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Source: Journal of Wildlife Diseases, 59(1) : 84-92

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/JWD-D-22-00092

DOI: 10.7589/JWD-D-22-00092

Journal of Wildlife Diseases, 59(1), 2023, pp. 84–92

LETHAL EFFECTS ON FLEA LARVAE OF FIPRONIL IN HOST FECES: POTENTIAL BENEFITS FOR PLAGUE MITIGATION

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ABSTRACT: Plague, caused by the bacterium *Yersinia pestis*, is a zoonotic disease of mammalian hosts and flea vectors. Fipronil baits have been used to suppress adult fleas for plague mitigation. The degree and duration of flea control may increase if fipronil also kills other stages in the flea life cycle. We fed grain treated with 0.005% fipronil by weight, or nontreated grain, to black-tailed prairie dogs (*Cynomys ludovicianus*), which excrete fipronil and metabolites in their feces after consuming fipronil in their diet. We presented prairie dog feces to 331 larval *Oropsylla montana* (Siphonaptera: Ceratophyllidae). When exposed to feces lacking fipronil or metabolites, 84% of larvae survived for 24 h. In contrast, survival declined to 42% for larvae contacting feces from fipronil-treated prairie dogs. Just 7% of larvae consuming feces from fipronil-treated prairie dogs survived. Fipronil and metabolites may persist in host feces for several months or longer in prairie dog burrows where flea larvae dwell and forage. The lethal effects of fipronil on adult and larval fleas (and perhaps other life stages) may help to explain why fipronil baits are capable of suppressing fleas on prairie dogs for $\geq$12 mo.

Key words: Black-footed ferret, *Cynomys*, fipronil, fleas, insecticide, *Mustela nigripes*, *Oropsylla montana*, Siphonaptera, *Yersinia pestis*.

INTRODUCTION

The plague bacterium *Yersinia pestis* is well known for causing almost global human morbidity and mortality (Barbieri et al. 2020). The pathogen is mostly sylvatic, circulating among rodent hosts and flea vectors, and spilling over to other mammals when conditions allow (Biggins and Kosoy 2001). In killing mammals, plague causes widespread ecological disruptions (Eads and Biggins 2015). Therefore, a One Health view of plague, recognizing that human, animal, and environmental health are linked, is encouraged (Vallès et al. 2020), and effective plague mitigation is an important goal (D'Ortenzio et al. 2018).

Adult fleas, the primary plague vectors, are relatively inefficient at transmitting *Y. pestis* (Lorange et al. 2005). Consequently, rates of *Y. pestis* transmission are expected to be low if adult fleas are scarce (Lorange et al. 2005; Eisen et al. 2009). In some studies, flea-borne *Y. pestis* transmission was reduced or eliminated in areas where rodent burrows were treated with insecticides for flea control (Biggins et al. 2010; Matchett et al. 2010; Goldberg et al. 2021). Yet, no particular insecticide has been revealed as a panacea to plague mitigation (Miarinjara and Boyer 2016; Eads et al. 2018; Goldberg et al. 2022). Continued study is needed to optimize treatments on a case-by-case basis (Tripp et al. 2016).

Fipronil, a phenylpyrazole insecticide, can be applied systemically to hosts via consumable baits. Fipronil and resulting metabolites (e.g., fipronil sulfone in mammals) are sequestered in host fat and released over time into blood, where the residues are available to hematophagous adult fleas until hosts have eliminated the chemicals (dos Santos et al. 2020). The chemicals block $GABA_A$ receptors and desensitize sensitive glutamate-gated chloride channels, thereby causing hyperexcitation, paralysis, and death (Page 2008). Host-fed fipronil has proven effective in controlling adult fleas on multiple occasions, suggesting that the chemical might

be used for plague mitigation (Poché et al. 2017, 2020; Rajonhson et al. 2017; Eads et al. 2019, 2020, 2021).

Prairie dogs (*Cynomys* spp., PDs) are colonial sciurids highly susceptible to *Y. pestis*, and insecticides are commonly used to protect PDs, as well as nearby human populations, against plague, especially at reintroduction sites for black-footed ferrets (*Mustela nigripes*), which are endangered obligate predators of PDs (Matchett et al. 2010; Eads and Biggins 2015, 2019). During multiyear, replicated field experiments, fipronil bait treatments suppressed adult fleas on PDs for 12–24 mo (Eads et al. 2019, 2020, 2021; Matchett et al. 2023). Fipronil grain bait is now being used for plague management at multiple black-footed ferret reintroduction sites. Continued study is underway to optimize treatments and assess potential effects on target and nontarget species.

Fipronil kills adult fleas, which usually comprise 1–5% of flea populations (Beck and Pfister 2004). The insecticide and metabolites might also kill other life stages. Flea larvae, which can comprise >35% of flea populations (Beck and Pfister 2004), develop in rodent nests and feed on organic matter, including host feces (Krasnov 2008). Prairie dogs commonly defecate in their nests (Hoogland 1995), and after consuming fipronil-laced baits, PDs excrete fipronil and fipronil sulfone in feces (Gunasekara et al. 2007). In the confines of PD nests (including chambers sometimes referred to as "latrines"; Hoogland 1995, p. 28), flea larvae might contact or consume fipronil residue in feces, potentially resulting in larval mortality (Hinkle et al. 1997; Davis 1999; Rust et al. 2014). Suppression of two life stages may severely diminish flea densities and dampen population recruitment (Mehlhorn et al. 1999), with attendant benefits for plague mitigation.

We compared 24-h survival rates for flea larvae exposed to feces from PDs treated or not treated with fipronil bait. We hypothesized that survival rates would be reduced for larvae contacting or consuming fipronil residue in feces.

MATERIALS AND METHODS

We studied black-tailed PDs (*C. ludovicianus*, BTPDs) under animal use and care guidelines of the American Society of Mammalogists (Sikes and Animal Care and Use Committee of the American Society of Mammalogists 2016). We collected fecal pellets from six BTPDs in captivity (Wang et al. 2019). The BTPDs had been live trapped in Colorado from a colony treated with 0.05% deltamethrin dust to suppress fleas (DeltaDust®, Bayer Environmental Science, North Carolina, USA). All BTPDs were treated with fluid insecticide upon capture to kill fleas (Pyranha® 0.55% pyrethrin, 5.50% piperonyl butoxide, 1.10% permethrin; Pyranha Incorporated, Houston, Texas, USA). The BTPDs were transported to the US Fish and Wildlife Service's National Black-Footed Ferret Conservation Center, Carr, Colorado, US, where they were housed individually in secure housing bins in a climate-controlled room. Each bin was furnished with 1.3-cm-deep pine shavings as bedding material and was treated with DeltaDust to kill any remaining fleas and to inhibit flies. The BTPDs had access to clean water and timothy hay (*Phleum pretense*) ad libitum, and a nest box and plastic tubing as refuge (Wang et al. 2019). Before feeding the BTPDs fipronil (or nontreated) grain, all BTPD fecal pellets were removed from the housing bins.

During a feeding trial, BTPDs had been presented with either nontreated grain (wheat) or fipronil-treated grain (0.005% fipronil by weight; Scimetrics Ltd. Corp., Wellington, Colorado, USA; EPA registration number 72500-28), in a ceramic dish. Two BTPDs received nontreated grain and four BTPDs received fipronil grain. Individual BTPDs may have consumed a mean of 10.5 g of grain per day (Wang et al. 2019). The four animals provided with fipronil-treated grain had consumed a maximum of 35–63 g grain (mean 48 g) or 0.00175–0.00315 g of fipronil (mean 0.002 g) (Wang et al. 2019). During each day of the trial, BTPD fecal pellets were collected, stored in sealable plastic bags, labeled by date and treatment, and frozen at −18 C.

Fecal pellets collected starting 24 h after the feeding trial began were placed in 1.5-mL centrifuge tubes, ground into morsels and powder (fine particles for the flea larvae to eat; Bland et al. 2017) with a disposable polypropylene pestle, separated as 0.5-mg subsamples into prelabeled centrifuge tubes, and frozen. Grinding of the fecal pellets simulated breaking of fecal pellets by BTPDs (with their paws, for instance) as they move within nesting chambers. Fipronil and sulfone metabolite concentrations in fecal pellets from the fipronil-treated BTPDs (four pellets per day from each of the 4 d starting 24 h after treated

grain was provided) were quantified by liquid chromatography-mass spectrometry at the Analytical Toxicology Laboratory, Colorado State University, Fort Collins, Colorado, USA. They contained mean 296.20 ng/g fipronil and 158.19 ng/g fipronil sulfone.

We used the flea species *Oropsylla montana* (Siphonaptera: Ceratophyllidae) as a proxy for *Oropsylla hirsuta* and *Oropsylla tuberculata cynomuris*. The latter two flea species parasitize BTPDs under natural conditions but have not been successfully maintained in captivity (Eisen et al. 2009; Miarinjara et al. 2022). *Oropsylla montana*, an important vector of *Y. pestis* (Eisen et al. 2009), parasitizes a variety of ground squirrels, albeit not BTPDs (Eisen et al. 2009). In captivity, *O. montana* reproduces readily, providing large numbers of eggs, larvae (Fig. 1), pupae, and adults for experimentation (Bland et al. 2017). There is no evidence in the literature to suggest insecticidal efficacy would vary considerably among these species.

The *O. montana* were colonized and cared for as described (Eisen et al. 2007). We used flea larvae with empty alimentary canals (guts) and larvae that appeared to be first or second stage. Testing small, young larvae helped to reduce the probability of larvae pupating during assays (McTier et al. 2003). Multiple types of assays were evaluated. Herein, we concentrate on two experiments, each conducted under methods similar to Chen et al. (2017), although we present some supplemental information from additional experiments to facilitate inference.

Flea larvae were assayed in Corning® 6-well microplates (first experiment) or 12-well microplates (second experiment). Small holes were made in the microplate caps, immediately above each well, to allow for air exchanges. Each well contained sterilized fine sand substrate (~1/4 to 1/2 of well depth) for larvae locomotion and refuge. During each day, wells in some of the test plates received fipronil residue in BTPD feces and wells in the remaining plates received nontreated feces (0.50 mg/well).

We collected flea larvae by pouring substrate from colony jars into a sieve, capping the sieve, and shaking the sieve to eliminate substrate. Soft paintbrushes were used to transport individual flea larva from the sieve to unique wells in the microplates. The *O. montana* larvae have limited or no exposure to light during their development in rodent burrows (Bland et al. 2017). Thus, in our study, the microplates were lidded, loosely covered with aluminum foil, and stored in a dark location for 24 h at approximately 23 C and 85% relative humidity.

After 24 h, the microplates were uncovered and opened. Probes were used to prod each larva for 2 s. Live larva responded by coiling (Byron 1987) and moving away from the prod. Larvae that did not respond within 2 s were prodded for an additional 5 s and considered "dead" if no movement was observed (Panella et al. 2005). During preliminary trials (before those reported herein), some "dead" larvae were held in vials for 12–24 h to see if they would recover, and none recovered (suggesting they were indeed dead). A 60–120× pocket microscope was used to determine if each larva consumed BTPD feces (yes=visible meal, colored like BTPD scat, in the gut; no=no visible meal in the gut). At the end of each experiment, larvae were placed in ethanol to ensure mortality and transferred to a biohazard waste container.

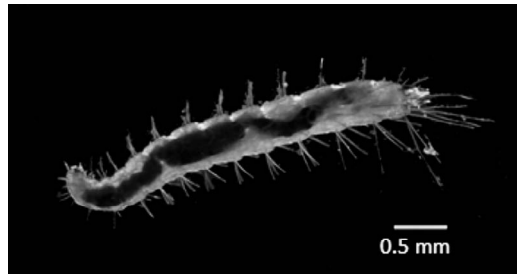

FIGURE 1. Larval flea, *Oropsylla montana*. Food is visible in the larva's alimentary canal (gut). Photo credit: B. J. Hinnebusch, National Institute of Allergy and Infectious Diseases, National Institute of Health.

We used logistic regression to examine the effect of fipronil residue in feces on 24-h survival of flea larvae (glm function in *stats* package, R x64 version 4.1.2, R Core Team 2021). Day of feces collection (on feeding Days 2, 3, 4, or 5) had no detectable effect on experimental outcomes and the variable was excluded from analysis. We concentrated on an interaction between categorical predictor variables for treatment and evidence of feces consumption (α=0.050). Consumption of fipronil residue in feces was expected to be more lethal than consumption of feces from BTPDs that had consumed nontreated grain. Data generated during this study are available as a US Geological Survey data release (Eads 2022).

RESULTS

Individual larva in treatment wells may have been exposed to mean 0.15 ng fipronil and 0.08 ng fipronil sulfone. On posttreatment inspection, most flea larvae were found submerged in substrate adjacent to the edges of wells. No larva escaped from the well plates and pupation was not observed. Overall, 59%

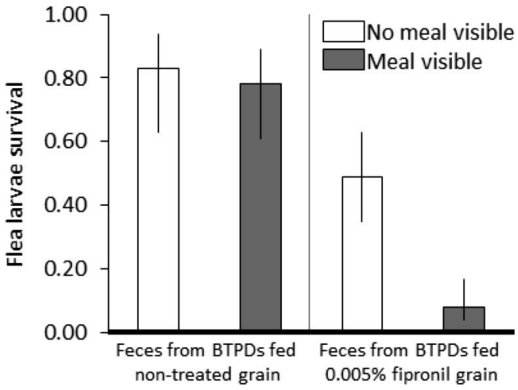

FIGURE 2. Survival rates (95% confidence intervals) for larval fleas (*Oropsylla montana*) that contacted but did not visibly consume, or contacted and visibly consumed, feces from black-tailed prairie dogs (*Cynomys ludovicianus*, BTPDs) treated with plain, nontreated grain, or grain treated with 0.005% fipronil by weight. Survival was assessed 24 h after the larvae were exposed to feces in well plates. Survival was estimated using logistic regression, with an interaction between type of prairie dog feces and meal status.

(n=196) of larvae exhibited evidence of consuming BTPD feces. Fipronil treatment had no detectable effect on the proportion of larvae with meals, of any size, in their guts (χ^2 test, P=0.728). For those larvae with evidence of fecal consumption, qualitative observations suggested that <25% of the gut was filled for most larvae that had consumed feces from fipronil-treated BTPDs, whereas >50% of the gut was commonly filled for larvae that had consumed feces from BTPDs fed nontreated grain.

In the first experiment, the two-way statistical interaction between treatment and evidence of fecal consumption was influential (P<0.016). Of the larvae exposed to control feces, 48/60 (80%) survived, with no statistical difference between larvae that had consumed or not consumed BTPD feces (Fig. 2). Among the larvae contacting but not consuming fipronil residue in feces, 22/45 (49%) survived. Only 6/76 (8%) larvae consuming fipronil residue in feces survived (Fig. 2).

In the second experiment, the two-way statistical interaction was influential (P<0.001). Of the larvae exposed to control feces, 44/50 (88%) survived, with no statistical difference between larvae that had consumed or not consumed BTPD feces. Among larvae contacting but not consuming fipronil residue in feces, 14/41 (34%) survived. Only 4/59 (7%) larvae consuming fipronil residue in feces survived.

DISCUSSION

We found that feces from BTPDs treated orally with fipronil insecticide (in this case, grain with 0.005% fipronil by weight) were lethal to larval *O. montana* if the larvae contacted the feces and especially if the larvae consumed the feces. Although larval mortality was assessed 24 h postexposure, during side experiments not detailed above, scat from fipronil-treated BTPDs killed flea larvae in an even shorter time: some of the treated *O. montana* larvae were killed within 2 h, further illustrating potential lethal effects on flea larvae of fipronil residue in host feces. The LD_{50} of fipronil or metabolites is unknown for adult or larval *O. montana* fleas. Rust et al. (2014) suggested the LD_{50} of fipronil, alone, is 0.11 to 0.40 ng per adult cat flea (*Ctenocephalides felis*); they cited multiple studies, including their own, that suggest fipronil and other insecticides are more lethal to larval than to adult insects.

The BTPD feces in these two experiments might have been contaminated by DeltaDust and Pyranha. During other experiments, in which we fed flea larvae a normal rearing diet (e.g., powdered blood, powdered milk, mouse chow, and adult flea feces), 24-h survival rates were typically >95%. During our experiments in which flea larvae were fed feces from BTPDs not treated with fipronil, 24-h survival rates were slightly lower, averaging 84%; this might reflect (weak) lethal effects of DeltaDust and/or Pyranha residues on flea larvae. Given that all BTPDs and housing bins in this study were treated similarly, any potential effect of DeltaDust or Pyranha residues should have been similar among larvae exposed to feces from fipronil-treated and nontreated BTPDs; thus, the relative comparisons and inference on fipronil were valid. Additionally, if any fecal pellets excreted by

BTPDs before treatment remained in the bins occupied by BTPDs treated with fipronil grain, we may have underestimated potential lethal effects of fipronil residue in BTPD feces on flea larvae.

The behaviors of larval *O. montana* in this study were consistent with prior investigations of flea larvae. The tendency for flea larvae to submerge into sand adjacent to the edges of wells is consistent with positive geotaxis (Rust and Dryden 1997), positive thigmotaxis (Yinon et al. 1967), and negative phototaxis (Crum et al. 1974; though light was limited in our experiment). Approximately 60% of flea larvae exhibited evidence of consuming BTPD feces, lending support to the hypothesis that host feces are a food source for flea larvae (Krasnov 2008). Qualitative observations suggested that larvae consumed less feces when exposed to scat from fipronil-treated than nontreated BTPDs, presumably due to morbidity and mortality caused by fipronil and metabolites in feces from fipronil-treated BTPDs. We did not detect evidence of larvae avoiding feces from BTPDs, fipronil-treated or not.

Traditionally, biologists and wildlife managers have attempted to mitigate plague on BTPD colonies by infusing insecticide dusts into burrows, with deltamethrin dust being the standard for the last 20+ yr (Seery et al. 2003; Biggins et al. 2010; Matchett et al. 2010; Tripp et al. 2016; Eads and Biggins 2019). Typically, BTPD burrows are deep and convoluted (Wilcomb 1954). Deltamethrin dust may not transfer to the depth of BTPD nests where young fleas develop (although BTPDs may carry dust on their paws and bodies to nesting chambers). In contrast, with fipronil bait treatments, BTPDs may ferry and deposit fipronil and metabolites directly into their nest and latrine chambers (in scat and perhaps urine), a potential benefit of oral insecticide treatments. This expectation probably applies to other species of PDs (e.g., *Cynomys gunnisoni*, *Cynomys leucurus*, and *Cynomys parvidens*).

Our results suggest that the degree and duration of flea control observed with fipronil grain treatments and PDs (e.g., Eads et al. 2019) may stem in part from lethal effects of fipronil and metabolites on adult fleas feeding on PD blood, but also to larval fleas contacting and consuming host feces in burrows. The effects on adult fleas are rapid (in the wild, adult flea control is evident within days; Eads et al. 2019) and may extend over 2–6 wk or more (until BTPDs have fully excreted fipronil and its metabolites).

The effects on larvae may be more prolonged. Fipronil and its metabolites remain relatively stable in the dark (Simon-Delso et al. 2015) and BTPD burrows, being dark (Wilcomb 1954), may function as "light shields" (Gunasekara et al. 2007). Fipronil products might persist for 200 d or more in BTPD burrows (Gunasekara et al. 2007). Such persistence within burrows may facilitate long-term flea control.

The fate of fipronil and its metabolites in burrow soils may be helpful for flea control. For example, fipronil can bind to soil particles, increasing retention rates and limiting penetration into the soil (Bonmatin et al. 2015). Flea larvae congregate at shallow depths in soils (Bland et al. 2017), a behavior that would presumably increase exposure to fipronil and metabolites. Organic matter such as PD feces and shed hair also help to reduce fipronil mobility in soils (Bonmatin et al. 2015), potentially increasing fipronil contact rates for flea larvae.

We hypothesize that nearly all flea life stages may be exposed to fipronil and metabolites in BTPD burrows in multiple ways. Young flea larvae, such as those we studied, might encounter these residues when contacting or consuming host feces (and perhaps urine, though mammals excrete little fipronil or associated metabolites in urine). Flea larvae might also encounter fipronil and metabolites when feeding on host skin and blood (Hinkle et al. 1991) or contacting host hair or skin (Mehlhorn et al. 1999). Older larvae may contact fipronil residues when using substrate to construct cocoons for pupation; some pupae are unable to spin cocoons and these "naked" pupae (Dryden and Smith 1994) would presumably be exposed to fipronil and metabolites in host feces. Adult fleas might excrete fipronil and

metabolites in their feces before death. Larvae might consume those feces (Silverman and Appel 1994; Hinkle et al. 1997; Davis 1999) or even scavenge adult fleas killed by fipronil (Wimsatt and Biggins 2009). Flea larvae feed on live and dead larvae (Krasnov 2008; Bland et al. 2017), providing additional routes of exposure. Adult fleas, some of which feed daily to avoid desiccation, are exposed to fipronil residues when feeding on host blood and could be exposed when moving through host hair and contacting host skin (e.g., via absorption through thin intersegmental membranes; Mehlhorn et al. 1999).

In contrast to other flea life stages, the egg stage may be somewhat protected against fipronil and metabolites in BTPD nests. The lethal effects of these compounds on flea larvae may reduce a significant source of mortality for flea eggs, because larvae may consume >20 flea eggs before pupation (Lawrence and Foil 2000); thus, fipronil treatments may allow more flea eggs to survive and transition to the larval stage. Regardless, because interference and exploitative competition, larvae survival is reduced at higher larval densities (Krasnov 2008). Moreover, larval fleas hatch within days of egg laying (Bland et al. 2017).

By reducing survival rates for adult, larval, and (perhaps) pupal fleas, fipronil treatments designed to control adult fleas systemically may dampen or eliminate flea population recruitment over prolonged periods (perhaps even after fipronil is effectively eliminated from the environment, for instance, via hydrolysis; Fent 2014). In doing so, fipronil may reduce future generations of adult fleas that are the primary vectors of plague bacteria, a potential long-term benefit for plague mitigation and conservation of many wildlife species.

In this study, we concentrated on scat from BTPDs fed grain laced with 0.005% fipronil by weight. Scat collected from treated BTPDs on Day 2 of grain consumption was lethal to flea larvae. After 24 h of access to treated grain, the treated BTPDs had consumed a mean of 5.5 g of grain, or 0.0003 g of fipronil (Wang et al. 2019). Fipronil has also been incorporated into bait pellets ("FipBits") that effectively suppress BTPD fleas for ≥12 mo (Eads et al. 2021). FipBits in current form each contain a mean of 0.0008 g fipronil, nearly three times more fipronil than BTPDs had consumed by Day 2 in the current study of fipronil grain. We suspect some BTPDs consume >1 FipBit under natural conditions. Thus, scat from FipBit-treated BTPDs also may kill several flea life stages, including larvae, helping to explain long-term flea control with FipBits (Eads et al. 2021; Matchett et al. 2023).

With fipronil grain or FipBits, biologists may choose to treat PDs annually. Repeated treatments might lead to accumulation of fipronil residues in PD burrows, potentially facilitating long-term flea control. Testing fleas and soils from PD burrows over time, on colonies with differing numbers of treatments, could assess this hypothesis. Prolonged insecticide exposure places strong selective pressure on fleas to evolve insecticide resistance. As an example, deltamethrin resistance has been detected among PD fleas at some sites treated annually (Eads et al. 2018). Thus, long-term persistence (and perhaps accumulation) of fipronil residues in PD burrows may not necessarily be beneficial in all contexts. To date, evidence of flea resistance to fipronil is scarce; some evidence suggests potential cross-resistance between dieldrin and fipronil, but the degree of fipronil resistance was relatively weak (Payne et al. 2001; Schenker et al. 2001; Bass et al. 2004; Rust 2016).

There have been questions about fipronil and metabolite bioaccumulation in hosts, with implications for predators, given repeated exposures may lead to acute toxicity or mortality in some vertebrates (Gunasekara et al. 2007). Bioaccumulation seems most probable for chemicals that persist in animals with little to no elimination, or animals repeatedly consuming fipronil over time. On BTPD colonies, fipronil baits are typically applied once annually and depleted within 3–7 d (Eads et al. 2019). The BTPDs and other mammals eliminate fipronil and metabolites over time, with full (or nearly full) elimination expected within 2 mo or less (and perhaps faster in mammals with higher metabolic

rates, such as the black-footed ferret). Thus, bioaccumulation in the context of fipronil baits and mammalian hosts is not expected with infrequent treatments and relatively rapid elimination rates, perhaps especially for relatively short-lived species. Both BTPDs and black-footed ferrets are considered to be short-lived, and the life spans of other ferret prey species, such as *Peromyscus* spp., are even shorter (Forrest et al. 1988; Hoogland 1995). Potential accumulation of fipronil residues in longer-lived BTPD predators (such as raptors) warrants investigation.

Deltamethrin treatments on PD colonies may reduce survival rates for some species (e.g., *Peromyscus* spp.) because of direct lethality or effects on arthropod prey (Goldberg et al. 2022). Fipronil bait treatments are thought to be more precise by delivering the insecticide directly to fleas on target hosts and may comparatively reduce impacts on nontarget organisms by reducing exposure for some animals (Eads et al. 2019, 2020, 2021). However, a variety of animals may encounter fipronil residues when exposed to host feces, and PDs excrete their feces both in burrows and aboveground. Fipronil bait treatments might influence a variety of organisms that move among and consume host feces in both environments. For example, effects on scarab beetles (e.g., *Onthophagus* spp.), some of which eat and nest in PD feces, seem probable.

Our results may inform conservation efforts for PDs and black-footed ferrets and, more broadly, inform efforts to mitigate plague in other areas where burrowing or ground-nesting rodents and their fleas play a significant role in maintaining the disease in nature. Continued study is needed to optimize fipronil bait treatments and minimize potential negative effects on nontarget species. We continue to evaluate fipronil safety with PDs and associated species, including a variety of arthropods, amphibians and small mammals, and the endangered black-footed ferret. As with any plague-mitigation approach, results may be evaluated in the context of how plague continues to disrupt ecosystem functions and limits endangered black-footed ferret conservation (Eads and Biggins 2015).

ACKNOWLEDGMENTS

Financial and logistical support were provided by US Geological Survey, Centers for Disease Control and Prevention, US Fish and Wildlife Service, and Colorado State University. Additional logistical support was provided by Scimetrics Limited Corporation and Genesis Laboratories, Inc. We thank J. Montenieri, R. Enscore, and K. Gage, who provided guidance on flea larvae sampling; R. Poché, who kindly provided fipronil-treated grain; Smith Environmental and Engineering, who donated BTPDs; G. Dooley, who completed LC-MS/MS assays; B. J. Hinnebusch, who graciously provided the photo in Fig. 1; and the Editor-in-Chief, Associate Editor, two anonymous journal reviewers, and T. Livieri and M. R. Matchett for helpful and constructive comments on the manuscript. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the US Government. The findings and conclusions in this article are those of the author(s) and do not necessarily represent the views of the US Fish and Wildlife Service.

LITERATURE CITED

Barbieri R, Signoli M, Chevé D, Costedoat C, Tzortzis S, Aboudharam G, Raoult D, Drancourt M. 2020. *Yersinia pestis*: The natural history of plague. *Clin Microbiol Rev* 34:e00044–19.

Bass C, Schroeder I, Turberg A, Field LM, Williamson MS. 2004. Identification of the *Rdl* mutation in laboratory and field strains of the cat flea, *Ctenocephalides felis* (Siphonaptera: Pulicidae). *Pest Manag Sci* 60:1157–1162.

Beck W, Pfister K. 2004. Recent investigations on the population dynamics of cat fleas (*Ctenocephalides felis*) and the concept of an integrated flea control. *Prakt Tierarzt* 85:555–563. [In German.]

Biggins DE, Godbey JL, Gage KL, Carter LG, Montenieri JA. 2010. Vector control improves survival of three species of prairie dogs (*Cynomys*) in areas considered enzootic for plague. *Vector-Borne Zoonotic Dis* 10:17–26.

Biggins DE, Kosoy MY. 2001. Influences of introduced plague on North American mammals: implications from ecology of plague in Asia. *J Mammal* 82:906–916.

Bland DM, Brown LD, Jarrett CO, Hinnebusch BJ, Macaluso KR. 2017. Methods in flea research. BEI Resources. https://www.beiresources.org/Catalog/VectorResources.aspx. Accessed January 2022.

Bonmatin JM, Giorio C, Girolami V, Goulson D, Kreutzweiser DP, Krupke C, Liess M, Long E, Marzaro M, et al. 2015. Environmental fate and exposure; neonicotinoids and fipronil. *Environ Sci Pollut Res* 22:35–67.

Byron DW. 1987. *Aspects of the biology, behavior, bionomics, and control of immature stages of the cat*

flea Ctenocephalides felis felis *(Bouché) (Siphonaptera: Pulicidae) in the domiciliary environment*. PhD Thesis, Virginia Polytech Institute and State University, Blacksburg, Virginia.

Chen YJ, Huang CG, Hsu JC, Wu WJ. 2017. Development of a larval bioassay method using 96-well microtiter plates for evaluation of susceptibility of the cat fleas (Siphonaptera: Pulicidae) to insecticides. *J Med Entomol* 54:377–381.

Crum GE, Knapp FW, White GM. 1974. Response of the cat flea, *Ctenocephalides felis* (Bouché), and the oriental rat flea, *Xenopsylla cheopis* (Rothschild), to electromagnetic radiation in the 300–700 nanometer range. *J Med Entomol* 11:88–94.

Davis RM. 1999. Use of orally administered chitin inhibitor (lufenuron) to control flea vectors of plague on ground squirrels in California. *J Med Entomol* 36: 562–567.

D'Ortenzio E, Lemaître N, Brouat C, Loubet P, Sebbane F, Rajerison M, Baril L, Yazdanpanah Y. 2018. Plague: Bridging gaps towards better disease control. *Med Mal Infect* 48:307–317.

dos Santos GCM, Rosado LHG, Alves MCC, de Paula Lima I, Ferreira TP, Borges DA, de Oliveira PC, de Sousa Magalhães V, Scott FB, Cid YP. 2020. Fipronil tablets: Development and pharmacokinetic profile in beagle dogs. *AAPS PharmSciTech* 21:9.

Dryden MW, Smith V. 1994. Cat flea (Siphonaptera: Pulicidae) cocoon formation and development of naked flea pupae. *J Med Entomol* 31:272–277.

Eads DA. 2022. Data on flea larvae survival following exposure to black-tailed prairie dog scat. US Geological Survey data release. https://doi.org/10.5066/P9005VC6.

Eads DA, Biggins DE. 2015. Plague bacterium as a transformer species in prairie dogs and the grasslands of western North America. *Conserv Biol* 29:1086–1093.

Eads DA, Biggins DE. 2019. Plague management of prairie dog colonies: Degree and duration of deltamethrin flea control. *J Vector Ecol* 44:40–47.

Eads DA, Biggins DE, Bowser J, Broerman K, Livieri TM, Childers E, Dobesh P, Griebel RL. 2019. Evaluation of five pulicides to suppress fleas on black-tailed prairie dogs: Encouraging long-term results with systemic 0.005% fipronil. *Vector-Borne Zoonotic Dis* 19:400–406.

Eads DA, Biggins DE, Bowser J, McAllister JC, Griebel RL, Childers E, Livieri TM, Painter C, Krank LS, Bly K. 2018. Resistance to deltamethrin in prairie dog (*Cynomys ludovicianus*) fleas in the field and in the laboratory. *J Wildl Dis* 54:745–754.

Eads DA, Livieri TM, Dobesh P, Childers E, Noble LE, Vasquez MC, Biggins DE. 2021. Fipronil pellets reduce flea abundance on black-tailed prairie dogs: Potential tool for plague management and black-footed ferret conservation. *J Wildl Dis* 57:434–438.

Eads DA, Yashin AC, Noble LE, Vasquez MC, Huang MHJ, Livieri TM, Dobesh P, Childers E, Biggins DE. 2020. Managing plague on prairie dog colonies: Insecticides as ectoparasiticides. *J Vector Ecol* 45:82–88.

Eisen RJ, Eisen L, Gage KL. 2009. Studies of vector competency and efficiency of North American fleas for *Yersinia pestis*: State of the field and future research needs. *J Med Entomol* 46:737–744.

Eisen RJ, Lowell JL, Montenieri JA, Bearden SW, Gage KL. 2007. Temporal dynamics of early-phase transmission of *Yersinia pestis* by unblocked fleas: Secondary infectious feeds prolong efficient transmission by *Oropsylla montana* (Siphonaptera: Ceratophyllidae). *J Med Entomol* 44:672–677.

Fent GM. 2014. Fipronil. In: *Encyclopedia of toxicology*. 3rd Ed. Wexler P, editor. Academic Press, London, pp. 596–597.

Forrest SC, Biggins DE, Richardson L, Clark TW, Campbell TM 3rd, Fagerstone KA, Thorne ET. 1988. Population attributes for the black-footed ferret (*Mustela nigripes*) at Meeteetse, Wyoming, 1981–1985. *J Mammal* 69:261–273.

Goldberg AR, Biggins DE, Ramakrishnan S, Bowser JW, Conway CJ, Eads DA, Wimsatt J. 2022. Deltamethrin reduces survival of non-target small mammals. *Wildl Res* doi: 10.1071/WR21153.

Goldberg AR, Conway CJ, Biggins DE. 2021. Effects of experimental flea removal and plague vaccine treatments on survival of northern Idaho ground squirrels and two coexisting sciurids. *Glob Ecol Conserv* 26: e01489.

Gunasekara AS, Truong T, Goh KS, Spurlock F, Tjeerdema RS. 2007. Environmental fate and toxicology of fipronil. *J Pest Sci* 32:189–199.

Hinkle NC, Koehler PG, Kern WH Jr, Patterson RS. 1991. Hematophagous strategies of the cat flea (Siphonaptera: Pulicidae). *Fla Entomol* 74:377–385.

Hinkle NC, Rust MK, Reierson DA. 1997. Biorational approaches to flea (Siphonaptera: Pulicidae) suppression: Present and future. *J Agric Entomol* 14:309–321.

Hoogland JL. 1995. *The black-tailed prairie dog: Social life of a burrowing mammal*. University of Chicago Press, Chicago, Illinois, 557 pp.

Krasnov BR. 2008. *Functional and evolutionary ecology of fleas: A model for ecological parasitology*. Cambridge University Press, Cambridge, UK, 593 pp.

Lawrence W, Foil LD. 2000. The effects of flea egg consumption on larval cat flea (Siphonaptera: Pulicidae) development. *J Vector Ecol* 25:98–101.

Lorange EA, Race BL, Sebbane F, Hinnebusch BJ. 2005. Poor vector competence of fleas and the evolution of hypervirulence in *Yersinia pestis*. *J Infect Dis* 191: 1907–1912.

Matchett MR, Biggins DE, Carlson V, Powell B, Rocke T. 2010. Enzootic plague reduces black-footed ferret (*Mustela nigripes*) survival in Montana. *Vector-Borne Zoonotic Dis* 10:27–35.

Matchett MR, Eads DA, Cordova J, Livieri TM, Hicks H, Biggins DE. 2023. Flea control on prairie dogs with fipronil bait pellets: Potential plague mitigation tool

for rapid field application and wildlife conservation. *J Wildl Dis* 59:71–83.

McTier TL, Evans NA, Martin-Short M, Gration K. 2003. Comparison of the activity of selamectin, fipronil, and imidacloprid against flea larvae (*Ctenocephalides felis felis*) in vitro. *Vet Parasitol* 116:45–50.

Mehlhorn H, Mencke N, Hansen O. 1999. Effects of imidacloprid on adult and larval stages of the flea *Ctenocephalides felis* after in vivo and in vitro application: A light-and electron-microscopy study. *Parasitol Res* 85:625–637.

Miarinjara A, Boyer S. 2016. Current perspectives on plague vector control in Madagascar: Susceptibility status of *Xenopsylla cheopis* to 12 insecticides. *PLoS Neglected Trop Dis* 10:e0004414.

Miarinjara A, Eads DA, Bland DM, Matchett MR, Biggins DE, Hinnebusch BJ. 2022. Reevaluation of the role of blocked *Oropsylla hirsuta* prairie dog fleas (Siphonaptera: Ceratophyllidae) in *Yersinia pestis* (Enterobacterales: Enterobacteriaceae) transmission. *J Med Entomol* 59:1053–1059.

Page SW. 2008. Antiparasitic drugs. In: *Small animal clinical pharmacology*. 2nd Ed. Maddison JE, Page SW, Church DB, editors. Elsevier, St Louis, Missouri, pp. 198–260.

Panella NA, Dolan MC, Karchesy JJ, Xiong Y, Peralta-Cruz J, Khasawneh M, Montenieri JA, Maupin GO. 2005. Use of novel compounds for pest control: Insecticidal and acaricidal activity of essential oil components from heartwood of Alaska yellow cedar. *J Med Entomol* 42:352–358.

Payne PA, Dryden MW, Smith V, Ridley RK. 2001. Effect of 0.29% w/w fipronil spray on adult flea mortality and egg production of three different cat flea, *Ctenocephalides felis* (Bouché), strains infesting cats. *Vet Parasitol* 102:331–340.

Poché D, Clarke T, Tseveenjav B, Torres-Poché Z. 2020. Evaluating the use of a low dose fipronil bait in reducing black-tailed prairie dog (*Cynomys ludovicianus*) fleas at reduced application rates. *Int J Parasitol Parasites Wildl* 13:292–298.

Poché DM, Hartman D, Polyakova L, Poché RM. 2017. Efficacy of a fipronil bait in reducing the number of fleas (*Oropsylla* spp.) infesting wild black-tailed prairie dogs. *J Vector Ecol* 42:171–177.

R Core Team. 2021. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/. Assessed January 2022.

Rajonhson DM, Miarinjara A, Rahelinirina S, Rajerison M, Boyer S. 2017. Effectiveness of fipronil as a systemic control agent against *Xenopsylla cheopis* (Siphonaptera: Pulicidae) in Madagascar. *J Med Entomol* 54:411–417.

Rust MK. 2016. Insecticide resistance in fleas. *Insects* 7: 10.

Rust MK, Dryden MW. 1997. The biology, ecology, and management of the cat flea. *Annu Rev Entomol* 42: 451–473.

Rust MK, Vetter R, Denholm I, Blagburn B, Williamson MS, Kopp S, Coleman G, Hostetler J, Davis W, et al. 2014. Susceptibility of cat fleas (Siphonaptera: Pulicidae) to fipronil and imidacloprid using adult and larval bioassays. *J Med Entomol* 51:638–643.

Schenker R, Humbert-Droz E, Moyses EW, Yerly B. 2001. Efficacy of nitenpyram against a flea strain with resistance to fipronil. *Suppl Compend Contin Edu. Pract Vet* 23:16–19.

Seery DB, Biggins DE, Montenieri JA, Enscore RE, Tanda DT, Gage KL. 2003. Treatment of black-tailed prairie dog burrows with deltamethrin to control fleas (Insecta: Siphonaptera) and plague. *J Med Entomol* 40:718–722.

Sikes RS, Animal Care and Use Committee of the American Society of Mammalogists. 2016. 2016 guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. *J Mammal* 97:663–688.

Silverman J, Appel AG. 1994. Adult cat flea (Siphonaptera: Pulicidae) excretion of host blood proteins in relation to larval nutrition. *J Med Entomol* 31:265–271.

Simon-Delso N, Amaral-Rogers V, Belzunces LP, Bonmatin JM, Chagnon M, Downs C, Furlan L, Gibbons DW, Giorio C, et al. 2015. Systemic insecticides (neonicotinoids and fipronil): Trends, uses, mode of action and metabolites. *Environ Sci Pollut Res* 22:5–34.

Tripp DW, Streich SP, Sack DA, Martin DJ, Griffin KA, Miller MW. 2016. Season of deltamethrin application affects flea and plague control in white-tailed prairie dog (*Cynomys leucurus*) colonies, Colorado, USA. *J Wildl Dis* 52:553–561.

Vallès X, Stenseth NC, Demeure C, Horby P, Mead PS, Cabanillas O, Ratsitorahina M, Rajerison M, Andrianaivoarimanana V, et al. 2020. Human plague: An old scourge that needs new answers. *PLoS Negl Trop Dis* 14:e0008251.

Wang K, Vasylieva N, Wan D, Eads DA, Yang J, Tretten T, Barnych B, Li J, Li QX, Gee SJ, Hammock BD. 2019. Quantitative detection of fipronil and fipronil-sulfone in sera of black-tailed prairie dogs and rats after oral exposure to fipronil by camel single-domain antibody-based immunoassays. *Anal Chem* 91:1532–1540.

Wilcomb MJ Jr. 1954. *A study of prairie dog burrow systems and the ecology of their arthropod inhabitants in central Oklahoma*. PhD Thesis, Graduate College, University of Oklahoma, Norman, Oklahoma, 172 pp.

Wimsatt J, Biggins DE. 2009. A review of plague persistence with special emphasis on fleas. *J Vector-Borne Dis* 46:85–99.

Yinon U, Shulov A, Margalit J. 1967. The hygroreaction of the larvae of the Oriental rat flea *Xenopsylla cheopis* Rothsch. (Siphonaptera: Pulicidae). *Parasitol* 57:315–319.

Submitted for publication 7 July 2022.
Accepted 8 September 2022