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Fipronil Pellets Reduce Flea Abundance on Black-tailed Prairie Dogs: Potential Tool for Plague Management and Black-footed Ferret Conservation

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ABSTRACT: In western North America, sylvatic plague (a flea-borne disease) poses a significant risk to endangered black-footed ferrets (*Mustela nigripes*) and their primary prey, prairie dogs (*Cynomys* spp.). Pulicides (flea-killing agents) can be used to suppress fleas and thereby manage plague. In South Dakota, US, we tested edible "FipBit" pellets, each containing 0.84 mg fipronil, on free-living black-tailed prairie dogs (*Cynomys ludivicianus*). FipBits were applied along transects at 125 per ha and nearly eliminated fleas for 2 mo. From 9–14 mo post-treatment, we found only 10 fleas on FipBit sites versus 1,266 fleas on nontreated sites. This degree and duration of flea control should suppress plague transmission. FipBits are effective, inexpensive, and easily distributed but require federal approval for operational use.

Key words: *Cynomys*, *Mustela nigripes*, pulicide, Siphonaptera, *Yersinia pestis*.

Yersinia pestis, the bacterial agent of sylvatic plague, is lethal to a variety of terrestrial mammals, including humans. Field research is needed to identify appropriate mitigation strategies. In this context we discuss prairie dogs (PDs; *Cynomys* spp.) and their endangered predators, black-footed ferrets (BFFs; *Mustela nigripes*).

Yersinia pestis was introduced to US Pacific ports in the early 1900s and had overtaken most of the PD range by the 1960s or earlier, expanding to the western edge of South Dakota at least. Plague reduced PD densities rapidly, transforming grassland ecosystems (Eads and Biggins 2015). Black-footed ferrets are specialized predators of PDs and use PD burrows for shelter. In the early 1990s, evidence from a variety of mustelids suggested that BFFs might be resistant to *Y. pestis*, until Williams et al. (1994) documented fatal plague in a captive BFF. In 1995, 30 captive BFFs were accidentally exposed to *Y. pestis*–infected meat; 27 were known or suspected to have died (Godbey et al. 2006). This showed that BFFs are highly susceptible to plague.

Since the late 1990s, wildlife managers have used pulicides (agents lethal to fleas) to suppress fleas and manage plague at BFF reintroduction sites. One pulicide, deltamethrin, proved effective in a dust formulation (Biggins et al. 2010). A vaccine against *Y. pestis* for BFFs (Rocke et al. 2004) also proved effective (Matchett et al. 2010) and, like deltamethrin, became (and still is) an essential tool for BFF conservation. Flea control with deltamethrin can be poor sometimes, due to technological limitations and human application error. Further, fleas may develop resistance to deltamethrin (Eads et al. 2018).

An immediate goal of BFF conservation is to mitigate plague under an integrated strategy (US Fish and Wildlife Service [USFWS] 2019). Toward this goal, we tested systemic 0.005% fipronil grain (EPA registration no. 72500-28) with black-tailed PDs (BTPDs; *C. ludovicianus*); this suppressed fleas for 12–24 mo (Eads et al. 2019).

Here we expand our evaluation, testing fipronil in edible pellet formulation (Fig. 1). The pellets, invented and named "FipBits" by M. R. Matchett (USFWS), were formulated from a flour-based mixture of food-grade ingredients. FipBits build upon existing pellets (Corro et al. 2017) and machinery for rapid field application (Kreiger and Matchett

FIGURE 1. Black-tailed prairie dog (*Cynomys ludovicianus*) handling a FipBit. (Photo credit: D.A.E.)

2019). In our study, each FipBit weighed 1.34 g and contained 0.84 mg of fipronil.

We sampled BTPDs at Conata Basin (43°45′N, 102°11′W), Buffalo Gap National Grassland (43°51′N, 102°03′W), and Badlands National Park (43°47′N, 102°08′W), South Dakota, US. We collected data at six sites (i.e., patches of juxtaposed burrows) among three BTPD colonies. A site near Big Foot Road (Buffalo Gap) had never been treated with an insecticide. Two sites at South Exclosure (Conata Basin) and three sites at Prairie Wind (Badlands) had not been treated with deltamethrin dust since 2015 and 2016, respectively. Effects of deltamethrin on BTPD fleas tend to diminish within 2 yr (Eads and Biggins 2019).

Just before sunrise (i.e., first BTPD morning emergence) on 4 and 7 August 2018, FipBits were applied along transects (125 per ha) at the two South Exclosure sites. FipBits were also applied on 4 August 2018 at the Big Foot Road site by placing three FipBits at each burrow opening. The Prairie Wind sites were left untreated and served as baselines.

We sampled fleas from live-trapped BTPDs during June–October 2018, May–October 2019, and July–August 2020. We anesthetized each BTPD with isoflurane and combed it (with a 89×51 mm fine-tooth comb) thoroughly for 30 s to dislodge fleas, which fell into a plastic bin (Eads et al. 2019). Fleas were counted and allowed to recover from anesthesia and placed back on BTPDs, to minimize any removal effect. We weighed BTPDs with spring scales (grams), measured their right hind feet with measuring tapes (millimeters), and estimated their age (adult or juvenile; Eads et al. 2016).

We compared flea abundance (i.e., counts including zeroes) on treated and nontreated sites before and after treatments. We limited post-treatment data to $\geq$10 d after treatments, an arbitrary but convenient cut-off given field effort. We ran negative binomial linear models, selecting a parsimonious form via Type III Wald chi-square tests (α=0.050 main effects, 0.100 interactions; R x64 version 3.6.1, glmmTMB; R Core Team 2019).

Using the 2018 data, we ran a model with effects for TREATMENT (FipBit or nontreated), PERIOD (Before treatment=June–July 2018, After=August–October 2018), and their interaction. From before to after treatment, flea abundance increased on nontreated sites but declined on FipBit sites (Fig. 2; PERIOD×TREATMENT $P<0.001$). After treatment, we found 2,521 fleas on nontreated sites and 42 fleas on FipBit sites (eight and 16 from transect treatments, 18 from burrow treatment).

To assess flea control from 2018 to 2019, we ran a model with TREATMENT, PERIOD, and their interaction (PERIOD, Before= June–July 2018, After-1=May–June 2019, After-2=July–August 2019, After-3=September–October 2019). From 9–14 mo post-treatment, we found 1,266 fleas on nontreated sites (683 combings), seven and three fleas on FipBit transect sites (169 and 119 combings), and 62 fleas on the burrow

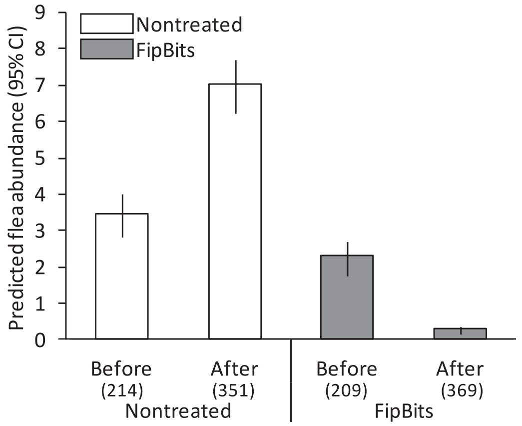

FIGURE 2. Predicted flea abundance on black-tailed prairie dogs (*Cynomys ludovicianus*), 2018 at sites in South Dakota, USA. Fleas were combed from anesthetized prairie dogs on sites treated with FipBits in early August 2018 and on nontreated sites. Data were collected in two periods: Before treatments (June–July) and After treatments (mid-August–October). Sample sizes are in parentheses. Predicted values are point estimates and 95% confidence intervals (CIs) from a negative binomial model (R x64 version 3.6.1, glmmTMB; R Core Team 2019).

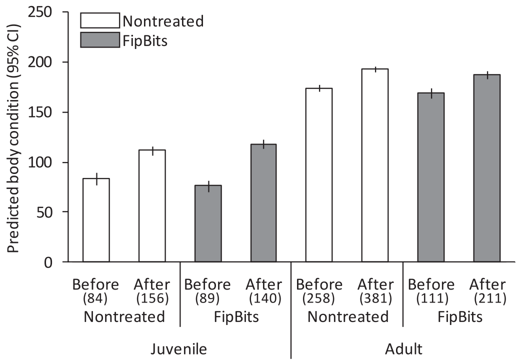

FIGURE 3. Predicted body condition (mass:foot) for juvenile and adult black-tailed prairie dogs (*Cynomys ludovicianus*), 2018 at sites in South Dakota, USA; mass in grams, foot in millimeters. Prairie dogs were sampled on sites treated with FipBits in early August 2018 and on nontreated sites. Data were collected during two periods: Before treatments (June–July) and After treatments (mid-August–October). Sample sizes are in parentheses. Predicted values are point estimates and 95% confidence intervals (CIs) from a Gaussian model (R x64 version 3.6.1, glmmTMB; R Core Team 2019).

treatment site (88 combings; PERIOD-TREATMENT $P<0.001$).

In July–August 2020, about 23 mo post-treatment, we found 197 fleas on the three nontreated sites (65 combings), zero fleas on the first FipBit transect site (37 combings), and 323 fleas on the second FipBit transect site (44 combings). We found zero fleas (39 combings) on the burrow treatment site.

Our results on long-term flea control, especially with transects, are compelling but assume that flea abundance "before" treatment reflects consistent year-by-year differences among sites. If flea abundance values are inconsistent within sites over time, which is sometimes the case, then greater site replication would be needed to confirm our findings; this is underway.

Fipronil and its metabolites (and photoproducts) may be toxic to some vertebrates (Gibbons et al. 2015). With BTPDs, the compounds might cause stress, lethargy, or anorexia; body condition might decline as a result. Conversely, by suppressing flea parasitism, fipronil might increase BTPD body condition (Neuhaus 2003). With the 2018 data on BTPD condition (mass:foot; Eads et al. 2016), we ran a Gaussian linear regression model, evaluating effects of TREATMENT, PERIOD, and AGE of BTPD and all possible interactions. The three-way interaction was supported ($P=0.038$). Juvenile BTPD body condition increased from before to after treatment on all sites, but the magnitude was stronger on FipBit sites (55%) than on nontreated sites (34%; Fig. 3). In contrast, adult BTPD body condition increased at similar rates on FipBit sites (110%) and nontreated sites (109%).

Considering BTPD condition from 2018 to 2019 (PERIOD=Before, After-1, After-2, and After-3), the three-way interaction was removed ($P=0.242$). All two-way interactions were retained ($P\leq 0.097$). Juveniles were in poorer condition on FipBit sites than on nontreated sites before treatments but were in better condition on FipBits sites 9–14 mo post-treatment. Juvenile condition increased from before to after treatments on all sites, but the magnitude of increase was stronger on

FipBit sites. Trends were similar for adult BTPDs (Fig. 4).

The degree and duration of flea control with FipBits was similar to that observed with deltamethrin dust or with fipronil grain. Control of adult fleas is expected when they take blood from a recently fipronil-treated PD, but larval fleas, which might feed on BTPD fipronil-laced feces in burrows, can also be killed (Eads et al. 2019). Fipronil products in PD burrows, away from sunlight, might persist for 200 d or more (Gunasekara et al. 2007).

In our study, less fipronil was applied with FipBits than would have been applied with fipronil grain. If ½ cup (113 g) of fipronil grain is dropped at each BTPD burrow opening (Eads et al. 2019) on 0.40 ha of habitat with 40 burrow openings, then 0.23 g of fipronil is applied. In contrast, if 50 FipBits are applied on transects at the same site, only 0.04 g of fipronil is applied.

FipBits were created for use with an automated delivery mechanism (Kreiger and Matchett 2019). In Montana, the delivery mechanism, attached to an all-terrain vehicle, was used to distribute 125 pellets/ha with a treatment rate of about 19 ha/hr. Similarly, one person distributed 125 pellets/ha at a rate of about 19 ha/hr in South Dakota. Those rates are 3.55 times faster than distributing fipronil grain on BTPD habitat with 99 burrow openings per ha.

Our results indicate that flea control by FipBits is likely to be sufficient to reduce plague transmission (as observed with deltamethrin; Biggins et al. 2010; Matchett et al. 2010). Compared to dust-based pulicides applied to burrows, FipBits may minimize nontarget effects and reduce the costs of flea control by 90% or more. Thus, Fipbits may be an effective tool for integrated plague management to benefit endangered BFFs. Work is underway to evaluate the degree and duration of flea control with varied fipronil application rates. Additional work on uptake rates in PDs and safety in BFFs is needed.

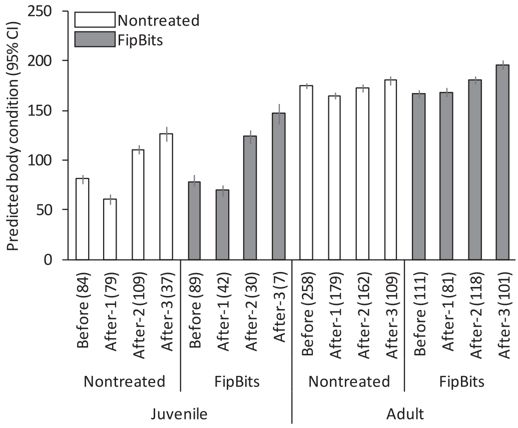

FIGURE 4. Predicted body condition (mass:foot) for juvenile and adult black-tailed prairie dogs (*Cynomys ludovicianus*), 2018–19 at sites in South Dakota USA; mass in grams, foot in millimeters. Prairie dogs were sampled on sites treated with FipBits in early August 2018 and on nontreated sites. Data were collected in four periods: Before treatments (June–July 2018), After-1 (May–June 2019), After-2 (July–August 2019), and After-3 (September–October 2019). Sample sizes are in parentheses. Predicted values are point estimates and 95% confidence intervals (CIs) from a Gaussian model (R x64 version 3.6.1, glmmTMB; R Core Team 2019).

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