Oral Sylvatic Plague Vaccine Does Not Adequately Protect Prairie Dogs (Cynomys spp.) for Endangered Black-Footed Ferret (Mustela nigripes) Conservation

Marc R. Matchett,1 Thomas R. Stanley,2 Matthew F. McCollister,1,* David A. Eads,2,* Jesse T. Boulerice,3,† and Dean E. Biggins2

Abstract

The plague bacterium Yersinia pestis is lethal to endangered black-footed ferrets (Mustela nigripes, BFF) and the prairie dogs (Cynomys spp., PD) on which they depend for habitat and prey. We assessed the effectiveness of an oral sylvatic plague vaccine delivered in baits to black-tailed PD (Cynomys ludovicianus, BTPD) from 2013 to 2017 on the Charles M. Russell National Wildlife Refuge (CMR) in northcentral Montana. We permanently marked BTPD on four paired vaccine (N = 1,349 individuals) and placebo plots (N = 926; 7,027 total captures). We analyzed capture–recapture data under a Cormack–Jolly–Seber model to estimate annual apparent survival. Overall, survival averaged 0.05 lower on vaccine plots than on paired placebo plots. Immediately before noticeable die-offs and detecting plague on pairs CMR1 and CMR2, 89% of BTPD sampled on vaccine plots had consumed at least one bait and the immune systems of 40% were likely boosted by consuming baits over multiple years. Survival to the following year was 0.16 and 0.05 on the vaccine plots and 0.19 and 0.06 on the placebo plots for pairs CMR1 and CMR2, respectively. These rates were markedly lower than 0.63, the overall average estimate on those same plots during the previous 3 years. PD populations subjected to such large die-offs would not be expected to sustain a BFF population. An overriding limitation to achieving sufficient protection rests with vaccine delivery constraints. Late summer/fall bait distribution results in the highest bait uptake rates. However, the PD birth pulse each spring can double the size of populations in most years, greatly reducing the proportion of vaccinates in populations and diminishing potential herd immunity benefits. In addition to nonvaccinated juveniles and PD that do not consume bait, incomplete vaccine protection and time required for immunity to develop leaves a large majority of PD populations vulnerable to plague for 6–7 months or more each year.

Keywords: vaccine, plague, Yersinia pestis, prairie dogs, black-footed ferrets

Introduction

Plague is a zoonotic disease caused by the primarily flea-borne bacterium Yersinia pestis (Poland and Barnes 1979). In North America, where Y. pestis was introduced ca 1900, the bacterium is the primary biological challenge limiting recovery of endangered black-footed ferrets (Mustela nigripes, BFF) and conservation of prairie dogs (Cynomys spp., PD) on which these specialized predators depend for habitat and prey (Barnes 1993, U.S. Fish and Wildlife Service 2013). Successful vaccination of PD against plague could protect BFF habitat and prey. In laboratory studies, many PD that ate sylvatic plague vaccine (SPV) baits containing recombinant raccoon poxvirus expressing plague antigens were protected from subcutaneous Y. pestis challenge (Rocke et al. 2010, 2014, 2015). If sufficiently

1Charles M. Russell National Wildlife Refuge, U.S. Fish and Wildlife Service, Lewistown, Montana, USA.
2Fort Collins Science Center, U.S. Geological Survey, Fort Collins, Colorado, USA.
3Wyoming Game and Fish Department, Laramie, Wyoming, USA.
4ORCID ID (https://orcid.org/0000-0002-4247-017X).
*Current affiliation: Big Cypress National Preserve, Ochopee, Florida, USA.
†Current affiliation: Wildlife Ecology Institute, Helena, Montana, USA.

© Marc R. Matchett et al. 2021; Published by Mary Ann Liebert, Inc. This Open Access article is distributed under the terms of the Creative Commons Attribution Noncommercial License [CC-BY-NC] (http://creativecommons.org/licenses/by-nc/4.0/) which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and the source are cited.
protective in the field, this vaccine could help mitigate the impacts of plague for the conservation of threatened and endangered species (Abbott et al. 2012). The primary driver behind SPV development has been to mitigate the effects of plague that are limiting BFF recovery (U.S. Fish and Wildlife Service 2013). If effective, SPV could also have conservation benefits for threatened Utah PD (C. parvidens, UPD) and Gunnison’s PD (C. gunnisoni, GPD), a species of concern.

Our assessments of SPV focus on its ability to maintain, and perhaps increase, PD survival and abundance for functional BFF conservation. There are numerous examples of BFF population extirpation when plague, or other stressors (e.g., drought), eliminate substantial portions of PD colony complexes (U.S. Fish and Wildlife Service 2019). Ideally, SPV treatment would protect PD populations from plague and result in PD survival rates sufficient to sustain relatively stable PD populations that could sustain BFF populations. Lacking that success, if SPV reduced mortality and prevented colony extirpation, PD populations might recover more quickly and support BFF populations in the future. However, such apparent partial successes must be interpreted with caution because fragments of PD colonies sometimes persist and undergo postepizootic rebound without plague mitigation treatments (Cully and Williams 2001, Pauli et al. 2006, Augustine et al. 2008, Cully et al. 2010, U.S. Department of Agriculture, U.S. Forest Service 2015).

If effective, SPV might increase PD survival during nonepizootic (enzootic) periods, and perhaps increase abundance during epizootic periods as well, as shown by Biggins et al. (2010) and Biggins et al. (2021b) using flea control. Enzootic plague, as defined by Biggins and Eads (2019), likely circulates frequently in plague endemic areas, including our study area in Montana (Hanson et al. 2007, Matchett et al. 2010), Hanson et al. (2007), Griffin et al. (2010), Matchett et al. (2010), St. Romain et al. (2013), Bron et al. (2019), and Liccioli et al. (2020), all reported detecting Y. pestis in fleas or carcasses from PD colonies that were not currently experiencing an epizootic. Near our study area, BFF that were vaccinated against plague and residing on areas without flea control had 266% higher annual reencounter rates compared with nonvaccinates during a time when black-tailed PD (C. ludovicianus, BTPD) populations appeared healthy and stable (Matchett et al. 2010). BFF rarely leave PD colonies (Biggins et al. 2006) suggesting exposure to plague occurred on colonies despite the absence of noticeable BTPD population declines characteristic of plague epizootics.

Carnivores have often been used for plague surveillance (Barnes 1982, Salkeld and Stapp 2006, Brown et al. 2011, Elbroch et al. 2020, Bevins et al. 2021). Coyotes (Canis latrans, N = 512) in southern Phillips County, Montana (where this study occurred) had positive plague antibody titers every year from 1993 to 2007 except one, 2003, with a sample of only six animals that year (Matchett et al. 2010). Positive antibody titers were detected in juvenile coyotes in all but 3 years (2002–2004), suggesting nearly continuous plague circulation. Many of those samples were collected on BTPD colonies occupied by BFF. Nearly 45,000 blood samples collected from a wide variety of wildlife from 2005 to 2018 found Y. pestis antibodies in 18 wildlife species in 17 Western states (Bevins et al. 2021). These observations are broadly supported by other studies demonstrating that Y. pestis can be maintained in varied ecosystems with low transmission and variable host mortality rates, with occasional epizootics (Lowell et al. 2015, Kosoy et al. 2017, Ramakrishnan 2017, Vogler et al. 2017, Biggins et al. 2021c, Goldberg et al. 2021).

The observation and expectation that Y. pestis frequently circulates at some level in our study system is important for two primary reasons. First, SPV efficacy cannot be evaluated if PD are not challenged with Y. pestis. Second, BFF are highly susceptible to plague and even low exposure to Y. pestis can have substantial impacts on BFF survival (Godbey et al. 2006, Rocke et al. 2006, Matchett et al. 2010). Field trials to evaluate SPV began in 2009 in Colorado (Tripp et al. 2014, 2015) and continued there through 2015 (Tripp et al. 2017). Rocke et al. (2017) reported results from a collaborative study evaluating the effectiveness of SPV using 29 paired vaccine and placebo plots. That study included data from Colorado (Tripp et al. 2017), Wyoming (Boulerice 2017), and this study in Montana from 2013 to 2015. Data presented herein include a portion of the 2013–2015 Montana data that were included in Rocke et al. (2017), plus continued data collection on four pairs of plots in 2016 and 2017. The 2 years of additional data doubled the number of survival intervals available to evaluate, enhanced our ability to estimate capture probabilities and encompassed a time when Y. pestis was detected and observed declines in BTPD abundance were striking.

Materials and Methods

This study began in May 2013 and ended in August 2017. The U.S. Geological Survey (USGS) National Wildlife Health Center (NWHC) completed an Environmental Assessment to satisfy National Environmental Protection Act requirements (https://www.usgs.gov/centers/nwhc/science/sylvatic-plague?qt-science_center_objects=0#qt-science_center_objects). Field use of vaccine was approved by the U.S. Department of Agriculture’s Center for Veterinary Biologics and the Montana Department of Livestock State Veterinarian. Montana Fish, Wildlife and Parks (MFWP) issued Scientific Collector’s permits annually.

Study area, plot establishment, and study design

Our study took place on the Charles M. Russell National Wildlife Refuge (CMR) located in southern Phillips County, Montana, USA (47° 38’ N, 107° 57’ W, Fig. 1). Data were collected on four experimental pairs of study plots (CMR1–CMR4) from 2013 through 2017. One plot from each pair was randomly chosen by the NWHC for SPV treatment and the other member of the pair received placebo baits without vaccine (Roke et al. 2017). As much as possible, we established plots where 100% of the BTPD-occupied area in distinct polygons could be treated and sampled. Short of that, we established plots on easily defined portions of larger areas occupied by BTPD that were of similar sizes within a pair. The outer extent of active BTPD burrows defined distinct polygons describing the areas occupied by BTPD. Each was mapped annually using Trimble Juno 3B GPS equipment (Sunnyvale, CA, 94085) and ArcPad software (ESRI, Redlands, CA).
Monitoring since 1979 indicates plague was the likely cause of multiple, sudden, and large BTPD declines on our study plots in the past. The CMR1 plots were located on opposite ends of what sometimes has been a near-single, contiguous colony situated along a narrow ridge and surrounded by virtually all non-BTPD habitat (Fig. 1 and Table 1) (Reading and Matchett 1997). There were only six BTPD colonies (totaling 98.8 ha; 0.5% of the surrounding habitat) within an 8 km radius of CMR1, two of which comprised the CMR2 pair (47.9 ha). Such isolation is an advantage for evaluating SPV effectiveness. Plague presumably caused the decline of BTPD-occupied area on CMR1 from 33 ha in 2004 to 9 ha in 2007. The separate colonies used for the CMR2 pair were similarly situated on a narrow ridge surrounded by extensive areas of non-BTPD habitat. Similar-sized plots were established on portions of each colony. Like the CMR1 pair, plague presumably caused a substantial decline in occupied area during 2007 from 47 to 1 ha and from 29 to 4 ha on the placebo and vaccine areas, respectively. The plots on the CMR3 pair were placed on portions of remaining BTPD-occupied fragments (33 ha) after a probable plague die-off of what was once a single contiguous colony that totaled 91 ha in 2010. Similarly, plots for the CMR4 pair were located on isolated remnants of what was once a single, contiguous colony that totaled 354 ha in 2006 before an apparent plague die-off from 2007 to 2008 that reduced its size to 33 ha. Plague interacts with many factors that cause PD colony declines (e.g., drought and winter length/severity; Biggins and Eads 2019), but we (and others; Barnes 1993) have not observed factors other than plague to cause BTPD declines like those described in this paragraph.

The NWHC produced and supplied all the vaccine and baits used in this study. Vaccine baits were periodically checked to confirm sufficient vaccine virus titers (Rocke et al. 2017). The cold chain in shipping and transporting baits was never broken during shipment from the NWHC until the morning of bait distribution. Frozen baits were distributed at a rate of 125/ha using a 9 × 9 meters grid pattern. Members of each pair were treated within 3 h of sunrise on the same morning, once annually, from 2013 to 2016 between June 17 and July 31 (Table 2). Field staff were blind to which member of a pair received SPV or placebo baits until 2016.
This study was originally scheduled to end in 2015. Results from the first 3 years indicated lower annual apparent survival (hereafter survival) on vaccine plots compared with placebo plots when accounting for imperfect capture probabilities. However, multiple metrics indicated greater BTPD abundance on vaccine plots compared with placebo plots at the beginning of, and during our study, which we postulated, might lead to underestimation of survival on vaccine plots due to trap competition (i.e., recaptures of previously marked BTPD on vaccine plots might be lower, causing underestimation of survival compared with placebo plots, where the ratio of BTPD to traps was lower because of the differences in BTPD density resulting in potentially not enough trap effort on vaccine plots to adequately recapture previously marked BTPD in comparison to the BTPD to trap ratio on placebo plots). Therefore, a fourth year of SPV treatment and BTPD mark-recapture data were collected during 2016 implementing 4.5 times the trapping effort compared with the previous 3 years. We conducted two trapping sessions in 2016, pre- and post-SPV treatments, and doubled the number of traps and trapping days during the post-treatment trapping session. We included all animals caught during 2016 in our survival analyses regardless of when they were caught during 2016.

BTPD die-offs were obvious and plague was detected during 2016 on three of the eight plots (two placebo, one vaccine). Hence, a fifth year of data collection was completed in 2017 to further evaluate SPV effectiveness, BTPD survival, and remaining population levels 1 year later. All previously marked animals caught in 2017 were recorded, but no additional animals were marked. This constraint precluded using a robust design analysis approach.

We calculated three indices of BTPD abundance. The first was catch per unit effort (CPUE), which was defined as the number of unique animals caught per plot each year divided by the actual number of trap days (atd; Rocke et al. 2017). The variable atd was defined as the product of the number of traps set and the number of days trapped, minus the number of closed traps found empty or with a nontarget species when checked. For the second index we used the “Huggins’ $p$ and $c$” selection in MARK (White and Burnham 1999) to obtain point and error estimates for population abundance on each plot during 3-day trapping efforts while accounting for imperfect capture ($p$) and recapture ($c$) probabilities. The area bounded by a 30-meter buffer around each trap location each year defined area trapped and was used to calculate BTPD density. The third index was trap success computed as the total number of captures divided by total trap days (Tripp et al. 2017).

BTPD are herbivorous, acquiring water and nutrients from plants. Normalized Difference Vegetation Index (NDVI), a measure of the absorbance of visible and near-infrared light by plants, was used to estimate the relative density and greenness of vegetation at each plot at the time of trapping with potential effects of NDVI on BTPD capture probabilities and survival. NDVI values from 16-day Moderate Resolution Imaging Spectroradiometer composites that included the days of trapping for the center point of each plot were extracted for each plot (data available from NASA Earthdata; https://modis.ornl.gov/data.html and https://modis.ornl.gov/cgi-bin/MODIS/global/subset.pl). In much of the BTPD range, plant production correlates positively with precipitation (Biggins et al. 2021a). We calculated

| Pair | Treatment | Area (ha) within 30 meters buffer of all traps (% of BTPD-occupied area trapped) | 2013 | 2014 | 2015 | 2016 | 2017 | Average | Difference |
|------|-----------|---------------------------------------------------------------------------------|------|------|------|------|------|---------|------------|
| CMR1 | Vaccine   | 10.1 (100)                                                                      | 9.5 (90) | 16.0 (100) | 19.4 (100) | 16.5 (100) | 14.3 (98) | 6.0        |
| CMR1 | Placebo   | 9.4 (94)                                                                        | 10.0 (66) | 17.3 (100) | 21.9 (100) | 16.8 (100) | 15.1 (92) | 6.0        |
| CMR2 | Vaccine   | 9.4 (39)                                                                        | 11.6 (75) | 13.7 (61) | 15.6 (75) | 13.7 (88) | 12.8 (67) | 6.0        |
| CMR2 | Placebo   | 7.3 (33)                                                                        | 10.0 (55) | 12.4 (39) | 13.2 (100) | 11.8 (82) | 11.0 (62) | 6.0        |
| CMR3 | Vaccine   | 9.4 (54)                                                                        | 9.8 (40) | 13.1 (28) | 23.7 (43) | 19.4 (35) | 15.1 (40) | -12.0      |
| CMR3 | Placebo   | 10.1 (63)                                                                       | 11.2 (55) | 12.0 (31) | 15.8 (50) | 14.4 (62) | 12.7 (52) | 9.0        |
| CMR4 | Vaccine   | 7.1 (80)                                                                        | 10.2 (78) | 13.5 (84) | 17.4 (100) | 16.3 (100) | 12.9 (89) | 2.0        |
| CMR4 | Placebo   | 9.1 (87)                                                                        | 9.1 (69) | 12.9 (100) | 14.3 (70) | 13.9 (80) | 11.9 (81) | 2.0        |
| Total (average) Vaccine | 36.1 (68) | 41.1 (71) | 56.2 (69) | 76.1 (80) | 65.9 (81) | 55.1 (74) | 2.0        |
| Total (average) Placebo | 36.3 (69) | 40.3 (61) | 54.7 (67) | 65.2 (80) | 56.9 (81) | 50.7 (72) | 2.0        |

*Positive values indicate a higher percentage of the vaccine plot was trapped compared with the placebo plot.

BTPD, black-tailed prairie dogs; CMR, Charles M. Russel National Wildlife Refuge, Montana, USA.
| Pair | Treatment | Bait date | Trap dates | Days since baiting | RB+ (%) | Difference a | Bait date | Trap dates | Days since baiting | RB+ (%) | Difference a | RB+ (%) | Difference a |
|------|-----------|-----------|------------|-------------------|---------|--------------|-----------|------------|-------------------|---------|--------------|---------|--------------|
|      |           |           |            |                   |         |              |           |            |                   |         |              |         |              |
| CMR1 | Vaccine   | 6/18      | 6/17, 6/30–7/3 | 12–15             | 76      | 19           | 6/24      | 7/8–10     | 14–16             | 83      | –3           | 6/24    | –3           |
|      | Placebo   |           |            |                   | 58      |              | 6/25      | 7/12–14    | 17–19             | 79      | 14           | 6/25    | 14           |
| CMR2 | Vaccine   | 7/11      | 7/8, 7/26–28 | 15–17             | 70      | 5            | 6/25      | 7/12–14    | 17–19             | 79      | 14           | 6/25    | 14           |
|      | Placebo   |           |            |                   | 64      |              | 7/8       | 7/22–24    | 14–16             | 71      | –11          | 7/8     | –11          |
| CMR3 | Vaccine   | 7/31      | 8/10–12    | 10–12             | 54      | –28          | 7/8       | 7/22–24    | 14–16             | 71      | –11          | 7/8     | –11          |
|      | Placebo   |           |            |                   | 82      |              | 7/11      | 7/26–28    | 15–17             | 84      | 1            | 7/11    | 1            |
| CMR4 | Vaccine   | 6/27      | 6/26, 7/14–16 | 17–19            | 66      | 6            | 7/11      | 7/26–28    | 15–17             | 84      | 1            | 7/11    | 1            |
|      | Placebo   |           |            |                   | 66      |              | 6/24–7/11 | 7/8–28     | 14–19             | 79      | 0            | 7/19–8/9 | 0            |
| ALL  | Vaccine   | 6/18–7/31 | 6/17–8/12  | 10–19             | 66      | 0            |           |            |                   |         |              |         |              |
|      | Placebo   |           |            |                   | 66      |              |           |            |                   |         |              |         |              |

*Positive values indicate a higher bait uptake rate on the vaccine plot compared with the placebo plot. Pair comparisons in bold were significantly different ($\chi^2$, $p<0.05$), otherwise there were no other significant differences detected. No baits were distributed in 2017.*
annual precipitation (appt) and growing season precipitation (March to July, gppt) from interpolated 4 km PRISM grid cells that intersected each plot for each year (data available from the PRISM Climate Group; http://prism.oregonstate.edu).

Capture and handling of BTPD*

*Correction added on December 15, 2021 after first online publication of November 10, 2021. The subheading has been corrected from: Capture and handling of PD to: Capture and handling of BTPD.

Procedures to catch, mark, and collect samples from BTPD generally followed Biggins et al. (2010), Rocke et al. (2017), and Tripp et al. (2017). Eighty to 160 single-door cage traps (Tru-Catch Traps, Belle Fourche, SD) were placed at active burrows distributed across each plot. Open, nonset traps were prebaited for 3–7 days with rolled corn, oat, barley, and molasses livestock feed. Trap locations were recorded with Garmin GPSMAP 64 Global Positioning Systems (Garmin International, Inc., Olathe, KS). Trapping procedures were simultaneous and identical within each trapping session within a pair. Traps were not moved during a trapping session.

Traps were generally set and baited near dusk, the night before each day, in a trapping session. Traps were checked once each day by mid-morning, closing open traps and transporting captured BTPD to a central processing site located off the plots. BTPD were anesthetized with isoflurane (Clipped Distributing Company, LLC, St. Joseph, MO) using a vaporizer (Anesthesia Equipment Service and Supply, Inc., Sanford, FL) and induction chamber. BTPD were then placed on an isoflurane mask, combed with a fine-toothed comb to remove fleas over a plastic tub (fleas were placed in cryovials on an isoflurane mask, combed with a fine-toothed comb to remove fleas over a plastic tub (fleas were placed in cryovials filled with saline and frozen), had whiskers and hair samples plucked and were weighed, sexed, and aged (based primarily on mass). Each animal was marked with a unique passive radiofrequency tag (GPT-12; Biomark, Inc., Boise, ID) implanted subcutaneously between the shoulder blades, marked with an ear tag in each ear (Monel tag 1005-1; National Band and Tag Co., Newport, KY), had blood drawn through medial saphenous venipuncture (dried and stored on Nobuto strips; Advantec, Cole Parmer, Vernon Hills, IL) and batch marked with a livestock marking crayon (LA-CO Industries, Inc., Elk Grove Village, IL) to facilitate immediate release if caught again later during a trapping session. BTPD were released at their capture location once fully recovered from anesthesia.

Bait uptake

Baits contained the biomarker rhodamine B that can be detected in hair and whiskers for animals that ate bait (Rb+) up to 60 days later, versus those that did not (Rb−). By examining them under a fluorescence microscope (Fernandez and Rocke 2011, Abbott et al. 2018). We collectively sequenced the capture histories, age, and bait uptake data from 2013 to 2016 (N = 1,937) and eliminated the records for all BTPD whose lifetime bait uptake history was not absolutely certain (e.g., an Rb−BTPD first caught as an adult, but not caught in any previous year, could have previously eaten a bait). We censored 85 records (4%) with uncertain bait uptake history leaving a confirmed, known bait uptake history for 1,360 individuals sampled on 1,852 occasions.

BTPD movements

To evaluate if there might have been unequal movement rates for vaccine versus placebo plots, which could affect comparisons of survival estimates on paired plots because our methods cannot distinguish between emigration and mortality, we calculated the linear distance between all capture locations for each BTPD with two or more captures (N = 1,416 BTPD, N = 11,409 distance measurements). Greater movement rates could indicate greater emigration rates that might influence survival estimates, greater predation risk, and suggest greater potential for mixing of vaccinated and nonvaccinated BTPD that could affect herd immunity benefits.

Disease surveillance

From 2013 to 2017, opportunistically finding dead animals in our overall study area, or euthanasia of obviously sick animals, produced 21 BTPD that were submitted to the NWHC for necropsy and Y. pestis and Franciscella tularensis (the causative agent for tularemia) testing using PCR (Rocke et al. 2017). Carcasses from desert cottontails (Sylvilagus audubonii, N = 10), deer mice (Peromyscus maniculatus, N = 9), and a northern grasshopper mouse (Onychomys leucogaster, N = 1) were also submitted and tested. Fleas collected from anesthetized BTPD were counted and identified to species at the NWHC and pooled by species and individual host, up to 10 fleas per pool, for Y. pestis testing (Russell et al. 2018).

Statistical analyses

Heavier PD have greater survival and reproductive output than lighter PD (Hoogland 2001, Facka et al. 2010, Stephens et al. 2018). To evaluate if mass indices might be different on vaccine or placebo plots, and potentially influence survival, we evaluated mass indices with two-tailed t-tests assuming unequal variances. To help evaluate if bait uptake indices might be biased high due to more frequent captures of Rb+ than Rb−animals (as found by Abbott et al. 2018), we compared single versus multiple captures within a trapping session between Rb+ and Rb−BTPD with chi-squared tests of independence. The odds ratio was calculated as Rb−single × Rb+multiple / Rb+single × Rb−multiple. We also tallied how frequently individual BTPD were assumed to be present, but not caught within a trapping session, and compared Rb+ and Rb−BTPD with chi-squared tests of independence. The assumption of presence was based on captures of an individual on any day before, and any day subsequent to, the day(s) without an intervening capture of that individual.

Y. pestis is primarily flea borne, and flea parasitism is a potential predictor of plague risk and mortality (Lorange et al. 2005, Eads et al. 2020). We evaluated differences in flea prevalence [hosts observed with flea(s)/hosts sampled] on vaccine and placebo plots for all years combined, and each year separately, with two-tailed Fisher’s exact tests (Ricezigel et al. 2019). Similarly, we evaluated differences in mean flea abundance (total fleas/hosts sampled) and mean
Our primary objective was to estimate BTPD survival. We analyzed BTPD capture-recapture data under a Cormack-Jolly-Seber (CJS) model (Cormack 1964, Jolly 1965, Seber 1965) to estimate annual apparent survival ($\Phi$), where apparent survival is the product of the probabilities of true survival and study area fidelity (Schaub and Royle 2014). Specifically, we used the “Live Captures (CJS)” selection in MARK (White and Burnham 1999) to construct candidate models and obtain point and error estimates while accounting for imperfect capture probabilities ($p$).

Because the set of models that can be constructed with the covariates we considered was large (Table 3), our approach was to start with the general $\text{group} \times \text{time}$ interaction model (where $\text{group} = \text{plot pair}$) for both $\Phi$ and $p$. We then constructed all subsets of this general model and identified the most parsimonious model from this set using Akaike’s Information Criterion adjusted for small sample size (AICc) (Burnham and Anderson 2002). We used that model as a starting point to identify a parsimonious model for $p$ using covariates. Covariates were integrated into models using a logit link and were used to model $\Phi$ and $p$ associated with the sampling occasions under the CJS model (Lebreton et al. 1992). Finally, conditioning on our most parsimonious model for $p$, we constructed a set of models for $\Phi$ using covariates, then ranked them using model weights ($w$). These model weights are equivalent to Bayesian posterior model probabilities (Burnham and Anderson 2002). Our inferences with respect to $\Phi$ are based on the top-ranked model for plot pairs. We show models with $\Delta \text{AIC} < 5$. We exponentiated the treatment coefficient estimated in our CJS model to calculate the treatment odds ratio.

No fleas were collected in 2017, so to enable use of the fleaprev covariate for modeling, we averaged the plot-specific values over the previous 4 years and set the 2017 values to those averages. When the covariates fleaprev, NDVI, appt, and gppt were used in models to estimate $\Phi$, the values were taken from the year marking the beginning of the interval, but when they were used to estimate $p$, they were taken from the year marking the end of the interval. We coded models this way because we expected flea prevalence and current vegetation conditions (quantity and greenness) to influence $\Phi$ over the course of the interval, whereas we expected them to influence $p$ during captures at the end of the interval.

Another method of assessing SPV efficacy is to compare data for Rb+ and Rb− BTPD within plots. We compared annual reencounter rates (the proportion of marked BTPD that were recaptured the following year) (Tripp et al. 2017) for BTPD relative to annual bait uptake (Rb+/Rb−) within vaccine and placebo plots. If SPV was protective and plague was circulating, BTPD reencounter rates should be higher for Rb+ than Rb− animals, on vaccine plots in particular. In a CJS modeling environment, uptake status cannot change between intervals. Therefore, we used logistic regression where reencounter was the response variable and uptake, mass, pair, and treatment were predictor variables. Abbott et al. (2018) reported that heavier PD had higher bait uptake rates. Generally, heavier PD also have higher survival rates (Facka et al. 2010, Stephens et al. 2018) so we were concerned about multicollinearity issues with uptake and mass evaluations. Indeed, in logistic regression models, uptake and mass were positively correlated in all cases ($p < 0.001$) while accounting for sex and age. We started by analyzing all annual intervals together and considered all possible combinations of uptake, treatment, pair, and two-way and three-way interactions. We did not consider mass in those models because mass and uptake were positively correlated. Instead, we expanded the model set, replacing uptake with mass, to include all possible combinations of mass, treatment, pair, and interactions. This allowed us to compare the relative importance of uptake versus mass while avoiding issues with multicollinearity. We ranked the models by AIC and compared model weights. We also analyzed the data by interval because plague activity increased toward the end of the study.

One of our goals was to compare our results with companion studies of SPV. Such comparisons have been difficult because analyses were done and reported differently. We found annual reencounter rates to be a simple, available, and easily understood common index to survival on which results could be compared in multiple ways.

### Results

#### Plot attributes

Cumulative area occupied by BTPD on all plots generally increased during 2013–2016. We responded by increasing areas treated and trapped (Table 1). Plot size was capped at 24 ha in 2016 to keep logistics manageable.

---

**Table 3. Definitions of Covariates Used to Model Annual Apparent Black-Tailed Prairie Dog Survival ($\Phi$) and/or Capture Probability ($p$) from 2013 Through 2017**

| Variable | Description |
|----------|-------------|
| age      | Age; adult (>1 year old) or juvenile (young of the year) at time of capture |
| appt     | Plot-specific annual precipitation for each year |
| atd      | Actual number of trap days; the number of traps times the number of days operated minus the number of closed traps found empty or with a nontarget species |
| fleaprev | Flea prevalence; the annual proportion of prairie dogs sampled for fleas that observably carried >0 fleas on each plot each year |
| gppt     | Plot-specific growing season (March to July) precipitation for each year |
| sex      | Sex: male or female |
| NDVI     | Plot-specific Normalized Difference Vegetation Index for each year |
| percr    | Current year recaptures as a percentage of the total number of prairie dogs caught during a year on each plot |
| pair     | Experimental pair; paired plots with one plot receiving vaccine baits and one plot receiving placebo baits |
| $t$      | Time; annual interval (2013–2014, 2014–2015, 2015–2016, 2016–2017) |
| $td$     | Total trap days; the number of traps times the number of days operated |
| totpdatd | Total number of prairie dogs captured divided by atd |
| treatment| Treatment; vaccine or placebo |
Several indices indicated BTPD were more abundant on vaccine plots compared with placebo plots at the beginning of this study in 2013 even though treatments were assigned randomly. Huggins' density estimates (N/ha) averaged 63% higher on vaccine plots (10.4) compared with placebo plots (6.4). Even though trap effort was the same, 49% more BTPD were initially marked on vaccine plots (281) than on placebo plots (203) at the start of our study. Juvenile mass was consistently and significantly higher on vaccine plots (x̄ = 548 g, N = 85) than for juveniles on placebo plots (x̄ = 483 g, N = 54, p = 0.002) throughout our study. Females weighed significantly more on vaccine plots (x̄ = 882 g, N = 2,368) compared with placebo plots (x̄ = 856 g, N = 1,758, p = 0.004).

The average mass of juvenile BTPD on vaccine plots (x̄ = 548 g, N = 85) was significantly higher (13%) than for juveniles on placebo plots (x̄ = 483 g, N = 54, p = 0.002) at the start of our study. Juvenile mass was consistently and significantly higher on vaccine plots (x̄ = 548 g, N = 663) than on placebo plots (x̄ = 513 g, N = 534, p ≤ 0.001) throughout our study. Females weighed significantly more on vaccine plots (x̄ = 839 g, N = 1,266) compared with placebo plots (x̄ = 806 g, N = 937, p = 0.001) throughout our study. There was no significant difference in male mass between paired plots. Overall, BTPD weighed significantly more on vaccine plots (x̄ = 882 g, N = 2,368) compared with placebo plots (x̄ = 856 g, N = 1,758, p = 0.004).

### Flea burdens and plague surveillance

Flea prevalence and abundance were both significantly lower on vaccine plots than on placebo plots at the beginning of our study in 2013 (Table 4). There were no other significant differences in flea prevalence, abundance or intensity from 2014 to 2016, or overall. However, prevalence trended toward lower rates on vaccine plots during 2016 and overall.

Twenty-one BTPD carcasses were submitted for testing, 10 of which were from our study plots. Four of those 10 tested positive for *Y. pestis*. The first was found on the CMR2 placebo plot on October 22, 2015. The colony appeared normal and healthy at the time. The other three were found on June 9, 2016 on the CMR1 placebo plot. Two carcasses were found on the CMR1 vaccine plot in 2016 and one tested presumptively positive for tularemia (liver and spleen were PCR positive, but culture negative). One of 10 desert cottontails (not found on study plots; 7 in 2015 and 3 in 2016) tested positive for tularemia in 2015. Tests were all *Y. pestis*-negative for all other mammals tested.

We found *Y. pestis*-positive fleas in 2016 on 12 (0.5%) of the 2,282 total BTPD sampled from 2013 to 2016. In total, 4,618 fleas (48% *Oropsylla hirsuta*, 32% *Pulex* spp., 20% *P. simulans*, and 1% other, including Aetheca wagneri, O. bruneri, O. tuberculata, and O. tuberculata cynomoritis) were collected across all plots from 2013 to 2016. The majority of fleas tested negative (99%) for plague, plague virus, and plague bacteria. One 10 of 10 desert cottontails was PCR positive for *Y. pestis* (liver and spleen were PCR positive, but culture negative).

### Table 4. Number of Black-Tailed Prairie Dogs Sampled, Flea Prevalence [Hosts Observed with Flea(s)/Hosts Sampled], Total Number of Fleas Collected (Fleas), Mean Flea Abundance (Total Fleas/Hosts Sampled), and Mean Flea Intensity (Total Fleas/Hosts with >0 Fleas) from 2013 to 2016 (Fleas Were Not Collected in 2017)

| Pair  | Treatment | No. of prairie dogs sampled | 2013 | 2014 | 2015 | 2016 | Total | 2013 | 2014 | 2015 | 2016 | Average |
|-------|-----------|----------------------------|------|------|------|------|-------|------|------|------|------|---------|
| CMR1  | Vaccine   | 58                         | 53   | 49   | 159  | 311  | 76    | 25   | 53   | 75   | 63   |
| CMR1  | Placebo   | 44                         | 53   | 50   | 108  | 255  | 80    | 15   | 48   | 77   | 59   |
| CMR2  | Vaccine   | 60                         | 58   | 64   | 76   | 258  | 45    | 43   | 38   | 76   | 52   |
| CMR2  | Placebo   | 51                         | 55   | 60   | 38   | 204  | 57    | 71   | 47   | 89   | 64   |
| CMR3  | Vaccine   | 50                         | 68   | 67   | 140  | 325  | 86    | 94   | 93   | 73   | 83   |
| CMR3  | Placebo   | 51                         | 60   | 60   | 122  | 293  | 82    | 75   | 73   | 75   | 76   |
| CMR4  | Vaccine   | 74                         | 55   | 59   | 152  | 340  | 54    | 91   | 71   | 57   | 64   |
| CMR4  | Placebo   | 57                         | 53   | 50   | 128  | 288  | 74    | 94   | 84   | 70   | 78   |
| Combined | Vaccine | 242                        | 234  | 239 | 527  | 1242 | 63    | 65   | 64   | 69   | 66   |
| Combined | Placebo | 203                        | 221  | 220 | 396  | 1040 | 73    | 64   | 63   | 75   | 70   |
| Fisher’s exact test | p value |                           |      |      |      |      |       | 0.033 | 0.922 | 0.771 | 0.055 | 0.087 |

### Table 4. Flea abundance and intensity

| Pair  | Treatment | Fleas | 2013 | 2014 | 2015 | 2016 | Average |
|-------|-----------|-------|------|------|------|------|---------|
| CMR1  | Vaccine   | 510   | 1.9  | 0.3  | 1.6  | 1.9  | 1.6     |
| CMR1  | Placebo   | 402   | 2.4  | 0.5  | 1.2  | 1.9  | 1.6     |
| CMR2  | Vaccine   | 385   | 1.0  | 0.9  | 0.7  | 3.0  | 1.5     |
| CMR2  | Placebo   | 315   | 1.2  | 1.6  | 0.9  | 2.9  | 1.5     |
| CMR3  | Vaccine   | 961   | 3.7  | 4.9  | 3.0  | 1.7  | 3.0     |
| CMR3  | Placebo   | 775   | 3.2  | 3.1  | 3.5  | 1.8  | 2.6     |
| CMR4  | Vaccine   | 579   | 1.0  | 3.4  | 2.3  | 1.2  | 1.7     |
| CMR4  | Placebo   | 691   | 2.5  | 3.7  | 3.5  | 1.4  | 2.4     |
| Combined | Vaccine | 2435  | 1.8  | 2.5  | 1.9  | 1.8  | 2.0     |
| Combined | Placebo | 2183  | 2.3  | 2.2  | 2.3  | 1.8  | 2.1     |
| t-Test | p value   | 0.043 | 0.319 | 0.231 | 0.987 | 0.715 | 0.284 | 0.249 | 0.255 | 0.255 | 0.218 |

Flea prevalence for vaccine and placebo plots was compared using Fisher’s exact test (p values shown for two-tailed tests). Flea intensity and abundance for vaccine and placebo plots was compared using t-test with 1,000 bootstrap samples.
were tested in 2,384 pools. Twenty-three pools (0.96%) containing 81 fleas (60 O. hirsuta, 12 P. simulans, and 9 P. spp.) were Y. pestis positive.

During 2016, Y. pestis-positive fleas were collected from 3 of 108 BTPD sampled on the CMR1 placebo plot (6 pools with 18 fleas) and none of 159 BTPD sampled on the CMR1 vaccine plot. The same year, Y. pestis-positive fleas were collected from 1 of 38 BTPD sampled on the CMR2 placebo plot (2 pools with 3 fleas) and 8 of 76 BTPD sampled on the CMR2 vaccine plot (15 pools with 60 fleas). Seven of those eight BTPD were first caught during the 2016 prebait trapping session (mid-June). Six of those seven animals were caught again, ~25 days later during the postbait trapping session and none of them observably carried any Y. pestis-positive fleas at that time.

### Bait uptake

Annual bait uptake rates averaged nearly 80% and were remarkably similar between vaccine and placebo plots, except for the CMR3 pair in 2013, 2014, and 2016, and on the CMR4 pair during 2016 (Table 2). Bait uptake on the CMR3 vaccine plot was significantly less than on the placebo plot, but was higher on the CMR4 vaccine plot compared with the placebo plot. We found no correlation between bait uptake and any of our BTPD abundance measures.

On average in 2015, after 3 consecutive years of SPV treatments, 99% of adult BTPD sampled on vaccine plots had consumed at least one bait during their lifetime and 43% had consumed baits over multiple years (Table 5). Such apparent boosting should further improve protection from plague (Rocke et al. 2014). On the CMR1 and CMR2 vaccine plots in 2015, 100% of sampled adults had consumed at least one bait and 50% had consumed baits in at least 2 years. Including juveniles that had not yet had an opportunity to consume multiple baits on those same plots, 89% of all sampled BTPD had consumed at least one bait and 40% had consumed baits over multiple years (Table 5). These results suggest near-complete treatment of the population and substantial boosting, presuming bait consumption equates to a protective immune response. This high level of bait consumption and boosting was documented just before detecting Y. pestis and observing substantial BTPD die-offs beginning in 2016.

BTPD that were captured multiple times within a trapping session were more likely to be Rb+ than those captured only once ($\chi^2 = 25.8$, df = 1, $p < 0.001$, odds ratio 1.75, 95% confidence interval [CI] 1.406 to 2.167, N = 1,938). Of the 427 Rb− BTPD, 46% were caught multiple times compared with 60% of 1,511 Rb+ BTPD.

Excluding single captures within a trapping session, 22% of Rb− BTPD (N = 192) were missed during a trapping session and 24% of Rb+ (N = 770) were missed ($\chi^2 = 0.192$, df = 1, $p = 0.661$) for 3-day sessions. For 5-day sessions, 54% of Rb− (N = 148) were missed and 56% of Rb+ (N = 318) were missed ($\chi^2 = 0.076$, df = 1, $p = 0.782$). For 6-day capture sessions, 53% of Rb− (N = 43) were missed and 67% of Rb+ (N = 232) were missed ($\chi^2 = 2.820$, df = 1, $p = 0.093$).

### Annual survival

Our top CJS model included plot×t, treatment, sex, and NDV×t for estimates of $\Phi$ and age, atd×t, and perc for estimates of $p$ while constraining $p$ to be equal over the first...
second, and fourth time intervals, but not the third (Table 6). Parameter estimates, standard errors and CIs are listed in Table 7. Trap effort was the lowest, and similar, for the first 3 years of our study (Table 8), but was increased 4.5 times in 2016 to address a postulated concern that trap competition may have been contributing to underestimated survival rates on vaccine plots because of greater BTPD abundance on vaccine plots compared to placebo plots. Trap effort was somewhat higher (33%) in 2017 than it was during the first 3 years.

The top three CJS models included the treatment variable for estimates of $\Phi$ (Table 6). The coefficient for this variable based on the most parsimonious model was negative ($-0.231$) indicating survival was lower on vaccine plots. The 95% CI did not cover zero ($-0.447$ to $-0.015$) suggesting treatment has explanatory value. Indeed, when we dropped the treatment variable from the top model and reanalyzed the data, we got a $\Delta AIC_c$ value of 2.35 and a model weight of 0.118 (Table 6). Because the model weights are equivalent to Bayesian posterior model probabilities (Burnham and Anderson 2002), and because the weight for the top model was 0.381, we interpret this to mean the top model that included the treatment variable is at least three times more likely to be the better model. The odds ratio for treatment was $<1$ (0.794) and its 95% CI did not cover one (0.640 to 0.985), which can be interpreted as evidence suggesting the vaccine treatment reduced survival.

Plague was not detected on the CMR1 pair from 2013 to 2015 and survival averaged 0.59 and 0.64 on the vaccine and placebo plots, respectively (Table 9 and Fig. 2). In 2016 on the CMR1 pair, when substantial BTPD losses were visually quite noticeable on both plots and $Y.\ pestis$-positive fleas and BTPD carcasses were found on the placebo plot, the survival estimates dropped to 0.16 and 0.19 on the vaccine and placebo plots, respectively. This drop followed 3 consecutive years of SPV treatment where in 2015, 88% of the sampled BTPD had consumed at least one bait and 42% had consumed baits over multiple years. $^*$The noticeable BTPD die-off that began in 2016 on CMR1 was the reason a fifth year of data was collected in 2017. Even with a fourth consecutive year of SPV treatment in 2016, survival estimates on CMR1 declined further in 2017 to 0.07 and 0.09 on vaccine and placebo plots, respectively.

Plague was not detected on the CMR2 pair from 2013 to 2015 and survival averaged 0.62 and 0.67 on the vaccine and placebo plots, respectively. In 2016 on the CMR2 pair, when substantial BTPD losses were also visually quite noticeable and $Y.\ pestis$-positive fleas were found on both plots, the survival estimates plummeted to 0.05 and 0.06 on the vaccine and placebo plots, respectively. This crash followed 3 consecutive years of SPV treatment where in 2015, 89% of the sampled BTPD had consumed at least one bait and 38% consumed baits over multiple years, likely boosting their protection.

**Correction added on December 15, 2021 after first online publication of November 10, 2021:** The sentence has been corrected from: Even with a fourth consecutive year of SPV treatment in 2016, survival estimates on CMR1 was the reason a fifth year of data was collected in 2017. to: The noticeable BTPD die-off that began in 2016 on CMR1 was the reason a fifth year of data was collected in 2017. Even with a fourth consecutive year of SPV treatment in 2016, survival estimates on CMR1 declined further in 2017 to 0.07 and 0.09 on vaccine and placebo plots, respectively.

**Reencounter rates of Rb+ and Rb− BTPD within plots**

If SPV provides protection and plague was circulating, we would have expected our logistic regression analysis to detect a significant interaction between treatment and bait uptake,

![Table 6. Model Selection Results](image)

| Models                                                                 | K   | $AIC_c$  | $\Delta AIC_c$ | w   |
|-----------------------------------------------------------------------|-----|----------|----------------|-----|
| $\Phi (plot \times t + treatment + sex + NDVI \times t)$, $p(1) = p2 = p4 + p3 + age + adt \times t + percr$ | 30  | 3987.38  | 0.00           | 0.381|
| $\Phi (plot \times t + treatment + sex + totpdadt + NDVI \times t)$, $p(1) = p2 = p4 + p3 + age + adt \times t + percr$ | 31  | 3989.06  | 1.69           | 0.164|
| $\Phi (plot \times t + treatment + sex + NDVI \times t)$, $p(1) = p2 = p4 + p3 + age + adt1415 + adt16 + adt17 + percr$ | 29  | 3989.61  | 2.24           | 0.125|
| $\Phi (plot \times t + sex + NDVI \times t)$, $p(1) = p2 = p4 + p3 + age + adt \times t + percr$ | 29  | 3989.73  | 2.35           | 0.118|
| $\Phi (plot \times t + treatment + sex + NDVI)$, $p(1) = p2 = p4 + p3 + age + adt \times t + percr$ | 27  | 3989.79  | 2.41           | 0.114|
| $\Phi (plot \times t + treatment + sex + totpdadt + NDVI \times t)$, $p(1) = p2 = p4 + p3 + age + adt1415 + adt16 + adt17 + percr$ | 30  | 3991.28  | 3.90           | 0.054|
| $\Phi (plot \times t + treatment + sex + NDVI)$, $p(1) = p2 = p4 + p3 + age + adt \times t + percr$ | 28  | 3991.73  | 4.35           | 0.043|

Only models within 5 $AIC_c$ units of the lowest $AIC_c$ model are shown. $\Delta AIC_c$ is the difference between the $AIC_c$ of the lowest $AIC_c$ model and the $AIC_c$ of the model referenced. $AIC_c$ weight ($w$) is the relative weight (equivalent to Bayesian posterior model probabilities) assigned to each model, and $K$ is the number of estimated parameters in the model. The notation $p(1) = p2 = p4$ indicates that capture probabilities were constrained to be equal during occasions 1 (2014), 2 (2015), and 4 (2017), and $p3$ (2016) was estimated separately. Trap effort was most similar during occasions 1, 2, and 4.

$AIC_c$, Akaike Information Criteria, corrected.
### Table 7. Parameter Estimates, Standard Errors, and 95% Confidence Intervals from the Top Model for Estimates of Annual Apparent Survival ($\Phi$) and Capture Probability ($p$) of Black-Tailed Prairie Dogs Monitored from 2013 to 2017

| Parameter | Estimate | SE  | Lower  | Upper  | Notes                   |
|-----------|----------|-----|--------|--------|-------------------------|
| $\Phi$    |          |     |        |        |                         |
| Intercept | -3.952   | 1.722 | -7.327 | -0.578 | CMR4 is reference       |
| $g_1$, CMR1 | -1.428 | 0.297 | -2.010 | -0.845 |                         |
| $g_2$, CMR2 | 0.623  | 0.260 | 0.114  | 1.132  |                         |
| $g_3$, CMR3 | 0.587  | 0.480 | -0.355 | 1.528  |                         |
| treatment  | -0.231  | 0.110 | -0.447 | -0.015 |                         |
| sex        | -0.421  | 0.084 | -0.587 | -0.256 |                         |
| NDVI 2013  | 0.000   | 0.001 | -0.002 | 0.002  |                         |
| NDVI 2014  | -0.001  | 0.001 | -0.002 | 0.001  |                         |
| NDVI 2015  | 0.002   | 0.001 | 0.000  | 0.003  |                         |
| NDVI 2016  | 0.001   | 0.001 | 0.000  | 0.002  |                         |
| $t_1$, 2013–14 | 5.304  | 3.691 | -1.931 | 12.539 | 2016–2017 is reference |
| $t_2$, 2014–15 | 5.884  | 2.466 | 1.050  | 10.718 |                         |
| $t_3$, 2015–16 | 0.285  | 0.562 | -4.686 | 5.255  |                         |
| $g_1 \times t_1$, CMR1 2013–14 | 1.207  | 0.562 | 0.105  | 2.309  | CMR1 2016–2017 is reference |
| $g_1 \times t_2$, CMR1 2014–15 | 1.638  | 0.577 | 0.507  | 2.769  |                         |
| $g_1 \times t_3$, CMR1 2015–16 | -0.338 | 0.526 | -1.369 | 0.693  |                         |
| $g_2 \times t_1$, CMR2 2013–14 | -0.372 | 0.449 | -1.252 | 0.508  | CMR2 2016–2017 is reference |
| $g_2 \times t_2$, CMR2 2014–15 | -0.561 | 0.463 | -1.469 | 0.346  |                         |
| $g_2 \times t_3$, CMR2 2015–16 | -3.711 | 0.479 | -4.649 | -2.772 |                         |
| $g_3 \times t_1$, CMR3 2013–14 | -1.121 | 1.040 | -3.160 | 0.918  | CMR3 2016–2017 is reference |
| $g_3 \times t_2$, CMR3 2014–15 | -1.561 | 0.652 | -2.839 | -0.282 |                         |
| $g_3 \times t_3$, CMR3 2015–16 | 0.053  | 0.743 | -1.403 | 1.509  |                         |
| $p$       |          |     |        |        |                         |
| Intercept | -8.471   | 2.415 | -13.203| -3.738 |                         |
| age       | 1.338    | 0.217 | 0.914  | 1.763  |                         |
| percrr    | 8.547    | 3.041 | 2.586  | 14.507 |                         |
| atd 2014  | 0.025    | 0.010 | 0.005  | 0.045  |                         |
| atd 2015  | 0.029    | 0.011 | 0.007  | 0.051  |                         |
| atd 2016  | -0.012   | 0.008 | -0.028 | 0.003  |                         |
| atd 2017  | 0.027    | 0.010 | 0.008  | 0.046  |                         |
| $t_3$, 2016 | 16.994  | 7.640 | 2.019  | 31.968 | 2014 = 2015 = 2017 is reference |

For categorical covariates we coded the design matrix for the linear predictor using dummy variables, which compare each level of the covariate with an omitted reference level that we specify in the Notes column.

CI, confidence interval.

### Table 8. Number of Trap Days (the Product of the Number of Traps and Number of Days Trapped) in Each Trapping Session Relative to Distributing Sylvatic Plague Vaccine and Placebo Baits from 2013 to 2017 (No Baits Were Distributed in 2017)

| Pair | Treatment | 2013-Pre | 2013-Post | 2014-Post | 2015-Post | 2016-Pre | 2016-Post | 2017-Post |
|------|-----------|----------|-----------|-----------|-----------|----------|-----------|-----------|
| CMR1 | Vaccine   | 31       | 320       | 240       | 240       | 160      | 800       | 320       |
| CMR1 | Placebo   | 31       | 320       | 240       | 240       | 160      | 800       | 320       |
| CMR2 | Vaccine   | 30       | 240       | 240       | 240       | 160      | 800       | 320       |
| CMR2 | Placebo   | 30       | 240       | 240       | 240       | 160      | 800       | 320       |
| CMR3 | Vaccine   | 0        | 240       | 240       | 240       | 240      | 960       | 320       |
| CMR3 | Placebo   | 0        | 240       | 240       | 240       | 240      | 960       | 320       |
| CMR4 | Vaccine   | 30       | 240       | 240       | 240       | 240      | 960       | 320       |
| CMR4 | Placebo   | 30       | 240       | 240       | 240       | 240      | 960       | 320       |
| Total| Vaccine   | 91       | 1040      | 960       | 960       | 800      | 3520      | 1280      |
| Total| Placebo   | 91       | 1040      | 960       | 960       | 800      | 3520      | 1280      |

*No baits were distributed in 2017. 2017-Post simply indicates trapping was completed in July and August, similar to the Post time periods in previous years.*
| Site/treatment | Year interval | 2012–2013 | 2013–2014 | 2014–2015 | 2015–2016 | 2016–2017 | Average | Net Difference | No. of captures | No. of reencounters | Reencounter rate | Net difference |
|----------------|---------------|------------|------------|------------|------------|------------|---------|----------------|----------------|------------------|----------------|---------------|
| Montana        |               |            | 0.57       | 0.60       | 0.16       | 0.07       | 0.35    | -0.04          | 313             | 89               | 0.28           | 0.08          |
|                |               |            | 0.62       | 0.66       | 0.19       | **0.09**   | 0.39    |                | 255             | 53               | 0.21           |              |
|                |               |            | 0.63       | 0.57       | **0.05**   | **0.38**   | 0.42    | -0.04          | 340             | 84               | 0.25           | -0.10         |
|                |               |            | 0.73       | 0.62       | 0.06       | **0.43**   | 0.46    |                | 245             | 85               | 0.35           |              |
|                |               |            | 0.49       | 0.32       | 0.67       | 0.37       | 0.46    | -0.05          | 574             | 142              | 0.25           | -0.10         |
|                |               |            | 0.55       | 0.37       | 0.72       | 0.42       | 0.52    |                | 372             | 128              | 0.34           |              |
|                |               |            | 0.62       | 0.55       | 0.52       | 0.24       | 0.48    | -0.05          | 483             | 154              | 0.32           | -0.11         |
|                |               |            | 0.67       | 0.61       | 0.58       | 0.29       | 0.54    |                | 362             | 154              | 0.43           |              |
| Average, Total-Vx |         | 0.59       | 0.51       | 0.35       | 0.26       | 0.43       | 0.48    | -0.05          | 1710            | 469              | 0.27           | -0.07         |
| Average, Total-Pcb |         | 0.64       | 0.56       | 0.39       | 0.31       | 0.48       |                | 1234            | 420              | 0.34           |              |
| Total          |               |            |            |            |            |            |         |                | 2944            | 889              | 0.30           |              |
| Wyoming        | B-Vx          |            | 0.63       | 0.52       | **0.33**   |          | 0.49    | 0.16           | 582             | 212              | 0.36           | 0.19          |
|                | A-Pcb         |            | 0.59       | 0.37       | **0.04**   |          | 0.33    |                | 488             | 85               | 0.17           |              |
|                | D-Vx          |            | 0.35       | 0.61       | **0.05**   |          | 0.34    | 0.04           | 386             | 73               | 0.19           | 0.03          |
|                | C-Pcb         |            | 0.43       | 0.45       | **0.03**   |          | 0.30    |                | 344             | 55               | 0.16           |              |
| Average, Total-Vx |         | 0.49       | 0.57       | 0.19       | 0.10       | 0.42    | 0.42    | 0.10           | 968             | 285              | 0.29           | 0.13          |
| Average, Total-Pcb |         | 0.51       | 0.41       | 0.04       |            | 0.32    | 0.32    |                | 832             | 140              | 0.17           |              |
| Total          |               |            |            |            |            |            |         |                | 1800            | 425              | 0.24           |              |
| Colorado       | MSR-Vx        | 0.25       | 0.06       | 0.37       |            | 0.22    | 0.12    |            | 203             | 12               | 0.06           | 0.06          |
|                | MSR-Pcb       | 0.32       | 0.00       | 0.00       |            | 0.11    |          |            | 134             | 0                | 0.00           |              |
|                | SNA-Vx        | 0.11       | 0.09       | 0.08       |            | 0.09    | 0.07    |            | 151             | 13               | 0.09           | 0.07          |
|                | SNA-Pcb       | 0.06       | **0.00**   | 0.00       |            | 0.02    |          |            | 137             | 2                | 0.01           |              |
| Average, Total-Vx |         | 0.18       | 0.08       | 0.22       |            | 0.16    | 0.10    |            | 354             | 25               | 0.07           | 0.06          |
| Average, Total-Pcb |         | 0.19       | 0.00       | 0.00       |            | 0.06    |          |            | 271             | 2                | 0.01           |              |
| Total          |               |            |            |            |            |            |         |                | 625             | 27               | 0.04           |              |
| Rocke et al. (2017) | Average, Total-Vx | nr       | nr         | nr         |            | nr      | nr      |            | 3997            | 970              | 0.24           | 0.01          |
|                | Average, Total-Pcb | nr       | nr         | nr         |            | nr      |          |            | 3258            | 768              | 0.24           |              |
| Total          |               |            |            |            |            |            |         |                | 7255            | 1738             | 0.24           |              |

Reencounter rate is defined as the proportion of marked prairie dogs that were recaptured the next year (Tripp et al. 2017). One pair of plots from Tripp et al. (2017) was omitted because plague was active there when that study began. “Net difference” is the net difference in average rate on the vaccine plot subtracted from the paired average placebo plot rate. Bold font indicates plague was detected as defined by Rocke et al. (2017). nr, not reported.
with a positive effect of uptake on vaccine plots (because SPV is providing protection from plague) and no effect of uptake on placebo plots (where there should be no protection from plague). When considering uptake and mass in separate, but comparable models, we failed to detect any substantial interaction between uptake and treatment; that is, any effect of uptake was similar for vaccine and placebo plots. Instead, mass gained considerably more support in models than uptake. The first model that included bait uptake had no AIC weight and was 45 AIC units away from the related mass model.

When analyzed by year interval, we found little consistent support for an uptake \( \times \) treatment interaction, and when we did detect the interaction in the final two intervals, the effect of uptake could not be separated from mass. We do note that in the final interval, there appeared to be a positive effect of uptake on reencounter rates on vaccine plots, yet almost all of the treatment effects (by pair due to a treatment \( \times \) pair interaction) suggested a negative effect of SPV on annual reencounters, consistent with the CJS analysis of survival.

### BTPD movements

BTPD movements were significantly less on vaccine plots than on placebo plots (Table 10). Except for six individual

| Pair   | Treatment | Average (meters) | Difference | N    | p     |
|--------|-----------|-----------------|------------|------|-------|
| CMR1   | Vaccine   | 39.0            | -9.6       | 1041 | 0.0002|
| CMR1   | Placebo   | 48.6            | 7.6        | 463  |       |
| CMR2   | Vaccine   | 35.0            | -18.4      | 795  | <0.0001|
| CMR2   | Placebo   | 53.4            | -18.4      | 874  |       |
| CMR3   | Vaccine   | 30.7            | -7.2       | 1771 | <0.0001|
| CMR3   | Placebo   | 37.9            | -7.2       | 1771 | <0.0001|
| CMR4   | Vaccine   | 32.1            | -3.1       | 2783 | 0.0009|
| CMR4   | Placebo   | 35.2            | 3.1        | 2783 |       |
| Average, total | Vaccine | 34.2          | -9.6       | 6390 | <0.0001|
| Average, total | Placebo | 43.8          | 9.6        | 5019 |       |

\( N \) = number of distance measures. Difference in means evaluated with two-tailed \( t \)-test assuming unequal variances (\( p \)). Difference was calculated as \( \text{Average}_\text{vaccine} - \text{Average}_\text{placebo} \) with negative values representing smaller average movement distances on vaccine plots.
long-range movements (417–7,600 meters) detected between plots over the course of the study, three emigrating from vaccine plots and three from placebo plots, no BTPD were caught at distances greater than 400 meters apart among 2,275 marked BTPD and 6,351 capture events from 2013 to 2017. These distance measures include all between-year movements and all capture locations for BTPD first caught as a juvenile and later caught as an adult. Average distance between captures was <100 meters for 96% and 93% of all distance measures on vaccine and placebo plots, respectively.

Discussion

Pathogen detection

The maxim: “absence of evidence is not evidence of absence” applies to all pathogens. We debated inclusion of a variable for Y. pestis detection in our models and ultimately excluded it. To include such a binary categorization, we felt its meaning and weight needed to be equal in both directions. Detection indicates presence, and presumably challenge to animals, but absence of detection does not mean absence of the pathogen or absence of challenge. The rarity of Y. pestis detections given our nontrivial sampling efforts, along with the rarity of Y. pestis detections from Holmes et al. (2006), Biggs et al. (2010), Matchett et al. (2010), Russell et al. (2018), and Bron et al. (2019), are among the reasons we did not include a plague detection variable in our evaluations (for additional discussion, see Eads and Biggins 2021, Eads et al. 2021).

Our plague detection probability from flea combing was likely low given our modest level of sampling; just ~50 BTPD on each plot annually during a few days in summer only. We found just 10 BTPD carcasses on our study plots over 5 years. Y. pestis was detected in 4 of them, but none of the other 37 carcasses found in the vicinity of our study plots. Many PD succumbing to disease likely die underground and scavengers quickly consume those animals that die on the surface (Boone et al. 2009, McTee et al. 2019), resulting in few carcasses being found by humans, even during plague epizootics with high researcher presence and opportunities to encounter carcasses.

Tularemia is a native, widespread disease in North America and has rarely been detected in BTPD outside of highly unnatural captive settings (Avashia et al. 2004, Petersen et al. 2004). The single BTPD carcass found on the CMR1 vaccine plot that was positive for tularemia was the only detection of tularemia among all of the SPV studies (Boulерice 2017, Rocke et al. 2017, Tripp et al. 2017). “Tularemia is rarely found in prairie dogs. In more than 20 years, CDC personnel have not found tularemia in prairie dogs even though tissues tested for plague were also tested for tularemia” (Barnes 1993:35–36). We know of no examples of tularemia causing large-scale PD losses, even when specifically searched for in association with an epizootic in voles (Microtus spp., Cherry et al. 2019). Serum samples from 42 adult BFF collected from 2004 to 2008 near our study sites tested positive for F. tularensis antibodies during four of those 5 years, suggesting common exposure and during a time when BTPD populations remained at healthy, normal levels. Nonetheless, diagnostic methods are poorly standardized and tularemia may be underdiagnosed (Maurin 2020). A strength of our paired plot study design, and that the paired plots were reasonably close together, is that plague, tularemia, predation, and a host of other mortality factors can be assumed to affect our paired plots similarly, regardless of documenting specific causes of mortality.

The BFF Recovery Plan (U.S. Fish and Wildlife Service 2013) states that plague in PD has been documented at, or within 40 km, of all but a few reintroduction sites. From a management perspective, assumption of Y. pestis presence is essential to effective BFF conservation. Any plague mitigation measure(s) need to maintain healthy and abundant PD populations regardless of plague detection. Numerous BFF reintroduction sites have failed with the collapse of PD colony complexes attributed to plague, most of which did not confirm Y. pestis as the agent that caused the die-offs (U.S. Fish and Wildlife Service 2019). We know varied and challenging weather conditions affect PD populations (Grassel et al. 2016, Stephens et al. 2018), but plague is the only disease known to cause near, or total colony collapses, and the disease poses persistent risks between epizootics.

Plague challenge

Y. pestis was detected on three plots on two of our four pairs where we are confident there was sufficient plague challenge to evaluate SPV effectiveness. It is likely there was some level of plague challenge on the other plots given our premise that plague routinely circulates, often undetected, in an enzootic state. Indeed, there is a long history of multiple, large-scale BTPD die-offs throughout Phillips County, Montana, and specifically on our study plots, consistent with recurring plague epizootics beginning in 1992 (Matchett 2002, Collinge et al. 2005a, 2005b, Augustine et al. 2008).

Widespread BTPD die-offs on many colonies in Phillips County were observed in 2007 (Matchett et al. 2010). Blood samples were collected that year from remaining BTPD on all of the colonies involved in this study. We detected 30 (N=73, 41%) with Y. pestis-positive antibody titers suggesting there were plague survivors residing on pairs CMR1, CMR2, and CMR4 (passive hemagglutination/inhibition testing at the Center for Disease Control and Prevention, Fort Collins, CO). Twelve BTPD on those same three pairs also had Y. pestis-positive antibody titers in 2008. Samples from 108 BTPD residing on 6 nearby colonies that did not experience die-offs were also tested and none were positive for Y. pestis antibodies. This prior knowledge influenced our selection of study sites to maximize the expectation that our study animals would be challenged with Y. pestis and we would be able to realistically evaluate SPV effectiveness.

Survival and capture probabilities

Throughout our study, we consistently observed survival rates on vaccine plots that averaged 5% lower than on placebo plots (Fig. 2). The low BTPD survival rates on vaccine plots (0.16 and 0.05) in this study when plague was detected in the vicinity would likely result in a BTPD population insufficient to sustain a BFF population. Such low survival rates on vaccine plots are especially troubling given 3 consecutive years of SPV treatment before plague detection and where confirmed SPV bait consumption histories indicated 89% of sampled BTPD had consumed at least one bait, and 40% had likely been boosted having consumed bait in multiple years. BFF population persistence would also be unlikely with the low PD survival rates on vaccine plots (Table 9) reported by Tripp et al. (2017) and Boulereice (2017).

Survival rate estimates during 2016–2017 on the CMR2 pair were among the highest observed on all pairs during that
interval and occurred immediately following the lowest survival estimates on any plots during the entire study (Table 9). We interpret that result as reflecting high survival of the very few plague survivors from the previous year, consistent with the numerous BTPD we found with *Y. pestis*-positive antibody titers post-die-offs in 2007. We suspect a similar scenario on the MSR-Vx (vaccine) plot in Colorado with 37% estimated survival during the 2014–2015 interval following a 6% survival estimate during the prior interval (Table 9) (Tripp et al. 2017).

The lack of SPV-induced improvement in BTPD survival is particularly troubling when, by multiple indicators, BTPD survival on our vaccine plots may have been higher to begin with compared with placebo plots. Greater BTPD abundance (advantageous in a social colonial system and an indicator of habitat quality), greater mass (better body condition is associated with higher survival) (Facka et al. 2010, Stephens et al. 2018) and lower flea burdens (lower flea-borne plague transmission) (Eads et al. 2016, Biggins and Eads 2019) at the beginning of our study on vaccine plots may have favored higher survival at the outset compared with placebo plots. Any beneficial SPV effects on survival should be additive to those potentially beneficial starting conditions.

Greater movement distances between capture locations on placebo plots, potentially contributing to higher predation risk (Lima and Dill 1990, Caro 2005), could also indicate greater emigration rates and potentially cause survival to have been underestimated on placebo plots. Despite these stacked potentials for higher comparative survival rates on vaccine plots compared with placebo plots, in addition to very high bait consumption and substantial boosting rates, our top model included a *treatment* effect that was negative with a 95% CI that did not overlap zero and we consistently observed lower survival on vaccine plots than on placebo plots.

The notion of insufficient trap effort contributing to underestimation of survival on vaccine plots was the reason for a fourth year of data collection and increasing our trap effort 4.5 times in 2016 compared with 2013–2015. That extra effort resulted in an increase of estimates of *p* (for all plots combined) from 0.67 and 0.61 in 2014 and 2015, respectively, to 0.91 in 2016. Severe drought in 2017, combined with somewhat increased trap effort (33%) compared with the first 3 years, contributed to an estimate of *p* = 0.97 in 2017. Because of the 2017 drought, forage was extremely limited and BTPD were highly attracted to the trap bait, resulting in very high BTPD “catchability.” In 2017, captures on the CMR3 vaccine plot averaged an amazingly high 97 BTPD/100 trap days and 91 BTPD/100 trap days on the CMR4 placebo plot. The extra trap effort in 2016 and trapping circumstances (drought) in 2017 resulted in estimates of *p* that approached 1.0, producing robust and reliable survival estimates.

While SPV experiments to date have not directly assessed BFF survival, the history of BFF recovery is replete with examples of BFF population extirpation when PD survival is low, for any reason. The small test plots herein, and in Rocke et al. (2017), Tripp et al. (2017), and Boulerice (2017), would not provide sufficient habitat for BFF populations, but we believe PD survival estimates resulting from those trials are translatable to what would be expected for BFF population persistence in the face of high PD mortality. Viable BFF populations are dependent on abundant, and generally stable PD populations; thus, PD survival rates are a sensitive attribute for assessing efficacy of plague management tools (see also Biggins et al. 2021b).

### Annual reencounter rates within plots

Comparison of annual reencounter rates between Rb+ and Rb− BTPD within treatments is an additional and potentially powerful way to evaluate the data, comparing presumably protected (Rb+) with nonprotected, nonvaccinated (Rb−) BTPD living together on the same plots, complementing our paired plot treatment design. We failed to find any consistent support for an *uptake × treatment* interaction, which should have been strong if SPV was providing protection from plague, especially during the latter years of the study. The relatively few Rb− BTPD on both vaccine (17%) and placebo (16%) plots overall, but especially during the last half of the study (Table 5), may have interfered with detection of an interaction due to sparse or unbalanced data. The last two intervals were the only times plague appeared to become more active however, no fleas were collected in 2017 to allow for much of an opportunity to detect plague.

Abbott et al. (2018) reported PD that were recaptured during a trapping session were more likely to be Rb+ than animals caught only once. This could bias bait uptake estimates high with Rb+, “trap happy” PD, being caught at higher rates than Rb− animals. Although statistically significant (likely because of our large sample size), we do not think 46% of all Rb− animals being caught multiple times is meaningfully different than 60% of all Rb+ animals being caught multiple times. Yes, those differences may bias our bait consumption indices somewhat higher than actual, but we maintain the vast majority of BTPD consumed at least one bait, and a substantial number were likely boosted by consuming baits over multiple years (Table 5).

We also looked at capture frequency within a trapping session, relative to Rb+/Rb−, for a “trap happy” effect that might bias bait uptake rate estimates high. The idea here is similar to the way capture histories are used to estimate *p* in capture–recapture survival analyses, looking at the number of occasions an animal was not caught (missed), for example, a capture history of “111” for a 3-day trapping session. The more days an animal was not caught during a single trapping session, the more “trap shy” that animal might be. None of those comparisons were significantly different, suggesting there was no bias in “trapability” between Rb+ and Rb− BTPD that might bias interpretation of bait uptake rates.

### Annual reencounter rate meta-analysis

Annual reencounter rates on our vaccine plots averaged 7% lower than on placebo plots (Table 9). Rocke et al. (2017) reported overall annual reencounter rates during 2013–2015 on vaccine plots that were 1% higher than on placebo plots (Table 9). To us, these effect sizes seem small, as do the 6% and 13% differences in annual reencounter rates observed on paired plots in Tripp et al. (2017) and Boulerice (2017), respectively (Table 9), although we note the differences were positive for a vaccine effect.

In a broader assessment, we combined the published data from Abbott et al. (2017) and Russell et al. (2019a) into a single data file, excluded data from pairs HEUT1, HEUT2, and HEUT4 for consistency with Rocke et al. (2017), and generated annual reencounter rates based on recurring animal ID numbers between years. These data (N = 9,271) are from PD sampled to assess bait uptake and/or flea burdens from 2013 to 2016. We found very small differences in annual
reencounter rates between vaccine and placebo plots while accounting for plague detection in various ways (Table 11). With a focus on comparing Rb+ PD during a year when Y. pestis was not detected, but was then detected the following year fostering the scenario where vaccine effectiveness should shine if protective, there were virtually no differences in reencounter rates between vaccine and placebo plots. All of these effect sizes are small, especially in comparison to the positive effects of flea control on PD populations during plague epizootics (Biggins et al. 2021b). Similar to results herein, Rb+ PD were reencountered at a 5% higher rate on both vaccine and placebo plots compared with Rb− PD. It is interesting the overall reencounter rates were higher on both vaccine and placebo plots, and the rate was 0.018 lower on vaccine plots than on placebo plots when including the CMR data (that accounts for 24% of the total data set), but overall reencounter rates were lower, and there was no difference in reencounter rates between treatments, when the CMR data were excluded (Table 11).

Possible reasons SPV did not improve PD survival or maintain BFF habitat

Insufficient SPV bait uptake by PD, and/or insufficient immune response after consuming bait, could result in low survival rates with plague exposure. It would seem difficult to increase bait uptake much more than described in Table 5. If SPV was protective, the small percentage of BTPD that did not consume vaccine should not have resulted in those very low survival rates, especially with the presumed level of boosting we documented with consumption of baits over multiple years.

Vacines rarely provide perfect immunity (Barnett and Civitello 2020). In the Rocke et al. (2014) study, 57% of BTPD died when challenged 30 days postvaccination after consumption of two SPV baits eaten 1 day apart, a result not statistically different from the placebo group. Forty percent died when challenged 270 days after consumption of two SPV baits eaten 1 day apart, probably the most similar comparison to operational field conditions. The effectiveness of a single SPV dose in laboratory challenges of GPD ranged from 80% in juveniles to 60% of young adults and 0% of adults surviving Y. pestis challenge 6 months postvaccination (Rocke et al. 2015). The substantial PD declines observed during epizootics on SPV-treated plots during this study, along with four others summarized by Biggins et al. (2021b): Table 2 are not unlike the results from laboratory studies, especially when typical PD population age structure and age-specific protection levels, along with bait consumption rates, are considered. *While PD population indices on vaccine plots in those four studies Biggins et al. (2021b) declined 14% less than on placebo plots, the average declines were still substantial (69%). On the two CMR pairs where we detected plague, survival declined from preplague detection an average of 82% on vaccine plots and 80% on placebo plots.

Possible reasons SPV did not improve PD survival or maintain BFF habitat

Insufficient SPV bait uptake by PD, and/or insufficient immune response after consuming bait, could result in low survival rates with plague exposure. It would seem difficult to increase bait uptake much more than described in Table 5. If SPV was protective, the small percentage of BTPD that did not consume vaccine should not have resulted in those very low survival rates, especially with the presumed level of boosting we documented with consumption of baits over multiple years.

Vacines rarely provide perfect immunity (Barnett and Civitello 2020). In the Rocke et al. (2014) study, 57% of BTPD died when challenged 30 days postvaccination after consumption of two SPV baits eaten 1 day apart, a result not statistically different from the placebo group. Forty percent died when challenged 270 days after consumption of two SPV baits eaten 1 day apart, probably the most similar comparison to operational field conditions. The effectiveness of a single SPV dose in laboratory challenges of GPD ranged from 80% in juveniles to 60% of young adults and 0% of adults surviving Y. pestis challenge 6 months postvaccination (Rocke et al. 2015). The substantial PD declines observed during epizootics on SPV-treated plots during this study, along with four others summarized by Biggins et al. (2021b): Table 2 are not unlike the results from laboratory studies, especially when typical PD population age structure and age-specific protection levels, along with bait consumption rates, are considered. *While PD population indices on vaccine plots in those four studies Biggins et al. (2021b) declined 14% less than on placebo plots, the average declines were still substantial (69%). On the two CMR pairs where we detected plague, survival declined from preplague detection an average of 82% on vaccine plots and 80% on placebo plots.

*Correction added on December 15, 2021 after first online publication of November 10, 2021: The sentence has been changed from: While PD population indices on vaccine plots in those four studies Biggins et al. (2021b) 14% less than on placebo plots, the average declines were still substantial (69%). to: While PD population indices on vaccine plots in those four studies Biggins et al. (2021b) declined 14% less than on placebo plots, the average declines were still substantial (69%).

Unequal BTPD dispersal from vaccine plots, perhaps resulting from intraspecific competition at greater BTPD densities on vaccine plots compared with placebo plots, could contribute to underestimating survival on vaccine plots because our methods cannot distinguish between emigration and mortality. Using average distance between all capture locations for

---

**Table 11. Sample Sizes and Annual Reencounter Rates for Prairie Dogs on Vaccine and Placebo Plots When Yersinia pestis Was, and Was Not Detected; When Yersinia pestis Was Not Detected in the Current Year, but Was Detected the Following Year; and Any Yersinia pestis Detection Status Followed by Yersinia pestis Detection the Next Year Based on Data from Abbott et al. (2017) and Russell et al. (2019a) from 2013 to 2016 (Excluding Data from Pairs HEUT1, HEUT2, and HEUT4 for Consistency with Rocke et al. 2017)**

| Plague detection | Vaccine | Placebo | Difference (vaccine − placebo) |
|-------------------|---------|---------|--------------------------------|
|                   | N | Annual reencounter rate | N | Annual reencounter rate | (vaccine − placebo) |
| Yes               | 391 | 0.161 | 378 | 0.138 | 0.024 |
| No                | 2900 | 0.181 | 2624 | 0.205 | −0.024 |
| Overall           | 3291 | 0.179 | 3002 | 0.196 | −0.018 |
| Overall without CMR data | 2481 | 0.138 | 2275 | 0.138 | 0.000 |

**Bait uptake**

- **No Yp detected followed by Yp detected**
  - Rb+ | 396 | 0.098 | 330 | 0.082 | 0.017 |
  - Rb− | 143 | 0.056 | 92 | 0.087 | −0.031 |
  - Difference (Rb+ − Rb−) | 0.043 | −0.005 |
  - Overall | 539 | 0.087 | 422 | 0.083 | 0.004 |

- **Any Yp detection status followed by Yp detected**
  - Rb+ | 635 | 0.131 | 581 | 0.117 | 0.014 |
  - Rb− | 282 | 0.085 | 217 | 0.088 | −0.002 |
  - Difference (Rb+ − Rb−) | 0.046 | 0.029 |
  - Overall | 917 | 0.117 | 798 | 0.109 | 0.008 |

Differences calculated as reencounter ratevacine − reencounter rateplacebo, and reencounter rateRb+ − reencounter rateRb−.

Yp, Yersinia pestis.
each BTPD as an index to movement rates, and potentially unequal emigration rates, we found greater movements on placebo plots compared with vaccine plots. We could not find any evidence that greater BTPD movements on vaccine plots contributed to survival underestimation there; perhaps the opposite, survival underestimation on placebo plots.

An inherent limitation to achieving high protection levels with this vaccine approach is the PD birth pulse each spring that typically doubles the size of the population (Crosby and Graham 1986, Hoogland 1995). This is a vaccine delivery problem; late summer and fall SPV treatments result in better uptake rates and have been encouraged (Tripp et al. 2014, Abbott et al. 2018). Even if SPV was distributed along transects in early summer after PD pup emergence with typically green vegetation, it is likely few juveniles would find baits as it takes maturation time for them to venture very far from natal burrows (Loughry 1992). It probably takes at least 2 months for protective titers to develop after bait consumption, additional time animals are left vulnerable to plague.

When assessed in combination, (1) nonvaccinated juveniles, (2) some percentage of the PD population that does not consume bait, (3) some level of less-than-sufficient immune response, (4) incomplete vaccine protection, and (5) time for immunity to develop, a large percentage of the population remains vulnerable to plague for at least 6–7 months each year. This is a time period in which the most common BTPD flea (O. hirsuta) typically increases in abundance (Wilder et al. 2008), thus increasing plague risk (Lorange et al. 2005, Tripp et al. 2009, Bosio et al. 2020, Eads et al. 2020, Biggins et al. 2021b; although see Brinkerhoff et al. 2010). The low survival rates observed on vaccine plots during active plague in this study, in Boulerice (2017) and in Tripp et al. (2017) should not be surprising given these constraints.

Exposure and plague infection severity in the wild involve a variety of interacting factors that may not be comparable to laboratory challenges. It is doubtful whether plague exposure in the wild would be less challenging than subcutaneous laboratory challenges, and the former may be more intense (Russell et al. 2019b). In addition to flea bites, consumption of tissues from animals that died of plague, inhalation, and multiple exposures over time are additional means of potential infection and disease transmission dynamics in a wild setting (Richgels et al. 2016, Russell et al. 2019b). A single subcutaneous injection in the laboratory, while adequate to cause mortality, may not be comparable to transmission by fleas in the wild. Hinnebusch et al. (2017:216) described: “Life stages in the arthropod [flea] are quite distinct, and it is the transmission stage of Y. pestis that is first encountered by the mammalian immune system in an intradermal microenvironment that cannot be duplicated by injecting cultured bacteria.”

The term “edge effects” has been used to describe two possible contributors to reduced SPV efficacy. On small colonies compared with larger colonies, a larger proportion of animals are living on the “edge” of the occupied area. Animals on small colonies, like our study plots, could possibly be exposed more frequently to external elements like plague. Second, when only a portion of a continuously PD-occupied area is treated, as was the case with two of our vaccine plots, edge effect may refer to nonvaccinated animals living adjacent to vaccine plots. Mixing of treated and nontreated PD along that edge could reduce our measures of treatment effect.

We are confident the potential influence of nontreated animals reducing treatment effects, be they nearby or immigrants from more distant areas, was minimal given the following: (1) the basic coterie social structure and behavioral ecology of colonial BTPD results in little movement, (2) the low rates of successful BTPD immigration reported in the literature, (3) 96% of the movement distances between individual BTPD capture locations on vaccine plots were <100 meters, and (4) our general lack of detecting interplot movements, even with plots fairly close together and with large sample sizes monitored over 5 years (see also Biggins et al. 2021b). This is especially true on the CMR1 and CMR4 pairs given their isolated locations in a large landscape of non-BTPD habitat. The CMR2 pair was also situated in a large landscape of non-BTPD habitat where influence of non-treated BTPD would be minimal, but there could have been some edge effect on the vaccine plot from treating the western portion of the BTPD-occupied area (average 67% treated) (Fig. 1 and Table 1).

Plague manipulation experiments with various treatments have improved survival for eight species of mammals in highly variable ecosystems (Biggins et al. 2010, 2021c, Matchett et al. 2010, Ramakrishnan 2017, Goldberg et al. 2021). Split area, paired plot study designs in such studies, where edge effects were likely substantial, still demonstrated improved survival from plague treatments. Thus, it seems unlikely that edge effects or study design flaws were responsible for our results demonstrating poor SPV performance.

Conclusions

For SPV to be considered a useful BFF conservation tool, treatment should result in substantially greater PD survival than no treatment, especially when considering annual SPV treatment cost about $67/ha ($0.54/bait) (U.S. Department of Agriculture Animal and Plant Health Inspection Service, Wildlife Services 2017). Labor and equipment to distribute the baits are additional costs. When plague was detected on our study plots, BTPD survival was low, often <10%, on paired vaccine and placebo plots alike. Such high BTPD mortality would jeopardize BFF population persistence. Similarly, low PD survival on vaccine plots was observed in Colorado (Tripp et al. 2017) and Wyoming (Boulerice 2017), indicating this result was not unique to our Montana study site. Plague may continue circulating as fleas are not controlled with SPV treatments, leaving BFF susceptible to plague. An overriding limitation to achieving high levels of protection with this vaccination approach rests with vaccine delivery constraints. Late summer/fall vaccine distribution results in the best bait uptake rates (Abbott et al. 2018), but with the PD birth pulse each spring, plus the time required for immunity to develop (and a variety of other factors), a large portion of a PD population is not vaccinated and remains vulnerable to plague for 6–7 months or more each year, including months in which plague flea vectors are typically most abundant on PD.

Acknowledgments

This study was a highly collaborative effort with the U.S. Fish and Wildlife Service (FWS), NWHC, the USGS Fort Collins Science Center (FORT), and the Western Association of Fish and Wildlife Agencies (WAFWA) with support from...
MFWP and World Wildlife Fund (WWF). This study would not have been possible without the many, many dedicated volunteers and seasonal field technicians over a 5-year period that persevered through intense heat, horrendous mosquitos, terrifying thunderstorms, and show-stopping Montana gumbo mud. Staff and logistical support from the CMR were essential ingredients to completing this study. Staff at the NWHC were instrumental in providing supplies, vaccine, baits, and completing all of the laboratory work. Staff at FORT helped with vehicle support, technician application review, and selection and logistics. The authors thank K. Gage (retired Center for Disease Control and Prevention); T. Rocke, D. Walsh, and K. Richgels (NWHC); D. Tripp (Colorado Parks and Wildlife); and two anonymous reviewers for helpful comments that improved the manuscript. While the authors greatly appreciate the valuable contributions from reviewers, by acknowledging them, the authors do not imply they agree with the authors’ interpretation of results. All capture and handling methods used in this study were in accordance with protocols approved by the NWHC Animal Care and Use Committee (Protocol No. EP130214). Data are available in two USGS data releases (Matchett et al. 2021a, 2021b).

Disclaimer

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

Author Disclosure Statement

No competing financial or conflicts of interest exist for any of the authors.

Funding Information

Funding was provided through the FWS, multiple USGS sources, grants from WAFWA, and support from MFWP and WWF.

References

Abbott RC, Osorio JE, Bunck CM, Rocke TE. Sylvatic plague vaccine: a new tool for conservation of threatened and endangered species? EcoHealth 2012; 9:243–250.
Abbott RC, Richgels KLD, Russell RE, Rocke TE. SPV field trials bait uptake data: U.S. Geological Survey data release. 2017. Available at https://doi.org/10.5066/F7X92968
Abbott RC, Russell RE, Richgels KLD, Tripp DW, et al. Factors influencing uptake of sylvatic plague vaccine baits by prairie dogs. EcoHealth 2018; 15:12–22.
Augustine DJ, Matchett MR, Toombs TP, Cully JF, Jr., et al. Spatiotemporal dynamics of black-tailed prairie dog colonies affected by plague. Landscape Ecol 2008; 23:255–267.
Avashia SB, Petersen JM, Lindley CM, Schriefer ME, et al. First reported prairie dog–to-human tularemia transmission, Texas, 2002. Emerg Infect Dis 2004; 10:483–486.
Barnes AM. Surveillance and control of bubonic plague in the United States. Symp Zool Soc London 1982; 50:237–270.
Barnes AM. A review of plague and its relevance to prairie dog populations and the black-footed ferret. In: Oldemeyer JL, Biggins DE, Miller BJ, Crete R, eds. Management of Prairie Dog Complexes for the Reintroduction of the Black-Footed Ferret. Washington, DC: U.S. Fish and Wildlife Service, Biological Report 1993; 13:28–37.
Barnett KM, Civitello DJ. Ecological and evolutionary challenges for wildlife vaccination. Trends Parasitol 2020; 36:970–978.
Bevins SN, Chandler JC, Barrett N, Schmit BS, et al. Plague exposure in mammalian wildlife across the Western United States. Vector Borne Zoonotic Dis 2021; 21:667–674.
Biggins DE, Eads DA. Prairie dogs, persistent plague, flocking fleas, and pernicious positive feedback. Front Vet Sci 2019; 6:1–12.
Biggins DE, Eads DA, Godbey JL. Plague transforms positive effects of precipitation on prairie dogs to negative effects. Int J Parasitol Parasites Wildl 2021a; 14:329–334.
Biggins DE, Godbey JL, Eads DA. Epizootic plague in prairie dogs: correlates and control with deltamethrin. Vector Borne Zoonotic Dis 2021b; 21:172–178.
Biggins DE, Godbey JL, Gage KL, Carter LG, et al. Flea control improves survival of three species of prairie dogs (Cynomys): evidence for enzootic plague? Vector Borne Zoonotic Dis 2010; 10:17–26.
Biggins DE, Godbey JL, Matchett MR, Livieri TM. Habitat preferences and intraspecific competition in black-footed ferrets. In: Roelle JE, Miller BJ, Godbey JL, Biggins DE, eds. Recovery of the Black-Footed Ferret—Progress and Continuing Challenges. U.S. Geological Survey Scientific Investigations Report 2005-5293, Reston, Virginia, USA: U.S. Geological Survey, 2006:129–142.
Biggins DE, Ramakrishnan S, Rocke TE, Williamson JL, et al. Enzootic plague reduces survival of Mexican woodrats (Neotoma mexicana) in Colorado. Ecosphere 2021c; 12:1–15.
Boone A, Kraft, JP, Stapp P. Scavenging by mammalian carnivores on prairie dog colonies: implications for the spread of plague. Vector Borne Zoonotic Dis 2009; 9:185–190.
Bosio CF, Jarrett CO, Scott DP, Fintzi J, et al. Comparison of the transmission efficiency and plague progression dynamics associated with two mechanisms by which fleas transmit Yersinia pestis. PLoS Pathog 2020; 16:e1009092.
Boulerice JT. Evaluation of oral sylvatic plague vaccine in white-tailed prairie dogs (Cynomys leucurus): 2013–2016 comprehensive report. In: Wyoming Game and Fish Department Nongame Program Annual Completion Report, Cheyenne, Wyoming, USA: Wyoming Game and Fish Department, 2017; 307–325.
Brinkerhoff RJ, Collinge SK, Ray C, Gage KL. Rodent and flea abundance fail to predict a plague epizootic in black-tailed prairie dogs. Vector Borne Zoonotic Dis 2010; 10:47–52.
Bron GB, Malavé CM, Boulerice JT, Osorio JE, et al. Plague-positive mouse fleas on mice before plague induced die-offs in black-tailed and white-tailed prairie dogs. Vector Borne Zoonotic Dis 2019; 19:486–493.
Brown HE, Levy CE, Enscore RE, Schriefer ME, et al. Annual seroprevalence of Yersinia pestis in coyotes as predictors of interannual variation in reports of human plague cases in Arizona, United States. Vector Borne Zoonotic Dis 2011; 11:1439–1446.
Burnham KP, Anderson DR. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. New York: Spring Science Business Media, 2002:488.
Caro TM. Antipredator Defenses of Birds and Mammals. Chicago, IL: University of Chicago Press, 2005:591.
Cherry CC, Kwit NA, Ohms RE, Hammesfahr AM, et al. Tularemia (Francisella tularensis) in a black-tailed prairie dog (Cynomys ludovicianus) colony. J Wildl Dis 2019; 55:944–946.

Collinge SK, Johnson WS, Ray C, Matchett R, et al. Testing the generality of a trophic-cascade model for plague. EcoHealth 2005a; 2:1–11.

Collinge SK, Johnson WS, Ray C, Matchett R, et al. Landscape structure and plague occurrence in black-tailed prairie dogs on grasslands of the western USA. Landscape Ecol 2005b; 20:941–955.

Cormack RM. Estimates of survival from the sighting of marked animals. Biometrika 1964; 51:429–438.

Crosby L, Graham R. Population dynamics and expansion rates of black-tailed prairie dogs. Proc 12th Vert Pest Conf 1986; 12:112–115.

Cully JF, Jr., Johnson TL, Collinge SK, Ray C. Disease limits populations: plague and black-tailed prairie dogs. Vector Borne Zoonotic Dis 2010; 1:7–15.

Cully JF, Jr., Williams ES. Interspecific comparisons of sylvatic plague in prairie dogs. J Mammalogy 2001; 82:894–905.

Eads DA, Abbott RC, Biggins DE, Rocke TE. Flea parasitism and host survival in a plague-relevant system: theoretical and conservation implications. J Wildl Dis 2020; 56:378–387.

Eads DA, Biggins DE. Utah prairie dog population dynamics on the Awaapa Plateau: precipitation, elevation, and plague. J Mammalogy 2021; gyab103. Available at https://doi.org/10.1093/jmammal/gyab103.

Eads DA, Biggins DE, Long DH, Gage KL, et al. Droughts may increase susceptibility of prairie dogs to fleas: incongruity with hypothesized mechanisms of plague cycles in rodents. J Mammalogy 2016; 97:1044–1053.

Eads DA, Matchett MR, Poje JE, Biggins DE. Comparison of flea sampling methods and Yersinia pestis detection on prairie dog colonies. Vector Borne Zoonotic Dis 2021; 21:753–761.

Elbroch LM, Vickers TW, Quigley HB. Plague, pumas and potential zoonotic exposure in the Greater Yellowstone Ecosystem. Environ Conserv 2020; 47:75–78.

Facka AN, Roemer GW, Mathis VL, Kam M, et al. Drought leads to collapse of black-tailed prairie dog populations reintroduced to the Chihuahuan Desert. J Wildl Manage 2010; 74:1752–1762.

Fernandez JRR, Rocke TE. The use of rhodamine B as a bio-marker for oral plague vaccination of prairie dogs. J Wildl Dis 2011; 47:765–768.

Godbey JL, Biggins, DE, Gareelle D. Exposure of captive black-footed ferrets to plague and implications for species recovery. In: Roelle JE, Miller BJ, Godbey JL, Biggins DE, eds. Recovery of the Black-Footed Ferret-Progress and Continuing Challenges. U.S. Geological Survey Scientific Investigations Report 2005-5293, Reston, Virginia, USA: U.S. Geological Survey, 2006: 233–237.

Goldberg AR, Conway CJ, Biggins DE. Effects of experimental flea removal and plague vaccine treatments on survival of northern Idaho ground squirrels and two coexisting sciurids. Glob Ecol Conserv 2021; 26:1–16.

Grassel SM, Rachlow JL, Williams CJ. Reproduction by black-tailed prairie dogs and black-footed ferrets: effects of weather and food availability. West N Am Nat 2016; 76:405–416.

Griffin KA, Martin DJ, Rosen LE, Sirochman MQ, et al. Detection of Yersinia pestis DNA in prairie dog–associated fleas by polymerase chain reaction assay of purified DNA. J Wildl Dis 2010; 46:636–643.

Hanson DA, Britten HB, Restani M, Washburn LR. High prevalence of Yersinia pestis in black-tailed prairie dog colonies during an apparent enzootic phase of sylvatic plague. Conserv Genet 2007; 8:789–795.

Hinnebusch BJ, Jarrett, CO, Bland, DM. Adaptations of Yersinia pestis to its insect vector that lead to transmission. Annu Rev Microbiol 2017; 71:215–232.

Holmes BE, Foresman KR, Matchett MR. No evidence of persistent Yersinia pestis infection at prairie dog colonies in North-central Montana. J Wildl Dis 2006; 42:164–169.

Hoogland JL. The Black-Tailed Prairie Dog: Social Life of a Burrowing Mammal. Chicago, IL: University of Chicago Press, 1995:562.

Hoogland JL. Black-tailed, Gunnison’s, and Utah prairie dogs reproduce slowly. J Mammalogy 2001; 82:917–927.

Jolly GM. Explicit estimates from capture-recapture data with both death and immigration-stochastic model. Biometrika 1965; 52:225–247.

Kosoy M, Reynolds P, Bai Y, Sheff K, et al. Small-scale die-offs in woodrats support long-term maintenance of plague in the U.S. Southwest. Vector Borne Zoonotic Dis 2017; 17:635–644.

Lebreton JD, Burnham KP, Clobert J, Anderson DR. Modeling survival and testing biological hypothesis using marked animals: a unified approach with case studies. Ecol Mono 1992; 62:67–118.

Liccioli S, Stephens T, Wilson SC, Mcpherson JM et al. Enzootic maintenance of sylvatic plague in Canada’s threatened black-tailed prairie dog ecosystem. Ecosphere 2020; 11:1–20.

Lima SL, Dill LM. Behavioral decisions made under the risk of predation: a review and prospectus. Can J Zool 1990; 68:619–640.

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

Loughry WJ. Ontogeny of time allocation in black-tailed prairie dogs. Ethology 1992; 90:206–224.

Lowell JL, Antolin MF, Andersen GL, Hu P, et al. 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 2015; 10:291–302.

Matchett MR. Plague management during black-footed ferret reintroductions in Montana. In: Brand CJ, ed. Landscape Ecology of Plague in the American Southwest. U.S. Geological Survey Information and Technology Report 2002-001, Reston, Virginia, USA: U.S. Geological Survey, 2002: 16–17.

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

Matchett MR, McCollister MF, Eads DA. Black-tailed prairie dog capture data from plots treated and not treated with oral plague vaccine from 2013–2017 in Montana. U.S. Geological Survey data release. 2021a. Available at https://doi.org/10.5066/P9JAZVE6

Matchett MR, McCollister, MF, Eads DA. Black-tailed prairie dog movement and bait uptake data from 2013–2017 in Montana. U.S. Geological Survey data release. 2021b. Available at https://doi.org/10.5066/P98EGZY1

Maurin M. Francisella tularensis, tularemia and serological diagnosis. Front Cell Infect Microbiol 2020; 10:1–20.

Mctee M, Hiller, B, Ramsey P. Free lunch, may contain lead: scavenging shoot small mammals. J Wildl Manag 2019; 83:1466–1473.
Pauli JN, Buskirk SW, Williams ES, Edwards WH. A plague epizootic in the black-tailed prairie dog (Cynomys ludovicianus). J Wildl Dis 2006; 42:74–80.
Petersen JM, Schriever ME, Carter LG, Zhou Y, et al. Laboratory analysis of tularemia in wild-trapped, commercially traded prairie dogs, Texas, 2002. Emerg Infect Dis 2004; 10: 419–425.
Poland JD, Barnes AM. Plague. In: Steele JF, ed. CRC Handbook Series in Zoonoses, Section A: Bacterial, Rickettsial, and Mycotic Diseases. Boca Raton, FL: CRC Press, 1979: 515–597.
Ramakrishnan S. Impact of enzootic plague on Neotoma mexicana in northern New Mexico. MS Thesis, New Mexico Highlands University, Las Vegas, NM, 2017:60.
Reading RP, Matchett R. Attributes of black-tailed prairie dog colonies in North-central Montana. J Wildl Manage 1997; 61: 664–673.
Reiczigel J, Marozi M, Fabian I, Rozsa L. Biostatistics for parasitologists—a primer to quantitative parasitology. Trends Parasitol 2019; 35:277–281.
Richgels KLD, Russell RE, Bron GM, Rocke TE. Evaluation of Yersinia pestis transmission pathways for sylvatic plague in prairie dog populations in the Western U.S. EcoHealth 2016; 31:415–427.
Rocke TE, Kingstad-Bakke B, Berlier W, Osorio, JE. A recombinant raccoon poxvirus vaccine expressing both Yersinia pestis F1 and truncated V antigens protects animals against lethal plague. Vaccines 2014; 2:772–784.
Rocke TE, Nol P, Marinari PE, Kreeger JS, et al. Vaccination as a potential means to prevent plague in black-footed ferrets. In: Roelle JE, Miller BJ, Godbey JL, Biggins DE, eds. Recovery of the Black-Footed Ferret-Progress and Continuing Challenges. U.S. Geological Survey Scientific Investigations Report 2005–5293, Reston, Virginia, USA: U.S. Geological Survey, 2006: 243–247.
Rocke TE, Pussini N, Smith SR, Williamson J, et al. Consumption of baits containing raccoon pox-based plague vaccines protects black-tailed prairie dogs (Cynomys ludovicianus). Vector Borne Zoonotic Dis 2010; 10:53–58.
Rocke TE, Tripp D, Lorenzsonn F, Falendysz E, et al. Age at vaccination may influence response to sylvatic plague vaccine (SPV) in Gunnison’s prairie dogs (Cynomys gunnisoni). EcoHealth 2015; 12:278–287.
Rocke TE, Tripp DW, Russell RE, Abbott RC, Tripp DW, Rocke TE. Sylvatic plague vaccine partially protects prairie dogs (Cynomys spp.) in field trials. EcoHealth 2017; 14:438–450.
Russell RE, Abbott RC, Tripp DW, Rocke TE. Local factors associated with on-host distributions on plague dog colonies. Ecol Evol 2018; 8:8951–8972.
Russell RE, Abbott RC, Tripp DW, Rocke TE. Sylvatic plague vaccine field trials flea data (ver. 2.0, July 2019): U.S. Geological Survey data release. 2019a. Available at https://doi.org/10.5066/F7TM79CK
Russell RE, Tripp DW, Rocke TE. Differential plague susceptibility in species and populations of prairie dogs. Ecol Evol 2019b; 00:1–10.
Salkeld DJ, Stapp P. Seroprevalence rates and prevalence of plague (Yersinia pestis) in mammalian carnivores. Vector Borne Zoonotic Dis 2006; 6:231–239.
Schaub M, Royle JA. Estimating true instead of apparent survival using spatial Cormack-Jolly-Seber models. Methods Ecol Evol 2014; 5:1316–1326.
Seber GAF. A note on the multiple recapture census. Biometrika 1965; 52:249–259.
St. Romain K, Tripp DW, Salkeld DJ, Antolin MF. Duration of plague (Yersinia pestis) outbreaks in black-tailed prairie dog (Cynomys ludovicianus) colonies of northern Colorado. EcoHealth 2013; 10:241–245.
Stephens T, Wilson SC, Cassidy F, Bender D, et al. Climate change impacts on the conservation outlook of populations on the poleward periphery of species ranges: a case study of Canadian black-tailed prairie dogs (Cynomys ludovicianus). Glob Change Biol 2018; 24:836–847.
Tripp DW, Gage KL, Montenieri JA, Antolin MF. Flea abundance on black-tailed prairie dogs (Cynomys ludovicianus) increases during plague epizootics. Vector Borne Zoonotic Dis 2009; 9:313–321.
Tripp DW, Rocke TE, Runge JP, Abbott RC, et al. Burrow dusting or oral vaccination prevents plague-associated prairie dog colony collapse. EcoHealth 2017; 14:451–462.
Tripp DW, Rocke TE, Streich SP, Abbott RC, et al. Apparent field safety of a raccoon poxvirus-vectored plague vaccine in free-ranging prairie dogs, Colorado, USA. J Wildl Dis 2015; 51:401–410.
Tripp DW, Rocke TE, Streich SP, Brown NL, et al. Season and application rates affect vaccine bait consumption by prairie dogs. J Wildl Dis 2014; 50:224–234.
U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services. Order Form 17, Pocatello Supply Depot, Pocatello, Idaho, USA, 2017; 1.
U.S. Department of Agriculture, U.S. Forest Service. Black-tailed prairie dog conservation assessment and management strategy for the Thunder Basin National Grassland. Douglas, WY, Steamboat Springs Colorado, USA: Medicine Bow-Routt National Forests, 2015: 74.
U.S. Fish and Wildlife Service. Recovery plan for the black-footed ferret (Mustela nigripes). Denver, CO: U.S. Fish and Wildlife Service, 2013:157.
U.S. Fish and Wildlife Service. Species status assessment report for the black-footed ferret (Mustela nigripes). Denver, CO: U.S. Fish and Wildlife Service, 2019:142. Available at https://ecos.fws.gov/ServCat/DownloadFile/169265?Reference=114857
Vogler AJ, Andrianaivoarimanana V, Telfer S, Hall CM, et al. Temporal phylogeography of Yersinia pestis in Madagascar: insights into the long-term maintenance of plague. PLoS Negl Trop Dis 2017; 11:e0005887.
White GC, Burnham KP. Program MARK: survival estimation from populations of marked animals. Bird Study 1999; 46(Suppl):120–138.
Wilder AP, Eisen RJ, Bearden SW, Montenieri JA, et al. Transmission efficiency of two flea species (Oropsylla tuberculosis cynomuris and Oropsylla hirsuta) involved in plague epizootics among prairie dogs. EcoHealth 2008; 5:205.

Address correspondence to:
Marc R. Matchett
Charles M. Russell National Wildlife Refuge
U.S. Fish and Wildlife Service
333 Airport Road, P.O. Box 110
Lewistown, MT 59457
USA

E-mail: randy_matchett@fws.gov