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# Prairie dog responses to vector control and vaccination during an initial *Yersinia pestis* invasion

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ABSTRACT

We evaluated the invasion of plague bacteria *Yersinia pestis* into a population of black-tailed prairie dogs (*Cynomys ludovicianus*; BTPDs) in South Dakota. We aimed to ascertain if *Y. pestis* invaded slowly or rapidly, and to determine if vector (flea) control or vaccination of BTPDs assisted in increasing survival rates. We sampled BTPDs in 2007 (before *Y. pestis* documentation), 2008 (year of confirmed invasion), and 2009 (after invasion). We estimated annual BTPD re-encounter rates on three 9-ha plots treated annually with deltamethrin dust for flea control and three 9-ha plots lacking dust. In 2007 and 2008, approximately half the adult BTPDs live-trapped were injected subcutaneously with either an experimental plague vaccine (F1–V fusion protein) or placebo formulation; the remaining individuals were not inoculated. From 2007 to 2009, we sampled 1559 BTPDs on 2542 occasions. During 2007–2008, the prevalence and intensity of fleas on BTPDs were 69–97% lower on the dusted vs. no dust plots. From 2007 to 2008, the annual re-encounter rate of non-inoculated BTPDs was 150% higher on the dusted vs. no dust plots. During the same interval on the dusted plots, the re-encounter rate was 55% higher for vaccinated adult female BTPDs vs. nonvaccinated adult females, but the annual re-encounter rate was 19% lower for vaccinated adult males. By late August 2008, BTPDs were nearly extirpated from the no dust plots. During 2007–2008 and 2008–2009 on the dusted plots, which persisted, the BTPD re-encounter rate was 41% higher for vaccinated vs. non-vaccinated adult females but 35% lower for vaccinated adult males. *Yersinia pestis* erupted with vigor as it invaded. Flea control enhanced BTPD survival but did not offer full protection. Flea control and F1–V vaccination seemed to have additive, positive effects on adult females. Annual re-encounter rates were reduced for vaccinated adult males; additional experimentation is needed to further evaluate this trend.

## 1. Introduction

The plague bacterium *Yersinia pestis* was introduced to western North America ca. 1900 and spread quickly from the Pacific Coast eastward, halting around the 103rd meridian and forming a "naturalized plague zone" in 17 western states (Abbott and Rocke, 2012; Mize and Britten, 2016). Therein, *Y. pestis* was (and remains) lethal to a variety of mammals and functions as an ecosystem transformer (Eads and Biggins, 2015). The fate of some mammal species hinges on effective plague mitigation, with accumulating evidence of negative *Y. pestis* effects on several imperiled species (Biggins et al., 2010, 2021a,b; Matchett et al., 2010; Goldberg et al., 2021, 2022), raising concern among ecologists and conservation biologists (Zeppelini et al., 2016; Eads et al., 2022a).

In populations of some rodents, *Y. pestis* causes enzootic mortality over prolonged periods (Biggins et al., 2010, 2021c; Kosoy et al., 2017; Goldberg et al., 2021, 2022) and occasional, explosive epizootics (Gage and Kosoy, 2005). For the purposes herein, we define plague epizootics within a given species as resulting in the deaths of >90% of individuals over a wide area within weeks to months (Biggins and Eads, 2019). We define enzootic plague as affecting lesser proportions of individuals, while acknowledging that enzootic plague can have strong ecological impacts (Zeppelini et al., 2016) with significant conservation implications (Biggins et al., 2010, 2021a,b; Matchett et al., 2010; Goldberg et al., 2021, 2022; Eads et al., 2022a,b).

In the western United States, most research on plague has been conducted in areas with regularly occurring *Y. pestis* transmission. In

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such plague "foci" (Antolin, 2008), *Y. pestis* may persist almost (or truly) indefinitely (Lowell et al., 2015), causing persistent enzootic mortality among hosts during some periods, and erupting in epizootic form when conditions allow (Biggins and Eads, 2019). Comparatively little research has been conducted in areas lacking historical evidence of plague, including some portions of the Great Plains of North America, such as western South Dakota, USA.

Plague was formerly rare in South Dakota and mostly limited to extreme western portions of the state (Mize and Britten, 2016). However, in 2005, colonies of black-tailed prairie dogs (*Cynomys ludovicianus*; BTPDs) on the Pine Ridge Indian Reservation, in southcentral South Dakota, experienced a plague epizootic (Rocke et al., 2008). That event caused concern, because it was detected ~44 km south of the Conata Basin, South Dakota, a site that was occupied by a successful reintroduced population of endangered black-footed ferrets (*Mustela nigripes*), which are exquisitely susceptible to *Y. pestis*. The easterly movement of plague posed a potential disaster for BTPDs and the ferrets they supported in Conata Basin and nearby Badlands National Park (hereafter Conata/Badlands). Proactive emergency dusting of BTPD burrows with deltamethrin powder, used to control fleas (the primary vectors of *Y. pestis*), began in late summer 2005 (Eads et al., 2018).

Two main approaches have been used to mitigate plague in *Cynomys* populations: flea control and host vaccination. Deltamethrin powder (herein referred to as dust) has been effective for flea control when applied to burrow openings of *C. ludovicianus*, *C. leucurus*, and *C. parvidens*. Applications of the dust reduced fleas by 96–98% after 1 month (Seery et al., 2003; Biggins et al., 2010) and 42–85% after 10 months (Biggins et al., 2010). In some cases, flea control was effective for 2 years (Eads and Biggins, 2019). Plague epizootics among *Cynomys* seem to have been halted with flea control in many cases (Seery et al., 2003; Hoogland et al., 2004), although not always (Tripp et al., 2017; Hoogland et al., 2018). Dusting improved annual apparent survival of prairie dogs and their population densities under enzootic and epizootic conditions (Biggins et al., 2010, 2021a,b) and preserved prairie dog genetic variability during an epizootic (Jones et al., 2012).

This study involved the use of deltamethrin dust for flea control and field application of an experimental, injectable F1–V plague protein vaccine to prairie dogs. The vaccine, developed by the U.S. Army Medical Research Institute of Infectious Diseases (Powell et al., 2005), had been previously tested in laboratory studies on black-footed ferrets with highly favorable results (Rocke et al., 2004, 2006, 2008) and on BTPDs with moderately favorable results (Rocke et al., 2010). In black-footed ferrets, vaccination with F1–V elicited very high antibody titers and excellent protection (up to 100%) against plague challenge, whereas in BTPDs, the rise in antibody titer was not as high as observed in ferrets, and only about 60% of challenged BTPDs survived.

This study was conceived in 2006 to evaluate the hypotheses that *Y. pestis* might first invade Conata/Badlands as an enzootic disease with relatively low rates of transmission among BTPDs, based on studies that detected such conditions in prairie dogs of several species (Biggins et al., 2010) and in black-footed ferrets (Matchett et al., 2010) in known plague foci. Alternative hypotheses might be 1) the ecological barriers presumed to have halted *Y. pestis* expansion into South Dakota historically would prevail and prevent easterly invasion into Conata/Badlands, or 2) plague would erupt vigorously as it invaded the optimal conditions of dense BTPD hosts and flea vectors in previously unoccupied habitat (Eskey and Haas, 1940) where plague-naïve BTPDs presumably would be highly susceptible to *Y. pestis*, as indicated by laboratory experiments (Rocke et al., 2012; Russell et al., 2019).

We live-trapped and sampled BTPDs in 2007, before documented *Y. pestis* had invaded the ecosystem, and 2008–2009, after plague had invaded, ultimately in epizootic form (Griebel, 2009; Shoemaker et al., 2014; Keuler et al., 2020). We compared annual re-encounter rates of BTPDs treated with the F1–V plague vaccine and BTPDs treated with placebo, under protocol inoculations administered to subsets of animals on dusted and no dust colonies. We also compared re-encounter rates for non-inoculated BTPDs on dusted and no dust colonies to see if attempted flea control assisted in plague mitigation. The experimental design allowed us to assess the effects of epizootic plague on BTPDs, the efficacy of flea control with dust, and the efficacy of F1–V vaccination. We hypothesized that flea parasitism would be lower on dusted than no dust plots, as found in many other studies of *Cynomys* (e.g., Biggins et al., 2010; Hoogland et al., 2018; Eads and Biggins, 2019; but see Eads et al., 2018) and both dust and vaccination would provide at least partial protection against plague (with a stronger positive effect of dust than vaccination, given the vaccine performed moderately well in laboratory trials).

## 2. Materials and methods

We conducted field research under Institutional Animal Care and Use Committee protocols (U.S. Geological Survey, Fort Collins Science Center, Colorado). Conata Basin (43°46′N, 102°18′W) is situated on Buffalo Gap National Grassland, South Dakota, and is administered by the U.S. Forest Service (USFS). The BTPD complex extends through Badlands National Park to the north of Conata Basin, an area administered by the National Park Service. Conata/Badlands is characterized by short-grass prairie, badland buttes, and drainages. Primary land uses include recreation and, on USFS-managed lands, cattle grazing (Livieri and Anderson, 2012).

We studied BTPDs on six plots, each 9-ha, in the Conata Basin. Three plots (>300 m apart from their nearest neighboring plot) were situated on the North Exclosure (213 ha in 2007), a colony treated (dusted) in its entirety once annually with dust since 2005 (Eads et al., 2018); the dusted colony was buffered (by > 1000 m) from no dust habitat by a seasonal water drainage and open grassland habitat. The remaining plots (>1900 m apart) were situated ~6–10 km southeast, on three separate colonies (167, 147, and 1769 ha) along Highway 44 and had not been treated with dust (herein functioning as no dust baselines). All plots were considered independent; BTPDs typically move over distances <40 m (Matchett et al., 2021).

Treatments on the dusted colony were accomplished by infusing each burrow with ~4–6 g of DeltaDust® (0.05% deltamethrin, Bayer Environmental Science, Research Triangle Park, North Carolina, USA; Biggins et al., 2010; Eads and Biggins, 2019). Dust treatment on the North Exclosure BTPD colony were accomplished in early- to mid-October 2006 (before this study began), mid-October 2007, mid-September 2008, and late-June 2009.

During July–October 2007–2009, we live-trapped and sampled BTPDs using Tomahawk live traps (Tomahawk Live Trap, Hazelhurst, Wisconsin, USA). Trapping was completed on each plot for at least 7 days (range = 7–43 days) in each year (except for the no dust plots, which were devastated by plague, as described further below). We collected Universal Transverse Mercator coordinates of BTPD trapping locations using hand-held global positioning units with ~5 m accuracy. We marked both ears of each BTPD with #1 monel fingerling fish tags for permanent identification (National Band and Tag Company, Newport, KY, USA). We classified each BTPD's age based on body mass and size as juvenile or adult (Biggins et al., 2010).

We anesthetized subsets of BTPDs each year on each plot, along with fleas on their bodies, with isoflurane and combed each BTPD for 30 s to dislodge and count fleas (Eads et al., 2023a,b). We did not identify flea species, but one species, *Oropsylla hirsuta*, was likely to comprise ≥99% of fleas detected; this species specializes on *Cynomys* hosts, transmits *Y. pestis*, and is commonly found on BTPDs and black-footed ferrets at Conata/Badlands (Harris et al., 2014; Eads et al., 2018; Russell et al., 2018). Fleas were sampled from only subsets of BTPDs to reduce potential negative effects of combing on flea survival and density, given the primary objective was to evaluate plague's potential and realized invasion.

Approximately half of the adult BTPDs live-trapped on each plot (dusted and no dust) were inoculated subcutaneously with 40 mg F1–V

vaccine, obtained from the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) and prepared as described previously (Rocke et al., 2004, 2006, 2008, 2010) or a placebo consisting of the diluent (adjuvant suspended in Dulbeco's medium) at 0.5 mL volume per individual for both vaccine and placebo. The remaining half of adult BTPDs were non-inoculated (Fig. 1). We alternately inoculated adult BTPDs with vaccine and placebo, so mean elapsed times from treatment in a given year to potential recapture in the following year would be similar for vaccinated and placebo-injected adult BTPDs. The remaining half of adult BTPDs captured were not inoculated. Captured juveniles were also not inoculated (Fig. 1). The vaccine and placebo formulations were prepared at the U.S. Geological Survey, National Wildlife Health Center, Madison, Wisconsin. The cold chain was maintained in the field using refrigerators, coolers, and ice packs (for chilling, but not freezing). All BTPD inoculations were completed within 2 weeks of vaccine/placebo preparation.

Some of the inoculated adult BTPDs received a booster of their initial solution, vaccine or placebo, if they were recaptured within 3–4 wks of initial inoculation. For the inoculated adults, the ratios of boosted vs. non-boosted individuals were similar for the vaccine (59%) and placebo groups (61%). During analyses of BTPD re-encounters, inclusion of a variable for boosting was significant for adult females and males. However, the effect was supported for the vaccine and placebo groups, suggesting no significant effect of actual vaccine boosting. Instead, BTPDs with booster inoculations had already survived an extra 3–4 weeks (in order to receive a booster) and may have been the most healthy and fit individuals in our study population. For parsimony, boosting is not considered further herein (results remained the same when controlling for boosting).

We implemented general linear models for analysis in R x64 4.1.2 ('glm' in 'stats' package), concentrating on (1) effects of dust on flea parasitism of BTPDs, (2) effects of dust on BTPD annual re-encounter rates (2007–2008 and 2008–2009), and (3) effects of F1–V fusion protein vaccination on BTPD annual re-encounter rates. In all cases, we reduced initial models containing variables of hypothesized importance to more parsimonious forms via backward elimination ($\alpha = 0.050$ for main-effects, 0.200 for interactions; 'Anova' in 'car' package; Fox et al., 2023). We assessed model goodness-of-fit using Hosmer-Lemeshow

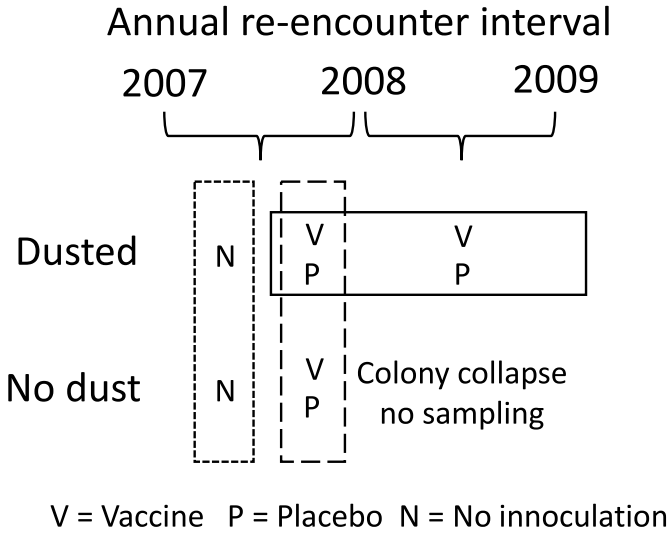


**Fig. 1.** Categories of flea vector control (deltamethrin dust) and F1–V fusion protein plague vaccination (V = vaccine, P = placebo, N = no inoculation) used for analyses of black-tailed prairie dog annual re-encounter rates (2007–2008 and 2008–2009) at Conata Basin, South Dakota. Annual re-encounter rates were compared for subsets of animals, here each enclosed by unique rectangles. Sample sizes are depicted in subsequent figures with results from multivariate analyses.

tests, with *P*-values $> 0.050$ as evidence of acceptable correspondence between the field data and model predictions ('hoslem.test' in 'ResourceSelection' package; Lele et al., 2022). For interpretation, we present model predictions and 95% confidence intervals (CIs) in figures with the associated sample sizes ('ggpredict' in 'ggeffects' package; Lüdecke et al., 2022).

To assess flea control, we ran two analyses (Fig. 1). We concentrated on an effect of dust treatment and compared flea prevalence (binomial family) and flea intensity (Gaussian family) among all BTPDs (vaccinated and non-inoculated) on the dusted and no dust plots. Prevalence is the proportion of BTPDs from which $\geq$1 flea was combed (Bush et al., 1997). Intensity is the number of fleas combed from BTPDs with $\geq$1 flea combed; we log transformed flea intensity for analysis. In both flea analyses, we considered dust treatment, year of sampling (for the first year of each annual re-encounter interval, as described below, 2007 [2007–2008] and 2008 [2008–2009]), BTPD age, BTPD sex, and an interaction between treatment and year. Flea parasitism on BTPDs varies seasonally at Conata/Badlands (Eads et al., 2019, 2022b), so we included a variable for Julian day.

As an index to BTPD annual survival, we used re-encounter rates (the proportion of marked BTPDs that were recaptured the following year; Tripp et al., 2017; Matchett et al., 2021; Eads et al., 2022b) analyzed with binomial logistic regression. This approach does not account for imperfect capture probabilities or potential emigration from study plots. That said, each year, trapping effort was distributed evenly among all plots. Moreover, vaccinated, non-vaccinated, and non-inoculated BTPDs (of all age/sex classes) occupied the same plots. Therefore, we suggest there may be little (to no) reason to believe that capture probabilities varied by treatment or age/sex class. In many prior studies of BTPDs, capture probabilities have been consistent between experimental treatments and age/sex classes (e.g., Biggins et al., 2010; Rocke et al., 2017; Matchett et al., 2021). BTPDs are highly territorial and move over short distances within colonies (Matchett et al., 2021) suggesting influences of emigration would be minimal (though some influences cannot be fully discounted; Hoogland, 2013).

The analyses of BTPD annual re-encounter rates, described below, could be influenced by elapsed time during intervals between captures in a year (e.g., 2007) and recaptures the following year (2008) if those elapsed times varied by treatment(s). For example, if mortality hazard rate is constant over time, and elapsed times between BTPD first captures and recaptures were longer on the dusted plots than no dust plots, we might underestimate any positive effect of dust treatment on BTPD annual survival (e.g., because a longer interval on the dusted plots would have allowed more time for BTPDs to die). For each interval and treatment of interest, we compared mean elapsed times between captures in the first year of the interval and recaptures in the following year using *t*-tests ('t.test' in 'stats' package). These analyses considered only those BTPDs that were recaptured in an interval (thus, elapsed time could not be included as a control variable in the re-encounter analyses).

We ran three analyses of BTPD annual re-encounter rates (Fig. 1). First, we assessed adult BTPD re-encounter rates from 2007 (before plague) to 2008 (plague invasion) considering potential effects of BTPD sex, dust treatment, vaccination, and all possible 2-way and 3-way interactions. Inclusion of the 2009 data was deemed inappropriate because epizootic plague had devastated the no dust plots by 2008, leaving few BTPDs on those plots to assess survival into 2009. We restricted this analysis to inoculated adult BTPDs that had received vaccine or placebo (Fig. 1). With plague's known invasion by 2008, we hypothesized that annual re-encounter rates from 2007 to 2008 would be higher for BTPDs inoculated with vaccine than BTPDs injected with placebo (though, given moderately favorable results in laboratory trials [Rocke et al., 2010], we thought any positive effect of the F1–V vaccine might be somewhat weak, i.e., considering biological importance; *sensu* Nakagawa and Cuthill, 2007).

Second, to concentrate specifically on the effect of vaccination, we limited the data to the dusted plots (to reduce influences of flea-borne

*Y. pestis* and to extend the data set by one year) and analyzed re-encounter data from inoculated adult BTPDs. Again, BTPDs persisted from 2008 to 2009 on the dusted plots, allowing us to consider two annual intervals (Fig. 1). We considered potential effects of BTPD sex, vaccination, interval (2007–2008 or 2008–2009), and all possible interactions. In this case, two outcomes seemed plausible: (1) survival rates might be similar for BTPDs injected with vaccine or placebo, because dust was effective in suppressing epizootic plague (as found during a study of ferrets under enzootic conditions; Matchett et al., 2010) or (2) survival rates might be at least somewhat higher for vaccinated BTPDs because the force of epizootic plague was sufficient to at least partly overwhelm positive effects of dust treatments on individual BTPDs (though, at a coarser scale, BTPD populations persisted on the dusted plots).

Lastly, to concentrate specifically on the effect of dust on BTPD annual re-encounter rates from 2007 to 2008, we analyzed data from non-inoculated BTPDs, thus eliminating effects of vaccine/placebo or, perhaps, inoculation on survival. This analysis included adult and juvenile BTPDs (only adults had been injected with vaccine or placebo). We considered potential effects of BTPD age, BTPD sex, dust treatment, and all possible interactions. We hypothesized that BTPD annual re-encounter rates would be higher on dusted than no dust plots.

For each flea and re-encounter rate analysis, we calculated effect sizes as percent differences between treatments (dust/no dust, vaccine/placebo), using point estimates with the following equation: (treatment estimate – no treatment [placebo] estimate)/no treatment [placebo] estimate (Goldberg et al., 2022).

## 3. Results

Data are available from Eads (2023). From 2007 to 2009, we captured and sampled 1559 individual BTPDs on 2542 processing occasions. Trapping effort per year, calculated as trap hours (i.e., trap days × hours/day) was 49,241 in 2007, 104,674 in 2008, and 115,208 in 2009. Numbers of captures varied by year and dust treatment, from 1312 captures in 2007 (52% on dusted plots) to 655 in 2008 (75% on dusted plots) to 575 in 2009 (100% on dusted plots, because BTPD populations on the no dust plots collapsed by 2009, precluding sampling on those plots). Numbers of individual BTPDs captured on the no dust plots declined from 2007 ($n = 377$) to 2008 (123) to 2009 (0 observed during multiple, temporally replicated observations over several months in daylight). BTPD numbers remained comparatively higher, and more stable, on the dusted plots (445, 331, and 430, respectively).

In 2007, fleas were combed from BTPDs 260–348 days after the 2006 treatment, and 6–12 days after the 2007 treatment. In 2008, fleas were combed 247–338 days after the 2007 treatment, and 1–24 days after the 2008 treatment. In the analysis of flea prevalence ($n = 464$ observations), the following variables were sequentially eliminated from the binomial model: dust treatment × year ($\chi^2 = 0.13$, $P = 0.714$), BTPD sex ($\chi^2 = 0.75$, $P = 0.388$), and BTPD age ($\chi^2 = 1.59$, $P = 0.207$). Flea prevalence increased with Julian day ($\chi^2 = 4.24$, $P = 0.040$) and was higher in 2007 than 2008 ($\chi^2 = 12.83$, $P < 0.001$). Flea prevalence was 97% lower on dusted plots than no dust plots (Fig. 2; $\chi^2 = 194.17$, $P < 0.001$). We failed to detect disparity between field and model predictions ($\chi^2 = 9.15$, $P = 0.330$).

In the analysis of flea intensity ($n = 136$ observations), the following variables were sequentially eliminated: dust treatment × year ($\chi^2 = 0.13$, $P = 0.719$), year ($\chi^2 = 0.04$, $P = 0.840$), and BTPD sex ($\chi^2 = 0.59$, $P = 0.441$). Flea intensity was higher on adult than juvenile BTPDs ($\chi^2 = 3.93$, $P = 0.048$) and increased with Julian day ($\chi^2 = 21.54$, $P < 0.001$). Flea intensity was 69% lower on the dusted plots (Fig. 2; $\chi^2 = 12.20$, $P < 0.001$). The 95% CI for flea intensity on the dusted sites is relatively wide because flea intensity includes only those BTPDs with at least 1 flea, and ≥1 flea was combed from very few BTPDs (12 of 282 combings) on the dusted sites (hence very low flea prevalence on those plots; Fig. 2). We failed to detect disparity between field data and model predictions ($\chi^2 = 12.20$, $P = 0.143$).

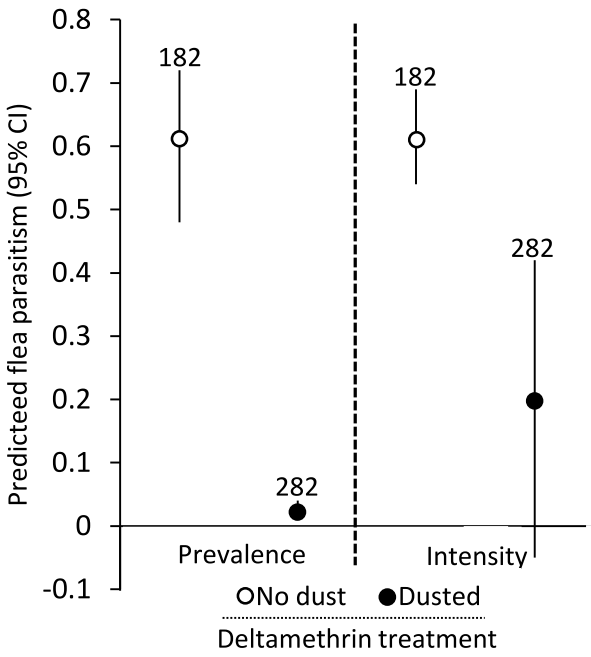


**Fig. 2.** Predicted flea parasitism (95% confidence intervals [CIs]) on black-tailed prairie dogs at the no dust and dusted plots, 2007–2008 at Conata Basin, South Dakota (prevalence on the left, intensity on the right). Prairie dog burrows on the dusted plots were treated annually with deltamethrin dust at ~4–6 g per burrow. Model predictions adjust (i.e., control) for year and Julian day (adjusted here as year 2008, and Julian day 212 for prevalence and 200 for intensity). Flea intensity data were log-transformed ($\log_{10}$) for analysis; hence, predicted flea intensity and 95% CIs could extend below 0. Sample sizes are depicted above the 95% CIs.

For vaccinated and placebo-injected BTPDs in the vaccine experiment that were first captured in 2007 and recaptured in 2008, mean elapsed times between capture in 2007 and recapture in 2008 were similar for the treatments (vaccine = 426.1 days, placebo = 429.8 days; $t = 0.15$, $P = 0.885$), providing evidence that interval length would not bias the annual re-encounter rate estimates of BTPDs inoculated with vaccine or placebo. For the non-inoculated BTPDs used to assess an effect of dust flea control on annual re-encounter rates, mean elapsed times between capture in 2007 and recapture in 2008 were longer for BTPDs on the dusted plots (dusted = 392.5 days, no dust = 335.5 days; $t = -7.14$, $P < 0.001$). Thus, the positive effect of dust on BTPD re-encounter rates (reported below) may have been underestimated.

In the annual re-encounter rate analysis of inoculated adult BTPDs from 2007 to 2008, the following variables were sequentially eliminated: dust treatment × vaccination × BTPD sex ($\chi^2 = 0.14$, $P = 0.710$), dust treatment × vaccination ($\chi^2 = 0.05$, $P = 0.822$), and dust treatment × BTPD sex ($\chi^2 = 0.79$, $P = 0.373$). BTPD re-encounter rate was 52% lower on the no dust plots ($\chi^2 = 10.58$, $P = 0.001$). The vaccination × BTPD sex interaction gained support ($\chi^2 = 2.22$, $P = 0.136$), and a trend was noted. The mean re-encounter rate was 55% higher for adult females receiving vaccine (vs. placebo) but 19% lower for adult males receiving vaccine compared to placebo (Fig. 3). We failed to detect evidence for lack of model fit ($\chi^2 = 0.97$, $P = 0.998$).

In the annual re-encounter rate analysis of inoculated adult BTPDs on dusted plots from 2007 to 2008 and 2008–2009, the following variables were sequentially eliminated: trapping interval × vaccination × BTPD sex ($\chi^2 = 0.02$, $P = 0.877$), and trapping interval × vaccination ($\chi^2 = 0.596$, $P = 0.440$). The vaccination × BTPD sex ($\chi^2 = 5.80$, $P = 0.016$) and trapping interval × BTPD sex ($\chi^2 = 1.80$, $P = 0.179$) interactions gained support (Fig. 4). In this case, two trapping intervals were

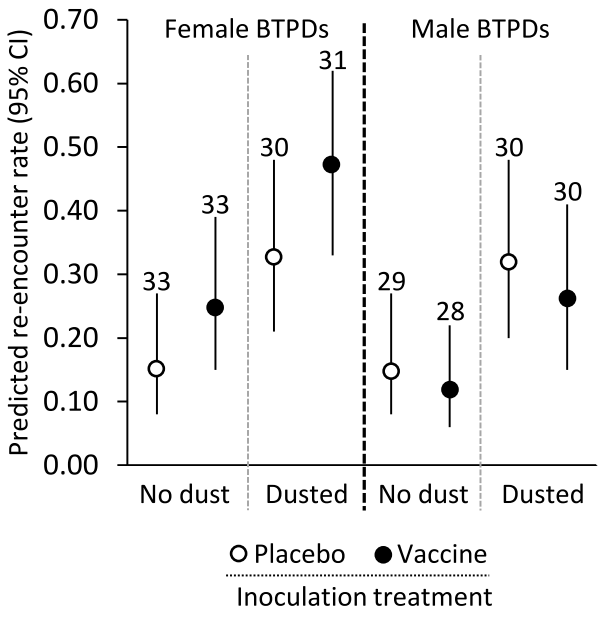


**Fig. 3.** Predicted re-encounter rates (95% confidence intervals [CIs]) over a single trapping interval (2007–2008) for adult female and male black-tailed prairie dogs inoculated at Conata Basin, South Dakota in 2007 with F1–V fusion protein vaccine or placebo on the no dust and dusted plots (the latter with flea control). Sample sizes are depicted above the 95% CIs.

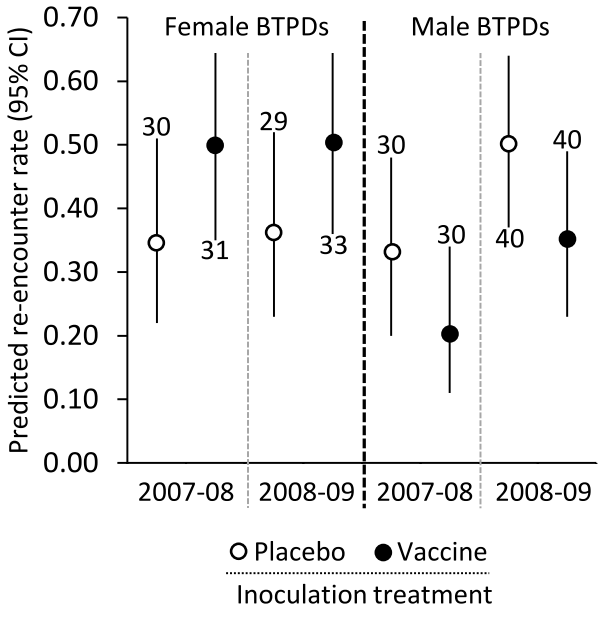


**Fig. 4.** Predicted re-encounter rates (95% confidence intervals [CIs]) over two trapping intervals (2007–2008 and 2008–2009) for adult female and male black-tailed prairie dogs in Conata Basin, South Dakota inoculated in 2007 or 2008 with F1–V fusion protein vaccine or placebo on the dusted plots (with flea control). Sample sizes are depicted above or below the 95% CIs.

included, increasing statistical power relative to the analysis above for dusted and no dusted plots 2007–2008. Moreover, this analysis concentrated on the dusted plots (with flea control) where plague transmission may have been reduced and any differential sex effects of vaccination should be most discernible. To further evaluate the trends noted above, we separated this analysis by sex. On the dusted plots, the annual re-encounter rate for adult females receiving vaccine was 43% and 39% higher ($\chi^2 = 2.61$, $P = 0.107$) during 2007–2008 and 2008–2009, respectively, than the placebo group. In contrast, on the dusted plots the re-encounter rates for adult males receiving vaccine were 39% and 30% lower ($\chi^2 = 3.89$, $P = 0.049$), respectively, than the placebo group. Again, the statistical interaction and differential sex responses are of primary interest. We failed to detect evidence for lack of model fit ($\chi^2 = 3.53$, $P = \sim 1.000$).

In the annual re-encounter rate analysis of non-inoculated adult and juvenile BTPDs from 2007 to 2008, the following variables were sequentially eliminated: dust treatment × BTPD age × BTPD sex ($\chi^2 = 0.52$, $P = 0.472$), dust treatment × BTPD age ($\chi^2 = 0.28$, $P = 0.599$), dust treatment × BTPD sex ($\chi^2 = 0.38$, $P = 0.540$), BTPD age × BTPD sex ($\chi^2 = 0.95$, $P = 0.329$), BTPD age ($\chi^2 = 0.02$, $P = 0.899$), and BTPD sex ($\chi^2 = 0.50$, $P = 0.480$). Annual re-encounter rate was 150% higher on the dusted plots vs. the no dust plots (Fig. 5; $\chi^2 = 33.46$, $P < 0.001$). We failed to detect evidence for lack of model fit ($\chi^2 = \sim 0.00$, $P = \sim 1.000$).

## 4. Discussion

### 4.1. Plague invasion

Because of previous studies that provided evidence of enzootic plague in *Cynomys* in other locations (e.g., Hanson et al., 2007; Biggins et al., 2010; Mize and Britten, 2016), we hypothesized that initial *Y. pestis* detection at Conata/Badlands might be in the form of enzootic plague at reduced rates of transmission. Instead, *Y. pestis* seemingly invaded our study colonies with the full force of an epizootic outbreak (Shoemaker et al., 2014; Keuler et al., 2020), perhaps because the bacterium invaded dense BTPD hosts and flea vectors in previously

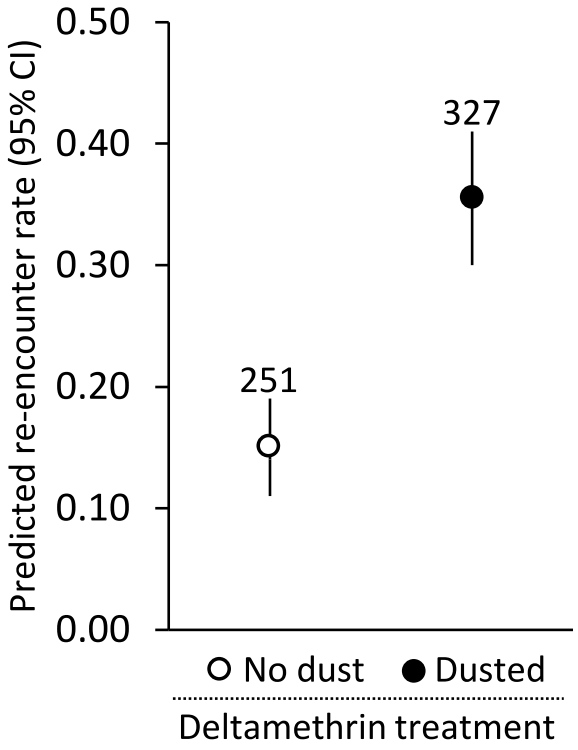


**Fig. 5.** Predicted re-encounter rates (95% confidence intervals [CIs]) over a single trapping interval 2007–2008 for non-inoculated adult and juvenile black-tailed prairie dogs on the no dust and dusted plots (the latter with flea control) at Conata Basin, South Dakota. Sample sizes are depicted above the 95% CIs.

unoccupied habitat (Eskey and Haas, 1940) and the resident, likely plague-naïve BTPDs were highly susceptible to *Y. pestis* as previously documented in laboratory challenge trials (Rocke et al., 2012; Russell et al., 2019). *Yersinia pestis* was first confirmed in Conata/Badlands in a dead BTPD found during May 2008 at a location near one of the no dust plots. Repeated re-mapping of areas of BTPD activity indicated plague was causing the greatest BTPD mortality during May–June 2008 (Griebel, 2008, 2009). In 2008, plague nearly extirpated BTPDs on nearly all 5665 ha of habitat that had not been treated annually with dust (Eads et al., 2018). By late summer 2009, 6534 ha of mapped BTPD colonies had become nearly devoid of BTPD activity due to plague, including the no dust plots in this study (Griebel, 2010). The dusted areas in our study were part of the 5319 ha of treated colonies, all of which continued to have relatively high levels of prairie dog activity into 2009 (Griebel, 2010) and continued to support black-footed ferrets.

### 4.2. Overall study results

Nearly complete collapses of the BTPD populations on the no dust plots compared to the dusted plots demonstrated the positive protection afforded by flea control, a finding previously documented in other studies (e.g., Biggins et al., 2010, 2021a,b; but see Tripp et al., 2017; Hoogland et al., 2018). In contrast, we found inconsistent effects of F1–V vaccination. Vaccinated adult female BTPDs survived at higher rates on both dusted and no dust plots compared to the placebo groups, whereas reduced re-encounter rates were detected in vaccinated adult male BTPDs on both dusted and no dust plots, possibly indicating the relative ineffectiveness of the F1–V vaccine, at least for that age-sex group. These findings differed from those of a previous field experiment using F1–V vaccine to immunize black-footed ferrets in Montana (Matchett et al., 2010), where F1–V vaccination or dust flea control alone provided equally high re-encounter rates in ferrets and thus, presumably, equal protection against plague infection.

### 4.3. Vaccination

In a previous laboratory experiment (Rocke et al., 2010), F1–V vaccination of BTPDs resulted in incomplete protection against plague; just 60% of F1–V vaccinated BTPDs survived a challenge of ~70,000 colony forming units of *Y. pestis*, but no difference was noted between males and females. Based on these results, we believed the vaccine would provide BTPDs with limited protection under natural conditions. Instead, we detected a trend that indicated a sexually disproportionate effect of vaccination, with a potential positive effect on adult female (but potentially negative effect on adult male) BTPD annual re-encounter rates on both dusted and no dust plots (Figs. 3 and 4). Other research findings have indicated *Y. pestis* is indiscriminate when infecting and killing adult male and female *Cynomys* (Biggins et al., 2010; Tripp et al., 2017; Rocke et al., 2017). Likewise, Matchett et al. (2021) and Rocke et al. (2017) did not detect disproportionate effects of a different (oral) plague vaccine in male BTPDs compared to females in Montana and elsewhere. The vaccine in those studies was an orally-delivered, virally-vectored vaccine expressing *Y. pestis* F1 antigen and a truncated form of V antigen (Rocke et al., 2017), which stimulates cellular immune responses and mucosal immunity. The injectable F1–V protein vaccine in contrast elicits a very different immune response (primarily humoral-mediated) with higher levels of IgG antibody (Rocke et al., 2010).

The potentially negative effect of F1–V vaccination in male BTPDs observed in this study might be explained by the effects of male sex hormones on immune responses, the mating behaviors of BTPDs and the type of immune response elicited by the F1–V protein vaccine. A large body of evidence (see Roved et al., 2017) has shown that sex hormones can affect immune responses; males with high testosterone levels have been shown to have lowered immunocompetence due to suppressed immune responses. In summary, male testosterone tends to have mixed effects on type 1 immune responses (mostly cellular mediated) but suppresses type 2 immune responses (mostly humoral-mediated), whereas female estrogen tends to lower type 1 responses and enhance type 2 responses (Roved et al., 2017). Moreover, the size of this disproportionate effect in immune response between the sexes may be dependent on mating behavior. Larger sexual differences have been predicted in species with polygynous mating systems, where males are more involved in fighting and have higher levels of testosterone (Hasselquist, 2007; Zuk, 1990). No or smaller differences are perhaps expected in species that are monogamous or have lower levels of sexual selection.

*Cynomys* are harem polygynous, and adult males are the primary defenders of family territories (Hoogland, 1995). The differing effects of male and female sex hormones on humoral-mediated immunity may explain the different rates of returns (re-encounter rates) for adult female and male BTPDs in our study, with a potential negative effect of vaccination on adult males that, at least hypothetically, may already suffer some fitness consequences of immune responses (Roved et al., 2017), and those costs might be magnified by F1–V vaccination and the associated humoral-mediated responses. This potential phenomenon in response to plague vaccination has not been reported previously in wild mammals, and further studies in *Cynomys* and other species affected by plague could be useful. Future experiments might also include vaccination of juvenile *Cynomys*. Age is known to affect response to vaccination in *Cynomys* inoculated with a virally-vectored vaccine (Rocke et al., 2015), with older individuals less responsive than juveniles or young adults.

Considering placebo-injected individuals on the dusted plots, adult female BTPD annual re-encounter rates were similar from 2007 to 2008 and 2008–2009. Re-encounter rates of placebo-injected adult males on the dusted plots were similar to adult females in 2007–2008 but had increased considerably into 2008–2009 (Fig. 4). The spread of plague seemed rapid during May–June 2008 but slower in late 2008 and 2009. In 2007–2008, plague and the potential negative effect of F1–V vaccine may have reduced adult male BTPD densities, even on the dusted plots, perhaps reducing the stresses of territorial defense by males and increasing the survival of placebo-injected adult males, in particular, from 2008 to 2009.

### 4.4. Flea control

In both years of the study, we detected an additive, positive effect of F1–V vaccination on adult female BTPDs on plots treated with dust. These results indicate flea control did not offer full protection for adult female BTPDs. The dust plots were not treated until mid-September 2008 (~11 mo after dusting in 2007) and late-June 2009 (~10 mo after the dusting in 2008). In some cases, flea populations on *Cynomys* colonies may begin to rebound <1-yr post-treatment (Biggins et al., 2010; Hoogland et al., 2018). In our study, perhaps flea populations in July–August 2008 were beginning to recover and able to sustain low levels of *Y. pestis* transmission on the dusted plots (e.g., on one dusted plot, we found 11 fleas on a BTPD, 30 July 2008). If so, the additive effect of vaccination on adult female BTPDs occupying dusted plots is a possible consequence.

If the above hypothesis is correct, with rebounding flea numbers in July–August 2008 on the dusted plots, we suspect the rebounds are unrelated to the evolution of flea resistance to deltamethrin. Existing data from Conata/Badlands (and areas of Wyoming) indicate BTPD fleas (*O. hirsuta*) may begin to develop resistance to deltamethrin after 6–10 years, or more, of consecutive annual treatments (Eads et al., 2018). In the current study, treatments on the dusted plots began in 2005, and we collected data from 2007 to 2009. That said, we cannot rule out this possibility. Potential rebounding flea numbers in Jul–Aug 2008 may relate to application errors such as underdosing and missed burrows. Underdosing has been detected in Conata/Badlands, highlighting the importance of recent technological innovations with machinery for

dusting of *Cynomys* burrows with insecticides (Tripp et al., 2021, 2022). Other modes of insecticide treatment, that do not require burrow dusting, are also influential in this context (e.g., edible baits for systemic flea control; Poché et al., 2017; Eads et al., 2019, 2021; Matchett et al., 2023).

### 4.5. Conclusions

Although vaccination using an injectable protein vaccine, like the F1–V fusion protein, is not considered a feasible plague management tool for *Cynomys* at landscape level applications, it is currently being used to protect endangered black-footed ferrets against plague with great success (Matchett et al., 2010) and has been considered for other endangered species affected by plague (e.g., pygmy rabbits; *Brachylagus idahoensis*). The results of our study in BTPDs may be considered carefully before use of F1–V protein in other species. Although we did not detect significant sex differences in captive BTPDs vaccinated with F1–V fusion protein in an initial plague challenge study (Rocke et al., 2010), the animals were of mixed sex and age and the power to detect differences between cohorts was very low. Moreover, the animals were vaccinated in October while in captive conditions, and they were not breeding or defending territories or harems. It is possible the effects of sex hormones on the immune response are dampened in BTPDs during non-breeding periods or under captive conditions, which argues for the necessity of evaluating response to vaccination (and perhaps different vaccine doses) in animals under natural conditions. One could argue, these types of studies might be best conducted prior to wide-scale application of any vaccine in wild species. As for black-footed ferrets, no difference between sexes has been reported in response to vaccination with F1–V fusion protein, either in captive or wild-caught animals, and its continued application has proven critical to the recovery of the species (Rocke, 2023), but a more systematic investigation of their response to vaccination by sex is warranted.

Results herein indicate flea control with deltamethrin does not offer *Cynomys* full protection against plague, suggesting flea control may not offer black-footed ferrets with full protection. Vaccination of predatory black-footed ferrets even on dusted prairie dog colonies is advised, given that ferrets are highly sensitive to even low rates of *Y. pestis* transmission among *Cynomys* and associated rodents and their fleas (Matchett et al., 2010), and ferrets can succumb to *Y. pestis* even after licking and/or sniffing an infectious rodent carcass (Rocke et al., 2006). Our study supports an additive positive effect of vaccination for female BTPDs on dusted colonies, supporting that recommendation.

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## References

Abbott, R.C., Rocke, T.E., 2012. Plague. U.S. Geological Survey Scientific Investigations Report 2012-1372. National Wildlife Health Center, Madison, Wisconsin.

Antolin, M.F., 2008. Unpacking β: within-host dynamics and the evolutionary ecology of pathogen transmission. Annu. Rev. Ecol. Evol. Syst. 39, 415–437.

Biggins, D.E., Eads, D.A., 2019. Prairie dogs, persistent plague, flocking fleas, and pernicious positive feedback. Front. Vet. Sci. https://doi.org/10.3389/fvets.2019.00075.

Biggins, D.E., Eads, D.A., Godbey, J.L., 2021a. Plague transforms positive effects of precipitation on prairie dogs to negative effects. Int. J. Parasitol. Parasites Wildl. 14, 329–334.

Biggins, D.E., Godbey, J.L., Gage, K.L., Carter, L.G., Montenieri, J.A., 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, D.E., Godbey, J.L., Eads, D.A., 2021b. Epizootic plague in prairie dogs: correlates and control with deltamethrin. Vector Borne Zoonotic Dis. 21, 172–178.

Biggins, D.E., Ramakrishnan, S., Rocke, T.E., Williamson, J.L., Wimsatt, J., 2021c. Enzootic plague reduces survival of Mexican woodrats (*Neotoma mexicana*) in Colorado. Ecosphere 12, e03371.

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. J. Parasitol. 83, 575–583.

Eads, D.A., 2023. Data on the Effects of F1-V Plague Antigen Vaccination and Deltamethrin Dust on Black-Tailed Prairie Dog Annual Survival and Flea Parasitism, South Dakota, 2007–2009. U.S. Geological Survey Science Base data release. https://doi.org/10.5066/P9SHY4KR.

Eads, D.A., Biggins, D.E., 2015. Plague bacterium as a transformer species in prairie dogs and the grasslands of western North America. Conserv. Biol. 29, 1086–1093.

Eads, D.A., Biggins, D.E., 2019. Plague management of prairie dog colonies: degree and duration of deltamethrin flea control. J. Vector Ecol. 44, 40–47.

Eads, D.A., Biggins, D.E., Bowser, J., McAllister, J.C., Griebel, R.L., Childers, E., Livieri, T.M., Painter, C., Krank, L.S., 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, D.A., Biggins, D.E., Bowser, J., Broerman, K., Livieri, T.M., Childers, E., Dobesh, P., Griebel, R.L., 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, D.A., Biggins, D.E., Wimsatt, J., Eisen, R.J., Hinnebusch, B.J., Matchett, M.R., Goldberg, A.R., Livieri, T.M., Hacker, G.M., et al., 2022a. Exploring and mitigating plague for One Health purposes. Curr. Trop. Med. Rep. 9, 169–184.

Eads, D.A., Huyvaert, K.P., Biggins, D.E., 2023a. Estimating parasite infrapopulation size given imperfect detection: proof-of-concept with ectoparasitic fleas on prairie dogs. Int. J. Parasitol. Parasites Wildl. 20, 117–121.

Eads, D.A., Livieri, T.M., Dobesh, P., Childers, E., Noble, L.E., Vasquez, M.C., Biggins, D. E., 2021. Fipronil pellets reduce flea abundance on black-tailed prairie dogs: potential tool for plague management and black-footed ferret conservation. J. Wildl. Dis. 5, 434–438.

Eads, D., Livieri, T., Tretten, T., Hughes, J., Kaczor, N., Halsell, E., Grassel, S., Dobesh, P., Childers, E., et al., 2022b. Assembling a safe and effective toolbox for integrated flea control and plague mitigation: fipronil experiments with prairie dogs. PLoS One 17, e0272419.

Eads, D.A., Tretten, T.N., Hughes, J.P., Biggins, D.E., 2023b. Lethal effects on flea larvae of fipronil in host feces: potential benefits for plague mitigation. J. Wildl. Dis. https://doi.org/10.7589/JWD-D-22-00092.

Eskey, C.R., Haas, V.H., 1940. Public Health Bulletin No. 254, Washington, D.C.. In: Plague in the Western Part of the United States. U.S. Public Health Service, p. 83.

Fox, J., Weisberg, S., Price, B., Adler, D., Bates, D., Baud-Bovy, G., Bolker, B., Ellison, S., Firth, D., Friendly, M., et al., 2023. Package 'car'. https://cran.r-project.org/web/packages/car/.

Gage, K.L., Kosoy, M.Y., 2005. Natural history of plague: perspectives from more than a century of research. Annu. Rev. Entomol. 50, 505–528.

Goldberg, A.R., Conway, C.J., Biggins, D.E., 2021. Effects of experimental flea removal and plague vaccine treatments on survival of northern Idaho ground squirrels and two coexisting sciurids. Glog. Ecol. Conserv. 26, e01489.

Goldberg, A.R., Eads, D.A., Biggins, D.E., 2022. Plague circulation in small mammals elevates extinction risk for the endangered Peñasco least chipmunk. Glog. Ecol. Conserv. 38, e02244.

Griebel, R.L., 2008. Wall Ranger District, Conata Basin, 2008 Plague Control Report. Nebraska National Forest, Buffalo Gap National Grassland, Wall, South Dakota.

Griebel, R.L., 2009. Wall Ranger District 2009 Plague Management Report. Nebraska National Forest, Buffalo Gap National Grassland, Wall, South Dakota.

Griebel, R.L., 2010. Conata Basin/Badlands Area 2010 Plague Management Report. Nebraska National Forest, Buffalo Gap National Grassland, Wall, South Dakota.

Hanson, D.A., Britten, H.B., Restani, M., Washburn, L.R., 2007. High prevalence of *Yersinia pestis* in black-tailed prairie dog colonies during an apparent enzootic phase of sylvatic plague. Conserv. Genet. 8, 789–795.

Harris, N.C., Livieri, T.M., Dunn, R.R., 2014. Ectoparasites in black-footed ferrets (*Mustela nigripes*) from the largest reintroduced population of the Conata Basin, South Dakota, USA. J. Wildl. Dis. 50, 340–343.

Hasselquist, D., 2007. Comparative immunoecology in birds: hypotheses and tests. J. Ornithol. 148, 571–582.

Hoogland, J.L., 1995. The Black-Tailed Prairie Dog: Social Life of a Burrowing Mammal. University of Chicago Press, Illinois.

Hoogland, J.L., 2013. Prairie dogs disperse when all close kin have disappeared. Science 339, 1205–1207.

Hoogland, J.L., Davis, S., Benson-Amram, S., Labruna, D., Goossens, B., Hoogland, M.A., 2004. Pyraperm kills fleas and halts plague among Utah prairie dogs. Southwestern Nat. 49, 376–383.

Hoogland, J.L., Biggins, D.E., Blackford, N., Eads, D.A., Long, D., Rodriguez, M.R., Ross, L.M., Tobey, S., White, E.M., 2018. Plague in a colony of Gunnison's prairie dogs (*Cynomys gunnisoni*) despite three years of infusions of burrows with 0.05% deltamethrin to kill fleas. J. Wildl. Dis. 54, 347–351.

Jones, P.H., Biggins, D.E., Eads, D.A., Eads, S.L., Britten, H.B., 2012. Deltamethrin flea-control preserves genetic variability of black-tailed prairie dogs during a plague outbreak. Conserv. Genet. 13, 183–195.
Keuler, K.M., Bron, G.M., Griebel, R., Richgels, K.L., 2020. An invasive disease, sylvatic plague, increases fragmentation of black-tailed prairie dog (*Cynomys ludovicianus*) colonies. PLoS One 15, e0235907.
Kosoy, M., Reynolds, P., Bai, Y., Sheff, K., Enscore, R.E., Montenieri, J., Ettestad, P., Gage, K., 2017. Small-scale die-offs in woodrats support long-term maintenance of plague in the US southwest. Vector Borne Zoonotic Dis. 17, 635–644.
Lele, S.R., Keim, J.L., Solymos, P., 2022. Package 'ResourceSelection'. https://cran.r-project.org/web/packages/ResourceSelection/index.html.
Livieri, T.M., Anderson, E.M., 2012. Black-footed ferret home ranges in Conata Basin, South Dakota. West N. Am. Nat. 72, 196–205.
Lowell, J.L., Antolin, M.F., Andersen, G.L., Hu, P., Stokowski, R.P., Gage, K.L., 2015. Single-nucleotide polymorphisms reveal spatial diversity among clones of *Yersinia pestis* during plague outbreaks in Colorado and the western United States. Vector Borne Zoonotic Dis. 15, 291–302.
Lüdecke, D., Aust, F., Crawley, S., Ben-Shachar, M.S., 2022. Package 'ggeffects'. https://cran.r-project.org/web/packages/ggeffects/index.html.
Matchett, M.R., Biggins, D.E., 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, M.R., Stanley, T.R., Mccollister, M.F., Eads, D.A., Boulerice, J.T., Biggins, D.E., 2021. Oral sylvatic plague vaccine does not adequately protect prairie dogs (*Cynomys* spp.) for endangered black-footed ferret (*Mustela nigripes*) conservation. Vector Borne Zoonotic Dis. 21, 921–940.
Matchett, M.R., Eads, D.A., Cordova, J., Livieri, T.M., Hicks, H., Biggins, D.E., 2023. Flea control on prairie dogs (*Cynomys* spp.). J. Wildl. Dis. Dis 59, 71–83.
Mize, E.L., Britten, H.B., 2016. Detections of *Yersinia pestis* east of the known distribution of active plague in the United States. Vector Borne Zoonotic Dis. 16, 88–95.
Nakagawa, S., Cuthill, I.C., 2007. Effect size, confidence interval and statistical significance: a practical guide for biologists. Biol. Rev. 82, 591–605.
Poché, D.M., Hartman, D., Polyakova, L., Poché, R.M., 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.
Powell, B.S., Andrews, G.P., Enama, J.T., Jendrek, S., Bolt, C., Worsham, P., Pullen, J.K., Ribot, W., Hines, H., et al., 2005. Design and testing for a non-tagged F1-V fusion protein as vaccine antigen against bubonic and pneumonic plague. Biotechnol. Prog. 21, 1490–1510.
Rocke, T.E., 2023. Plague and distemper: threats to black-footed ferret conservation. In: Jessup, D.A., Radcliffe, R.W. (Eds.), Wildlife Disease and Health in Conservation. Johns Hopkins University Press, Baltimore, Maryland, pp. 217–235.
Rocke, T.E., Mencher, J., Smith, S.R., Friedlander, A.M., 2004. Recombinant F1-V fusion protein protects black-footed ferrets (*Mustela nigripes*) against virulent *Yersinia pestis* infection. J. Zoo Wildl. Med. 35, 142–146.
Rocke, T.E., Nol, P., Marinari, P.E., Kreeger, J.S., Smith, S.R., Andrews, G.P., Friedlander, A.W., 2006. Vaccination as a potential means to prevent plague in black-footed ferrets. Reston Virginia. In: Roelle, J.E., Miller, B.J., Godbey, J.L., Biggins, D.E. (Eds.), Recovery of the Black-Footed Ferret: Progress and Continuing Challenges. U.S. Geological Survey Scientific Investigations Report 2005–5293, pp. 243–247.
Rocke, T.E., Smith, S.R., Stinchcomb, D.T., Osorio, J.E., 2008. Immunization of black-tailed prairie dog against plague through consumption of vaccine-laden baits. J. Wildl. Dis. 44, 930–937.
Rocke, T.E., Pussini, N., Smith, S.R., Williamson, J., Powell, B., Osorio, J.E., 2010. Consumption of baits containing raccoon pox-based plague vaccines protects black-tailed prairie dogs (*Cynomys ludovicianus*). Vector Borne Zoonotic Dis. 10, 53–58.
Rocke, T.E., Williamson, J., Cobble, K.R., Busch, J.D., Antolin, M.F., Wagner, D.M., 2012. Resistance to plague among black-tailed prairie dog populations. Vector Borne Zoonotic Dis. 12, 111–116.
Rocke, T.E., Tripp, D.W., Russell, R.E., Abbott, R.C., Richgels, K.L., Matchett, M.R., Biggins, D.E., Griebel, R., Schroeder, G., et al., 2017. Sylvatic plague vaccine partially protects prairie dogs (*Cynomys* spp.) in field trials. EcoHealth 14, 438–450.
Rocke, T.E., Tripp, D., Lorenzsonn, F., Falendysz, E., Smith, S., Williamson, J., Abbott, R., 2015. Age at vaccination may influence response to sylvatic plague vaccine (SPV) in Gunnison's prairie dogs (*Cynomys gunnisoni*). EcoHealth 12, 278–287.
Roved, J., Westerdahl, H., Hasselquist, D., 2017. Sex differences in immune responses: hormonal effects, antagonistic selection, and evolutionary consequences. Horm. Behav. 88, 95–105.
Russell, R.E., Abbott, R.C., Tripp, D.W., Rocke, T.E., 2018. Local factors associated with on-host flea distributions on prairie dog colonies. Ecol. Evol. 8, 8951–8972.
Russell, R.E., Tripp, D.W., Rocke, T.E., 2019. Differential plague susceptibility in species and populations of prairie dogs. Ecol. Evol. 9, 11962–11971.
Seery, D.B., Biggins, D.E., Montenieri, J.A., Enscore, R.E., Tanda, D.T., Gage, K.L., 2003. Treatment of black-tailed prairie dog burrows with deltamethrin to control fleas (Insecta: Siphonaptera) and plague. J. Med. Entomol. 40, 718–722.
Shoemaker, K.T., Lacy, R.C., Verant, M.L., Brook, B.W., Livieri, T.M., Miller, P.S., Fordham, D.A., Resit Akçakaya, H., 2014. Effects of prey metapopulation structure on the viability of black-footed ferrets in plague-impacted landscapes: a metamodelling approach. J. Appl. Ecol. 51, 735–745.
Tripp, D.W., Rocke, T.E., Runge, J.P., Abbott, R.C., Miller, M.W., 2017. Burrow dusting or oral vaccination prevents plague-associated prairie dog colony collapse. EcoHealth 14, 451–462.
Tripp, D.W., Emslie, A.C., Sack, D.A., Zieschang, M., 2021. A prototype insecticide applicator and quality control monitoring for plague management on prairie dog colonies. Wildl. Soc. Bull. 45, 176–183.
Tripp, D.W., Sullivan, A.E., Sack, D.A., Emslie, A.C., Drake, M.K., 2022. A low-pressure compressed air insecticide applicator to manage plague on prairie dog colonies using all-terrain vehicles. Wildl. Soc. Bull. 47, e1402.
Zeppelini, C.G., de Almeida, A.M.P., Cordeiro-Estrela, P., 2016. Zoonoses as ecological entities: a case review of plague. PLoS Neglected Trop. Dis. 10, e0004949.
Zuk, M., 1990. Reproductive strategies and disease susceptibility: an evolutionary viewpoint. Parasitol. Today 6, 231–233.