al., 2021).

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https://doi.org/10.1016/j.crpvbd.2023.100124
Received 6 April 2023; Received in revised form 11 May 2023; Accepted 15 May 2023

Deltamethrin is capable of suppressing PD fleas for at least two years (Eads & Biggins, 2019). However, annual treatments are sometimes needed to sustain flea control (Eads & Biggins, 2019) and PD fleas can evolve deltamethrin resistance, rendering treatments ineffective (Eads et al., 2018).

Previously, we reported on experimental testing of host-fed grain bait formula, laced with 0.005% fipronil (50 mg/kg) and applied at ½ cup or ¼ cup per PD burrow (or along grid transects). Results were encouraging with black-tailed PDs (*C. ludovicianus*, BTPDs). During 2016–2019, flea control was significant for at least 12 months on five sites (i.e. patches of BTPD burrow openings) distributed among three BTPD colonies (Eads et al., 2019, 2020, 2022b, 2022c). In the laboratory, we found that larval fleas were killed by feces from fipronil-treated PDs (Eads et al., 2023). As of this writing, we have tested "fipronil grain bait" (*sensu* Poché et al., 2020) with colleagues on more than 30 sites distributed among more than 10 BTPD colonies, with similar levels of flea control in most cases (see also Poché et al., 2017, 2020). When fipronil grain bait was registered in February 2018 as a Restricted Use Pesticide (EPA Registration #72500–28), wildlife managers began using fipronil grain bait at larger scales for plague mitigation research and landscape-scale BFF conservation (Eads et al., 2022c).

We have also reported on experimental testing of "FipBit" pellets, each containing 0.84 mg fipronil. From 2018 to 2020, FipBits applied along transects (at 9 m spacing; 125 FipBits/ha) suppressed BTPD fleas for 12 months and up to 24 months at two sites on a single colony (Eads et al., 2021a). At this time, transect FipBit treatments have been tested on 12 sites distributed among six PD colonies (including a colony of Gunnison's PDs, *C. gunnisoni*), with similar levels of flea control in most cases (Eads et al., 2021a; Matchett et al., 2023). FipBits are another potential game changing tool to significantly increase affordability and practicality with estimated cost savings of 90% or more relative to deltamethrin dust or fipronil grain bait treatments (Matchett et al., 2023). Conditions for compounding and distributing FipBits at larger scales are being developed with the Food and Drug Administration's Center for Veterinary Medicine. Research on fipronil grain bait may inform the use of fipronil baits in general.

Currently, at least 10 PD complexes supporting BFF reintroduction are using fipronil grain bait (~4249 ha) and additional complexes are considering fipronil bait treatments. Annual treatments are the norm, mostly because prior experiments have concentrated on flea control at 10–12 months post-treatment (Eads et al., 2019, 2020, 2022b, 2022c). Herein, we investigate flea control from 639 to 1084 days post-treatment, comparing flea loads on BTPDs at small-scale sites treated with fipronil grain bait and non-treated sites.

We also present a preliminary investigation of secondary flea control on BFFs, which prey upon PDs and other grain-consuming rodents, analyzing data collected 188–984 days after fipronil grain bait treatments. Flea reductions on PDs may help to reduce flea loads on predatory BFFs, which can acquire fleas from the prey they kill, and from PD burrow environments. Preliminary laboratory data indicate BTPDs fully eliminate fipronil and associated metabolite (fipronil sulfone) within four to eight weeks. Within this window of time, BFFs may consume treated BTPDs (and other treated rodents), thereby consuming fipronil and residues which may kill hematophagous adult fleas biting the BFFs.

2. Materials and methods

2.1. Study areas

We studied BTPDs and BFFs at Buffalo Gap National Grassland (43°45′N, 102°03′W), Conata Basin (43°45′N, 102°11′W), and Badlands National Park (43°48′N, 102°07′W), South Dakota, USA. The Buffalo Gap is administered by the U.S. Department of Agriculture Forest Service. Badlands is administered by the National Park Service. All three areas are situated adjacent to each other. Predominant vegetation included western wheatgrass (*Pascopyrum smithii*), blue grama (*Bouteloua gracilis*), buffalo grass (*Buchloe dactyloides*), pricklypear cactus (*Opuntia polyacantha*), and various species of forbs. Primary land uses included cattle grazing and human recreation (Livieri & Anderson, 2012).

2.2. Black-tailed prairie dog (BTPD) sampling, treatment and analysis

TPD data collected partly with funding from the U.S. Geological Survey, are available at https://doi.org/10.5066/P9PDS9DC). We studied BTPDs from June-September 2020–2022. BTPDs were combed for fleas at 39 sites (i.e. patches of BTPD burrows, ranging in size from ~0.75 to 4 ha) distributed among 9 BTPD colonies, including 21 treated sites (previously receiving 0.005% fipronil grain bait, Scimetrics Limited Corp., Wellington, Colorado, USA) and 18 non-treated sites (Table 1, Fig. 1). Treated sites were either within a colony that was treated in entirety or a colony without treatment and thus we treated those sites and included 50-m treatment buffers in an attempt to reduce edge effects on flea control (which have been detected; Matchett et al., 2023). All sampling colonies were occupied by BFFs.

In 2020, we sampled 6 sites within 2 colonies entirely treated during October 2018, and 2 sites within non-treated colonies; the latter sites were treated with a 50-m buffer in July 2018 (Table 1). In 2021, within 2 non-treated colonies, we sampled 5 sites that we treated with a 50-m buffer (Table 1). In 2022, 5 sites were on a colony treated in entirety, in February 2020 (Table 1). The remaining treated sites in 2022 were on a colony treated in entirety, in September 2020. The 2022 non-treated sites were on 2 separate colonies with 4 and 1 site(s) each.

The 2018 treatments were completed by applying ¼ or ½ cup of fipronil grain bait to individual BTPD burrow openings on each treated site (Eads et al., 2019, 2020; Poché et al., 2020). In 2019, 2 sites were treated at ¼ cup of fipronil grain bait per BTPD burrow opening; the other 3 sites were treated along transects, each 10 m apart with ½ cup fipronil grain bait applied every 10 m (grid transects; Eads et al., 2020). The 2020 treatments were completed by applying ¼ cup of fipronil grain bait to individual BTPD burrow openings.

Adult fleas were combed out from the bodies of live-trapped BTPDs at each site. Animals were anesthetized with isoflurane and combed (using a 89 × 51 mm fine-tooth comb) as thoroughly as possible for 30 s to dislodge fleas, which fell into a plastic bin (Eads et al., 2019, 2021a). Fleas were counted, allowed to recover from anesthesia, and placed back on the BTPD to minimize a removal effect (Eads et al., 2021a). BTPDs were allowed to fully recover from anesthesia and then released at site of capture. Existing data from the present study areas (Eads et al., 2018; Russell et al., 2018) indicate most or all fleas from BTPDs were likely to have been *Oropsylla hirsuta*, an ectoparasitic specialist on PDs and known vector of plague bacteria (Wilder et al., 2008).

The BTPD data for 2020, 2021 and 2022 were analyzed separately, concentrating on an effect of treatment (fipronil grain bait or non-treated) on flea abundance (i.e. counts including zeroes) using negative binomial generalized linear models with a log-link (R x64 version 4.1.2, *glmmTMB* package; Brooks et al., 2023). The Julian day of each combing event was included as a temporal control variable, given flea abundance on BTPDs changes seasonally (Tripp et al., 2009; Wilder et al., 2008). For interpretation, we assessed *z*-tests for variables and extracted model predictions of flea abundance and 95% confidence intervals ('predictions' function, *ggeffects* package; Lüdecke et al., 2023).

We ran a similar analysis to evaluate an effect of "days post-treatment", as a continuous variable, on flea abundance, limiting the data to treated sites (similar to an analysis in Eads & Biggins, 2019). In this case, we explicitly investigated changes in flea abundance over time; thus, Julian day was not included as a control. We found 100 fleas and 440 fleas on the treated sites in 2020 and 2022, respectively, but only 20 fleas on the treated sites in 2021. Therefore, we limited this assessment to the 2020 and 2022 data, which provided more insight into how quickly flea burdens may rebound on BTPDs after fipronil bait treatments. Use of the 2020 and 2022 data, with more fleas, produces a bleaker view of the duration and degree of flea control than would occur if we included the 2021 data; we take this cautious approach given the BFF's status as

Table 1
Black-tailed prairie dog (BTPD) sampling year, treatment type (fipronil grain bait or non-treated), treatment method (burrows or transects), treatment spatial scale (sites surrounded by non-treated habitat or sites on colonies treated in entirety), and flea sampling information, including days post-treatment, number of BTPD combings, number of fleas collected, and flea abundance. Data were collected on 39 sites during 2020–2022, with sampling occurring 639–1084 days after some sites were treated with 0.005% fipronil grain bait. Non-treated sites functioned as experimental baselines.

Treatment year	Sampling year	Treatment	Treatment method (scale)	BTPD flea combing			
				Days post-treatment	No. of combings	No. of fleas	Flea abundance
2018	2020	Fipronil grain bait	Burrows (colony)	639–649	19	0	0
			Burrows (colony)	639–649	16	0	0
			Burrows (colony)	639–649	7	0	0
			Burrows (colony)	687–745	50	11	0.22
			Burrows (colony)	706–774	54	0	0
			Burrows (colony)	712–779	69	6	0.09
			Burrows (site)	729–791	131	49	0.37
			Burrows (site)	731–793	61	34	0.56
		Non-treated			31	33	1.06
					56	103	1.84
					27	63	2.33
					32	87	2.72
					27	76	2.81
					28	124	4.43
2019	2021	Fipronil grain bait	Transects (site)	698–719	47	0	0
			Transects (site)	698–719	32	0	0
			Burrows (site)	700–716	4	15	3.75
			Burrows (site)	1065–1084	18	1	0.06
			Transects (site)	1065–1083	15	4	0.27
		Non-treated			93	28	0.30
					82	41	0.50
					28	39	1.39
					59	88	1.49
					14	30	2.14
					29	80	2.76
					23	119	5.17
2020	2022	Fipronil grain bait	Burrows (colony)	644–666	61	220	3.61
			Burrows (colony)	644–666	45	172	3.82
			Burrows (colony)	644–666	35	31	0.89
			Burrows (colony)	860–885	22	0	0
			Burrows (colony)	860–885	10	6	0.60
			Burrows (colony)	860–885	44	0	0
			Burrows (colony)	860–885	55	0	0
			Burrows (colony)	860–885	18	11	0.61
		Non-treated			14	25	1.79
					13	17	1.31
					10	0	0
					5	13	2.60
					57	5	0.09

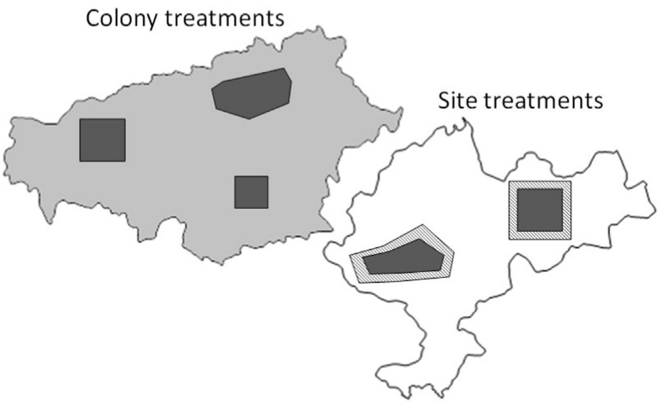

Fig. 1. Illustrative schematic of the black-tailed prairie dog colony treatments and, within the colonies, site treatments with 0.005% fipronil grain bait (see also Table 1). Colony treatments were completed throughout entire colonies (light gray) including, in this example, three sampling sites (dark gray). Site (i.e. patches of prairie dog burrows) treatments were completed within non-treated colonies (white), in this example with 2 sites (dark gray) including 50-m buffers for treatment to reduce edge effects on flea control. We sampled prairie dogs for fleas on the sites, which varied in shape and ranged in size from ~0.75 to 4 ha.

endangered under the Endangered Species Act.

2.3. Black-footed ferret (BFF) sampling, treatment and analysis

BFF data were collected by Prairie Wildlife Research and colleagues. We studied BFFs on BTPD colonies treated in entirety with 0.005% fipronil grain bait and colonies remaining non-treated as baselines. BFF data were collected in August-September and November 2020, August and October-November 2021, and August-November 2022.

In 2020, data from treated areas were collected on a colony that had received fipronil grain bait (¼ cup per BTPD burrow) in September-October 2018, and a second colony that received fipronil grain bait in February 2020. In 2021, data were collected at the colony that received fipronil grain bait in February 2020. In 2022, data were collected at the colony that received fipronil grain bait in February 2020, a colony that received fipronil grain bait in September 2021, and half of a colony that received fipronil grain bait in November 2021 (the other half was left non-treated).

BFFs were detected *via* spotlight surveys (Biggins et al., 2006) and adult fleas were combed out from the bodies of live-trapped, anesthetized BFFs (Harris et al., 2014) during annual population monitoring surveys. Each anesthetized BFF was combed as thoroughly as possible for 20 s to dislodge and count fleas, which were stored in 95% ethanol for subsequent identification. BFFs were allowed to fully recover from anesthesia and released at site of capture. Existing data from the present study areas

(Harris et al., 2014) indicate most fleas from BFFs were likely to have been *O. hirsuta*.

Data analysis and interpretation for BFFs mirrored the aforementioned methods for BTPDs, except we combined the 2020–2022 BFF flea data, given smaller sample sizes with this endangered species. In a first modeling exercise, we concentrated on the effect of treatment on flea abundance, with Julian day of each combing event as a temporal control. In a second analysis, we evaluated the effect of "days post-treatment", limiting the data to colonies treated with fipronil grain bait and excluding the Julian day variable.

3. Results

3.1. Black-tailed prairie dogs

With BTPDs, flea control was significant for 10–12 months (see Eads et al., 2019, 2020, 2022b, c). In the current assessment of longer-term flea control, we analyzed the 2020, 2021, and 2022 data, including 1441 combings in which we detected 1531 fleas. Sample sizes ranged from 4 to 131 combings per site, per year ($\bar{x}$ = 37, Table 1).

In the first modeling exercise, Julian day was supported for all years ($P < 0.001$), reflecting a trend for increasing flea abundance on non-treated sites during June-September (Eads et al., 2018, 2019, 2020, 2021a, 2022b, 2022c). The treatment effect was evident for all 3 years (*z*-test P values ≤ 0.010; Fig. 2). Fleas were less abundant on the fipronil grain bait sites (than non-treated sites) in 2020 and 2021, but slightly more abundant on the fipronil grain bait sites in 2022. Overall, from 639 to 885 days post-treatment, we detected 971 fleas during 628 BTPD combings on non-treated sites *vs* 555 fleas during 780 combings on treated sites. In total, 423 (76%) of the fleas on the fipronil treated sites came from 3 sites on the same BTPD colony in 2022, where 12-month flea control was strong, but longer-term flea control was relatively weak. If those 3 sites were removed from the analysis for 2022, reducing the data from 8 treated sites to 5 on a different colony, fleas were less abundant on the fipronil grain bait sites ($P = 0.002$, Fig. 2). We did not detect any fleas on 9 treated sites during 2020–2022 (Table 1). In 2021, we sampled BTPDs on 2 sites treated 1065–1084 days earlier, detecting 5 fleas during 33 BTPD combings (*c.*425 fleas during 328 combings on non-treated sites in 2021; Table 1).

Burrow and transect treatments were mostly effective over the long-term. In 2020, two-year flea control was least effective at 2 treated sites buffered by 50 m within non-treated habitat; though, flea abundance was still < 1 flea per BTPD (Table 1). In 2021, two-year flea control appeared weak at 1 site, but only 4 BTPDs were combed on that site and recreational shooting of BTPDs (which can increase flea burdens; Biggins & Eads, 2019) on that site was common in 2020 and 2021. For 2020 and 2021, flea control was strong on the remaining 6 sites located on BTPD colonies treated in entirety (Table 1). In 2022, flea control was relatively weak on 2 sites treated in entirety in September 2020 and stronger on the remaining 5 sites treated in February 2020 (Table 1).

With the 2020 data, days post-treatment was influential ($P < 0.001$). Flea abundance increased curvilinearly from 639 to 793 days post-treatment. Fleas were still relatively scarce (predicted as < 1 flea/BTPD) about 2.17 years after treatments (Fig. 3). Too few fleas were found in 2021 for a meaningful analysis. In an analysis of the 2022 data, which included 3 treated sites with inefficient flea control (Table 1), days post-treatment was unsupported ($P = 0.151$); we did not conduct an analysis of the 2022 data with those 3 plots excluded, because the remaining data were collected from 860 to 885 days post-treatment (a relatively short timeframe of 26 days).

3.2. Black-footed ferrets

The 2020–2022 data included 110 BFF combings in which we detected 422 fleas. Sample sizes ranged from 1 to 25 combings per colony, per year ($\bar{x}$ = 6.88, Table 2). In the first modeling exercise, Julian day was nearly supported ($P = 0.053$) and retained as a temporal control; flea abundance declined on BFFs from August-November (*vs* an increase on BTPDs from June-September). From 188 to 984 days post-treatment, we detected 249 fleas during 57 BFF combings on treated colonies *vs* 173 fleas during 53 combings on non-treated colonies. The treatment effect was relatively weak ($P = 0.157$); when accounting for the influence of Julian day, flea abundance on BFFs tended to be somewhat lower on the treated colonies than the non-treated colonies (Fig. 2). On the treated colonies, 86% of fleas were found on 19% of the BFFs, i.e. fleas were not widely distributed among BFFs on the colonies treated with fipronil grain bait (Fig. 4).

In the second modeling exercise, days post-treatment was influential ($P < 0.001$). Flea abundance on BFFs increased curvilinearly from 188 to 984 days post-treatment and increased at a faster rate than observed for

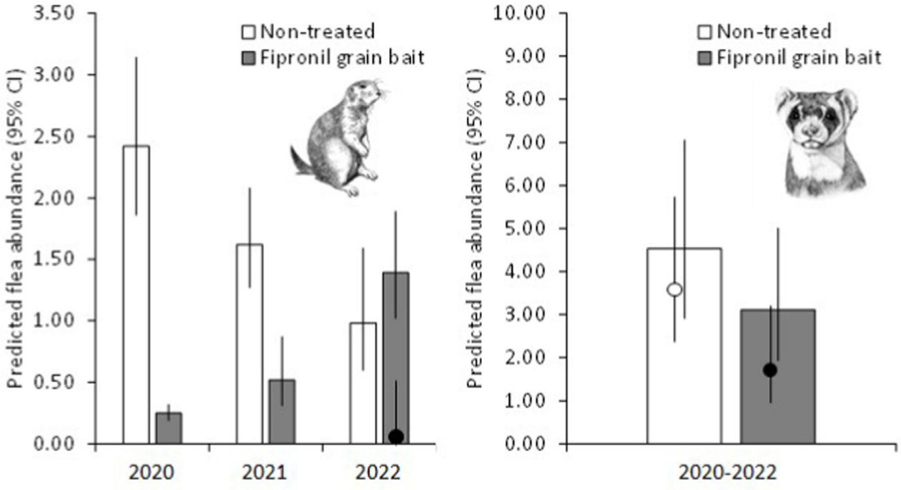

Fig. 2. Left: predicted flea abundance (95% confidence intervals, CIs) on black-tailed prairie dogs (BTPDs) sampled ($n = 1441$) in 2020, 2021 and 2022 on non-treated sites and other sites treated with 0.005% fipronil grain bait relative to days post-treatment in 2020 (639–793 days), 2021 (698–1084 days), and 2022 (644–885 days). For 2022, the black dot and associated 95% CI within the bar for fipronil grain bait represent predicted flea abundance if we excluded data from 3 treated sites, on the same BTPD colony, where 12-month flea control was strong, but longer-term flea control was weak (this limited the 2022 data to 5 sites on the same colony, where long-term flea control was strong). BTPD sketch by D. Crawford. Right: predicted flea abundance (95% CIs) on black-footed ferrets (BFFs) sampled ($n = 110$) during 2020–2022 on non-treated sites and other sites treated with 0.005% fipronil grain bait relative to days post-treatment in 2020 (188–705 days), 2021 (550–555 days), and 2022 (302–984 days). The dots and associated 95% CIs within the bars represent predicted flea abundance if we excluded data from a treated colony, collected > 900 days post-treatment, thereby concentrating data from 188 to 717 days post-treatment. Notice differences in the scale of vertical axes for BTPDs (0–3.5 fleas/BTPD) and BFFs (0–10 fleas/BFF). BFF sketch by H. Branvold.

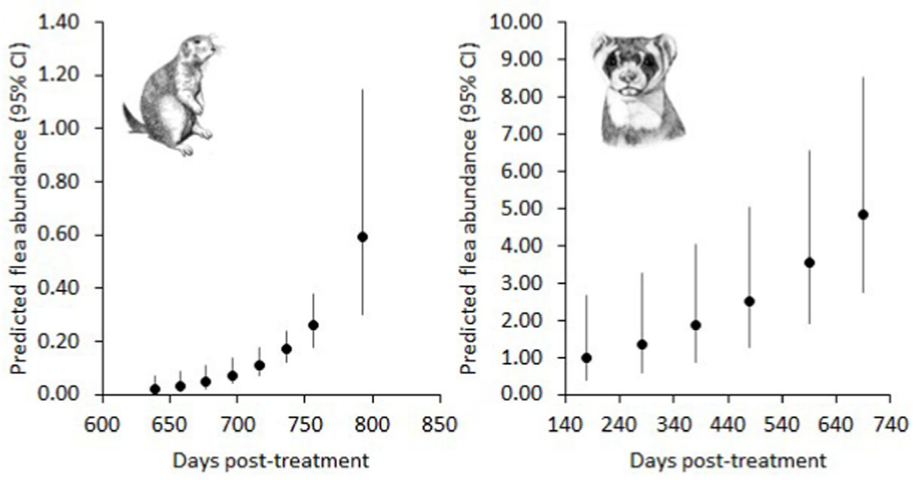

Fig. 3. Left: predicted flea abundance (95% confidence intervals, CIs) on black-tailed prairie dogs (BTPDs) sampled (n = 407) in 2020 on sites treated with 0.005% fipronil grain bait relative to days post-treatment (639–793 days). BTPD sketch: D. Crawford. Right: predicted flea abundance (95% CIs) on black-footed ferrets (BFFs) sampled (n = 57) during 2020–2022 on sites treated with 0.005% fipronil grain bait relative to days post-treatment (188–984 days). Notice differences in the scale of vertical axes for BTPDs (0–1.4 fleas/BTPD) and BFFs (0–10 fleas/BFF). BFF sketch: H. Branvold.

Table 2

Treatment type (fipronil grain bait or non-treated), treatment year, black-footed ferret (BFF) sampling year, and flea sampling information, including days post-treatment, numbers of BFF combings, numbers of fleas collected, and flea abundance. Data were collected on 4 treated black-tailed prairie dog (BTPD) colonies and 8 non-treated colonies, with sampling occurring 188–984 days after some of the colonies were treated with 0.005% fipronil grain bait. Non-treated colonies functioned as experimental baselines. This table differs from Table 1, because the BTPD and BFF experimental designs differed (due to varying research objectives over time).

Treatment	Treatment year	Sampling year	BFF flea combing			
			Days post-treatment	No. of combings	No. of fleas	Flea abundance
Fipronil grain bait	2018	2020	705	1	19	19.00
Fipronil grain bait	2020	2020	188–269	12	2	0.17
Non-treated		2020		7	35	5.00
Non-treated		2020		5	5	1.00
Non-treated		2020		3	2	0.67
Fipronil grain bait	2020	2021	550–555	6	55	9.17
Non-treated		2021		3	21	7.00
Fipronil grain bait	2020	2022	907–984	14	151	10.79
Fipronil grain bait	2020	2022	714–717	9	21	2.33
Fipronil grain bait	2021	2022	302–337	15	1	0.07
Non-treated		2022		25	70	2.80
Non-treated		2022		1	0	0
Non-treated		2022		2	19	9.50
Non-treated		2022		1	0	0
Non-treated		2022		5	21	4.20
Non-treated		2022		1	0	0

BTPDs; flea abundance on BFFs was predicted to be $>$ 1 flea/BFF within about 240 days post-treatment (Fig. 3).

Both BFF analyses were heavily influenced by data collected on a treated colony in August 2022, >900 days post-treatment (Table 2, days post-treatment = 907–984, with $>$ 10 fleas detected on 6 of 14 BFFs, all found *via* spotlight on the eastern portion of the colony). If all data from that treated colony, $>$ 900 days post-treatment, were excluded, thereby concentrating data from 188 to 717 days post-treatment, the treatment effect in the first analysis was much stronger (Fig. 2; $P = 0.021$). Using the restricted dataset for treated sites, days post-treatment was influential ($P < 0.001$); flea abundance on BFFs was predicted to be $>$ 1 flea/BFF within about 365 days post-treatment, implying that annual treatments might be needed to protect BFFs against fleas.

4. Discussion

During prior research, we found that fipronil grain bait suppressed fleas on BTPDs for at least 10–14 months (Eads et al., 2019, 2020) and may have caused increases in BTPD body condition and reproduction (Eads et al., 2022c). FipBits, which are functionally similar to fipronil grain bait, may suppress fleas on BTPDs for two years or more (Eads et al., 2021a; Matchett et al., 2023). Results herein demonstrate significant flea control on BTPDs for two to three years with fipronil grain bait, with some exceptions (Table 1). This degree and duration of flea control is expected to diminish flea-borne *Y. pestis* transmission to BTPDs (Biggins et al., 2010). During a study in Colorado, fipronil grain bait protected BTPDs and BFFs on treated habitat (Eads et al., 2022c).

In combination, multiple factors may help to explain the degree and duration of flea control observed in this study, and others, and the ability of fipronil bait treatments to protect BTPDs and BFFs against plague:

- Following consumption of fipronil baits, PDs metabolize fipronil into fipronil sulfone, and both compounds are sequestered mostly in host fat stores, released to the bloodstream over time, and eliminated through feces and (to a lesser extent) urine. Adult fleas acquire fipronil/sulfone from host blood. Fipronil/sulfone disrupt the flea central nervous system, causing hyperexcitation, paralysis, and death. This route of flea control may operate until PDs have mostly or fully eliminated fipronil/sulfone (~4–8 weeks with BTPDs according to laboratory experiments; see also Poché et al., 2023).
- Even if an individual PD does not consume fipronil bait, adult fleas on that PD can transfer to other PDs that consumed fipronil bait, with flea transfers occurring (for instance) during social interactions aboveground and when PDs are nesting underground in burrows. Adult fleas may die after moving to and feeding on (or simply touching the skin/hair of) treated PDs (Mehlhorn et al., 1999).

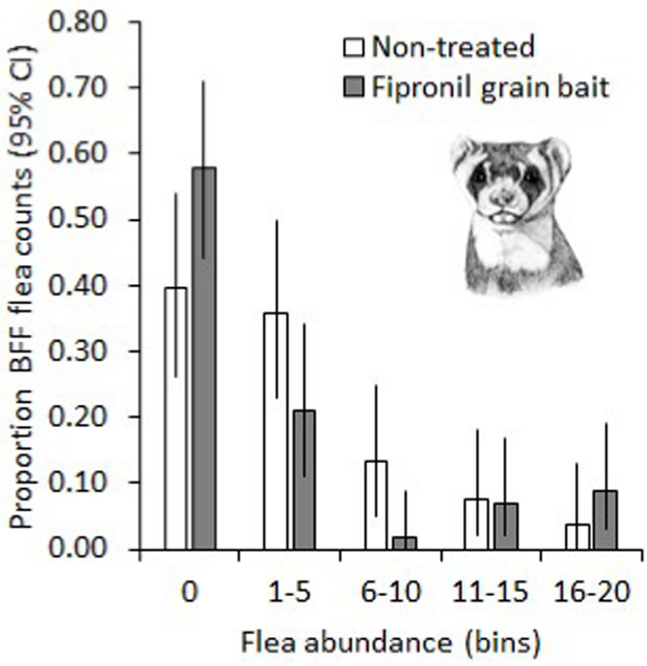

Fig. 4. Proportions of 110 black-footed ferrets (BFFs) (95% confidence intervals, CIs) with different flea abundance counts (in bins) on non-treated colonies and other colonies treated with 0.005% fipronil grain bait relative to days post-treatment (188–984 days), 2020–2022. *Note*: although not depicted here, 36 fleas and 34 fleas were found on two different BFFs in 2022, on a colony treated 907 and 912 days earlier, respectively. BFF sketch: H. Branvold.

Coloniality of PDs, which are some of the most social of sciurid rodents (Hoogland, 1981), may facilitate this process.

- Adult fleas might excrete fipronil/sulfone in their feces before death, and larvae might consume the feces (Silverman & Appel, 1994) or even scavenge adult fleas killed by fipronil/sulfone (Wimsatt & Biggins, 2009). Adult fleas contacting fipronil residues in their own feces, or feces from other adult fleas, might be re-exposed on treated hosts, or even on non-treated hosts that acquired adult fleas from treated hosts.
- Flea larvae are thought to develop in PD nests and may contact (and sometimes consume) fipronil/sulfone when interacting with feces excreted by treated hosts, resulting in larval mortality (Eads et al., 2023). Flea larvae might also contact skin and/or hairs shed by treated mammalian hosts and, perhaps as a result, contact fipronil/sulfone (Marchiondo et al., 2013).
- Older larvae might contact fipronil/sulfone when using substrate to construct cocoons for pupation, resulting in their deaths.
- PD families nest in burrow chambers that contain their scat and urine (Hoogland, 1995). As PDs move and interact in burrows, they can physically contact their feces and urine, providing an opportunity for transfer of fipronil/sulfone to PD hair and skin, producing yet another route of exposure for adult (and perhaps even larval and pupal) fleas (for a similar example, see Diepens et al., 2023).
- PDs also drop scat and urine aboveground on soils around their burrow openings (Eads et al., 2016) and they interact with those soils when maintaining "burrow mounds" that provide vantage points to enhance predator detection (Hoogland, 1995), providing another potential route of fipronil/sulfone exposure for fleas.
- If PD hair and skin become contaminated with fipronil/sulfone, for instance as described above, or if these compounds are excreted from sebaceous glands to the exterior skin and hair, the compounds may transfer to PD mouths and saliva (e.g. when they oral groom; Eads et al., 2017) and the animals may spread saliva (Ritter & Epstein, 1974) and the compounds over their body when oral grooming, perhaps assisting in flea control. PDs might even consume fipronil residues when oral grooming, potentially re-dosing themselves.
- In some cases, PDs may feed on fipronil baits for several days, sometimes feeding on grain exposed to sunlight for several days. Over time in sunlight, fipronil photodegrades (primarily into fipronil desulfinyl). In a laboratory study, more than 70% fipronil in water/ethanol was transformed into photoproducts after 170 hours exposure to UV light. It was reported that "photodegradation of fipronil leads to the initial accumulation of a mixture of products that retain a high level of insecticidal activity" (Raveton et al., 2006), indicating the photoproducts may assist in flea control.
- One of the treatments in this study occurred in mid to late February. When treatments occur shortly before or during adult female BTPD lactation (which can occur during mid-March to early May; Hoogland, 2003), fipronil/sulfone may transfer to the milk of treated, lactating adult females, allowing for indirect treatment of juvenile BTPDs *via* their mother's (or aunts', Hoogland, 1995) milk (Le Faouder et al., 2007), which may assist in controlling adult fleas on juvenile BTPDs.

In our experience with fipronil grain bait, flea control is oftentimes effective when field personnel apply the grain to PD burrows or along transects (Eads et al., 2019, 2020, 2022b, 2022c). Treatment of burrow openings is perhaps recommended. Unlike mice, PDs have not been observed to cache food items; by placing fipronil baits at PD burrows, biologists cache grain for PD consumption. PDs encounter the grain when emerging from burrows for daily activities and readily consume the grain (reducing potential search times). Transect treatments might increase search times but might be particularly useful in areas where vegetation is thick and tall, which reduces the ability of biologists to detect and treat PD burrows. When treating on foot, transect treatments are about 3-times faster than burrow treatments (Eads et al., 2020). Future experiments might evaluate flea control when fipronil grain bait is deposited in BTPD burrow tunnels (which might reduce fipronil contact rates for other animals, but also influence effects of fipronil photodegradates).

Like fipronil baits, deltamethrin dust is an effective tool for flea control on PD colonies. If > 3 g of deltamethrin dust is infused into each PD burrow, flea abundance may be reduced to ≤ 0.5 fleas per PD for ~1 year (Eads and Biggins, 2019). The suppressing effect of deltamethrin wanes over time, and individual BTPDs may harbor > 1 flea or more, on average, ≥~360 days post-treatment (Eads & Biggins, 2019). Herein, fipronil grain bait tended to suppress fleas to < 0.5/BTPD for > 700 days, indicating that fipronil baits might prove more effective for longer durations than deltamethrin dust with BTPDs. However, replication is needed to refine the curves in Fig. 2. Two-year (~1.8-year) BTPD flea control was obviously weak in 2 cases (> 3 fleas/BTPD; Table 1).

Fipronil bait treatments appeared more effective in controlling fleas on BTPDs than BFFs. If this pattern holds in future studies, several hypotheses might explain the phenomenon:

- PDs and other rodents are the targets of fipronil bait treatments, not BFFs. We suspect BFFs consume no (to very little) fipronil bait. Instead, BFFs acquire fipronil/sulfone (and photoproducts) from fipronil-treated prey. Perhaps flea control, in degree and duration, is less effective with the indirect route of treatment. Alternatively, BFFs may simply benefit from reduced spillover of fleas from PDs on treated sites, as discussed below.
- With BTPDs, long-term flea control was effective on most but not all treated sites; at least some fleas were detected on BTPDs at 57% of treated sites. BFFs inhabit and move within (much) larger home ranges than BTPDs; Hoogland (1995) reported an average territory size of 0.33 ha for BTPDs and, in comparison Livieri & Anderson (2012) reported average home range sizes (95% fixed kernels) of 64.7 ha and 131.8 ha for adult female and male BFFs, respectively. Far-ranging movements may provide BFFs with more opportunities to acquire fleas, for instance in "pockets" of PD habitat where fipronil bait proved, for some reason(s), less effective in controlling PD fleas.

- There is evidence to suggest that after fipronil bait treatments, flea spillover occurs from non-treated habitat to the edges of treated habitat, thus dampening the degree and duration of flea control in edge areas (Matchett et al., 2023). Currently, fipronil grain bait treatments end at PD colony boundaries. Fleas might persist near colony edges where PD foraging is reduced and vegetation is taller and, therefore, biologists can miss, and not treat, PD burrows. BFFs, and maternal BFFs in particular, preferentially use areas of high active burrow density that may occur near the edges of PD colonies (Eads et al., 2011) and treatment edge effects on flea control may increase the rate of flea exposure for BFFs in those areas (suggesting potential utility in applying fipronil baits along transects at the edges of PD colonies as a precaution, similar to the 50-m treatment buffers around BTPD sites herein).
- BFFs sometimes kill multiple PDs in a single night, and observations suggest they may kill multiple PDs within the same burrow in a single night. Mortality of PDs encourages fleas on them (if present) to quest for new, live hosts (Biggins & Eads, 2019). Frequent BFF killing of PDs (due to a fast metabolic rate of BFFs) and questing of fleas from dead PDs to BFFs may increase flea burdens on the BFFs. This seems particularly likely if BFFs kill multiple PDs within the same burrow in a single night, and then expropriate that burrow as a nest (e.g. Hillman, 1968; Paunovich & Forrest, 1987; Jachowski, 2007).
- In this study, the BTPD data included an abundance of adult and juvenile BTPDs. In contrast, most of the BFF data (> 85%) came from juvenile BFFs (i.e. kits). Moreover, 78% of the BFF kit samples were collected in August and September, when the kits may have been fully or somewhat reliant on their mothers for prey (Eads et al., 2012) and when prey requirements increase for growing kits (Biggins et al., 1993). Kits may acquire fleas from recently killed PD prey provided by their mother, and flea exposure may be greatest when mothers are provisioning increasing numbers of prey items. Note, in the South Dakota study area, flea burdens on BTPDs tend to increase from August through early October, which may increase the degree of flea exposure for kits as they grow toward, and reach, natal dispersal in September-October. Flea burdens declined somewhat on BFFs from October to November; perhaps flea exposure for BFF kits declines as they become independent of their mothers and littermates, and are no longer exposed to an influx of dead PDs provisioned by their mothers.

Continued study is needed to determine if fipronil bait treatments are, indeed, more effective in controlling fleas on BTPDs than BFFs. Confirmation is perhaps more important than identification of the underlying mechanisms; the phenomenon may guide the use of fipronil baits for plague mitigation at BFF reintroduction sites. Insecticide treatments and BFF vaccination to protect against plague, in combination (e.g. Matchett et al., 2010), provide a “two-pronged” approach to plague mitigation for BFF conservation. If flea control is weaker with BFFs than PDs, and fleas rebound faster on BFFs than PDs (Fig. 3), the “two-pronged” approach may be most useful for BFF conservation; even a single infectious flea bite may be sufficient for *Y. pestis* to infect a non-vaccinated BFF (Godbey et al., 2006). That said, in some cases, effective flea control on PDs, and overall flea population reductions, may be sufficient to protect BFFs against flea-borne *Y. pestis*. Potential negative effects of fipronil bait treatments on non-targets, if existent, might be lower with biennial than annual treatments.

No plague mitigation tool is perfect, and the mode, timing, and intensity of treatments influence outcomes (Rocke et al., 2017; Tripp et al., 2017; Matchett et al., 2021; Eads et al., 2022a). With deltamethrin and fipronil treatments, flea control is not always effective for > 10 months. If feasible, flea populations can be surveyed to determine when/where more frequent treatments seem useful (Eads et al., 2021b).

5. Conclusions

In conclusion, 0.005% fipronil grain bait proved mostly effective in controlling fleas on BTPDs for 639–1084 days, although there were exceptions (Table 1). Fipronil grain bait treatments with BTPDs helped to reduce flea burdens on predatory BFFs, but the degree and duration of flea control were both weaker with BFFs than BTPDs (suggesting BFF vaccination, in a “two-pronged” approach, may be useful). Results herein may inform treatments with fipronil grain bait and FipBits alike. Continued research is underway to refine and optimize fipronil bait treatments for flea control, plague mitigation, and wildlife conservation. Human and wildlife safety continue to be important considerations (Eads et al., 2022a, 2022b, 2022c).

Funding

Funding and logistical support were provided by the U. S. Geological Survey (USGS); Prairie Wildlife Research (PWR); National Park Service; U. S. Forest Service; U. S. Fish and Wildlife Service (USFWS); World Wildlife Fund; Defenders of Wildlife; and Dakota Zoo.

Ethical approval

BTPD research was conducted under extended IACUC protocol 2015–07 (USGS, Fort Collins Science Center, Colorado). BFF research was conducted by PWR under Endangered Species Recovery permit #TE064682-1 and approved USFWS protocols.

Disclaimer

The findings and conclusions in this article are those of the author(s) and do not necessarily represent the views of the USFWS. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U. S. Government.

CRediT authorship contribution statement

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Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The black-tailed prairie dog data supporting the conclusions of this article are included within the article; raw data are available online *via* USGS ScienceBase (https://doi.org/10.5066/P9PDS9DC). Black-footed ferret data are not publicly available (contact Prairie Wildlife Research for further information).

Acknowledgments

We thank many colleagues and field technicians for help and camaraderie during BTPD and BFF sampling, including S. Forrest, Defenders of Wildlife, and P. Roghair. We thank M.R. Matchett for suggestions on study design, and M.R. Matchett, R. Poché, D. Poché, and T. Tretten for discussions on fipronil and fleas. We thank D. Crawford and H. Branvold for graciously allowing the use of their artwork. We thank two anonymous reviewers, the Editor-in-Chief, and B. Windell for constructive comments on the manuscript.

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