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APPARENT FIELD SAFETY OF A RACCOON POXVIRUS-VECTORED PLAGUE VACCINE IN FREE-RANGING PRAIRIE DOGS (*CYNOMYS* SPP.), COLORADO, USA

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ABSTRACT: Prairie dogs (*Cynomys* spp.) suffer high rates of mortality from plague. An oral sylvatic plague vaccine using the raccoon poxvirus vector (designated RCN-F1/V307) has been developed for prairie dogs. This vaccine is incorporated into palatable bait along with rhodamine B as a biomarker. We conducted trials in August and September 2012 to demonstrate uptake and apparent safety of the RCN-F1/V307 vaccine in two prairie dog species under field conditions. Free-ranging prairie dogs and other associated small rodents readily consumed vaccine-laden baits during field trials with no apparent adverse effects; most sampled prairie dogs (90%) and associated small rodents (78%) had consumed baits. Visual counts of prairie dogs and their burrows revealed no evidence of prairie dog decline after vaccine exposure. No vaccine-related morbidity, mortality, or gross or microscopic lesions were observed. Poxviruses were not isolated from any animal sampled prior to bait distribution or on sites that received placebo baits. We isolated RCN-F1/V307 from 17 prairie dogs and two deer mice (*Peromyscus maniculatus*) captured on sites where vaccine-laden baits were distributed. Based on these findings, studies examining the utility and effectiveness of oral vaccination to prevent plague-induced mortality in prairie dogs and associated species are underway.

Key words: Black-tailed prairie dog, *Cynomys gunnisoni*, Gunnison's prairie dog, plague, raccoon pox, RCN-F1/V307, rhodamine B, vaccine.

INTRODUCTION

Epizootic and enzootic plague caused by the bacterium *Yersinia pestis* can result in direct mortality of prairie dogs (*Cynomys* spp.) and other species, disrupting the ecology of North American grassland and shrub-steppe ecosystems (Gage and Kosoy 2005; Augustine et al. 2008; Biggins et al. 2010). Moreover, multiple imperiled wildlife species rely on prairie dogs for habitat or prey, including the endangered black-footed ferret (*Mustela nigripes*). The long-term persistence of these species is jeopardized by declines in prairie dog abundance caused by plague (Antolin et al. 2002). Therefore, a sustainable and long-term approach for managing plague at landscape scales remains an important conservation need (Creekmore et al. 2002; Seglund and Schnurr 2010; Abbott et al. 2012).

Consequently, an experimental raccoon poxvirus-vectored sylvatic plague vaccine (SPV) for prairie dogs has been developed (Rocke et al. 2010a, b). Safety and efficacy of this oral vaccine have been demonstrated in laboratory trials (Mencher et al. 2004; Rocke et al. 2008; Rocke et al. 2010a). Palatable bait containing biomarker (rhodamine B) has been developed (Fernandez and Rocke 2011), and field studies have shown that high bait (and presumably vaccine) consumption can be achieved in free-ranging prairie dogs (Tripp et al. 2014). Collectively, these laboratory and field studies have provided the foundation for planning field trials to evaluate the effectiveness of SPV for controlling plague in free-ranging prairie dogs.

Here, we describe a pair of field safety trials conducted in 2012 as the first step in a long-term field evaluation of this exper-

imental SPV. Our specific objectives were to measure bait uptake by two species of prairie dogs and associated small rodents on paired treatment (vaccine) and control (placebo) plots, assess vaccine effects on abundance and rodent species diversity, look for lesions, morbidity, and mortality related to vaccine exposure, and screen prairie dogs and associated small rodents for the presence of poxviruses.

MATERIALS AND METHODS

Field trials were conducted by the Colorado Division of Parks and Wildlife (CPW) in collaboration with the US Geological Survey (USGS) National Wildlife Health Center (NWHC). Study protocols were reviewed and approved by the CPW Animal Care and Use Committee (file 05-2012) and were also approved by US Department of Agriculture's Center for Veterinary Biologics (USDA CVB). An environmental assessment was completed by the USGS (2012) with a finding of no significant impact.

Study areas

We conducted field trials at sites in south-central and north-central Colorado, US. Study sites for Gunnison's prairie dogs (*Cynomys gunnisoni*) located in Gunnison County (38°40.686′N, 106°59.986′W) included a 10.9-ha, vaccine-treated colony on a state wildlife area owned by CPW and a 10.4-ha, placebo-treated control colony on land administered by the US Bureau of Land Management. Black-tailed prairie dog (*Cynomys ludovicianus*) study sites located in northern Larimer County (40°52.184′N, 104°59.405′W) included 8-ha vaccine-treated and control plots within colonies located on land owned by the City of Fort Collins. The paired treatment and control areas at each study site were of similar size and prairie dog density; vaccine-treated sites were signed and closed to public access. Three months prior to the study, burrows on the treatment and control sites were treated with 5 g of powdered 0.05% deltamethrin formulation (DeltaDust®, Bayer Crop Science, Research Triangle Park, North Carolina, USA) to control fleas and suppress potential plague transmission during vaccine safety evaluation (Seery et al. 2003; Biggins et al. 2010).

Vaccine and placebo baits

The vaccine used was a recombinant raccoon poxvirus (RCN-F1/V307) engineered to express two immunologically important *Y. pestis* antigens (Rocke et al. 2014), herein designated SPV. Prior to field use, vaccine lots were tested and approved by the USDA CVB. The vaccine baits (~5 g each) consisted of an edible polymer with peanut butter added as an attractant (FoodSource Lures, Alabaster, Alabama, USA) and rhodamine B (0.25%) incorporated as a biomarker (Fernandez and Rocke 2011; Tripp et al. 2014). Placebo baits were identical to vaccine baits in composition but did not contain SPV. All vaccine and placebo baits were made at NWHC.

Treatment application and bait monitoring

On 5 August 2012 (study day 0), we distributed baits containing one dose of SPV ($\sim 2\times10^7$ plaque-forming units [pfu]) on the Gunnison's prairie dog treatment colony. We distributed vaccine baits ($\sim 4\times10^7$ pfu) on 7 September 2012 (study day 0) on the black-tailed prairie dog treatment plot (Fig. 1). Placebo baits were distributed on respective control areas on study day 0. We delivered all baits by hand along transects spaced 10 m apart at a bait density of 130 baits/ha (Tripp et al. 2014). This equated to a total placement of 1,426 vaccine baits on the Gunnison's prairie dog treatment colony and 1,362 placebo baits on the control colony; black-tailed prairie dog treatment and control plots received 1,060 vaccine or placebo baits, respectively. Bait distribution on all study sites occurred between 0700 and 1200 hours. The location of each dropped bait was recorded via a hand-held GPS unit, and 100 baits on each colony were randomly selected and marked with a labeled flag to estimate bait removal. We rechecked marked baits daily until all had disappeared. We also observed prairie dogs on the colonies on days 1, 2, and 3 after the bait distribution.

Prairie dog and small rodent species monitoring design

To assess vaccine effects on prairie dogs and associated small rodent species, we made visual and burrow counts and physically examined captured individuals before and after bait distribution (Fig. 1). We made visual counts (details in upcoming text) of Gunnison's prairie dogs on days −12 to −9 as an index of baseline prairie dog abundance; baseline counts for black-tailed prairie dog sites were done on days −7 and −3 to −1. We repeated these counts on days 1–3 (Gunnison County) or on days 1–3 and 13 (Larimer County) after bait distribution for comparison to baseline estimates. Data from visual and burrow counts would allow us to detect a

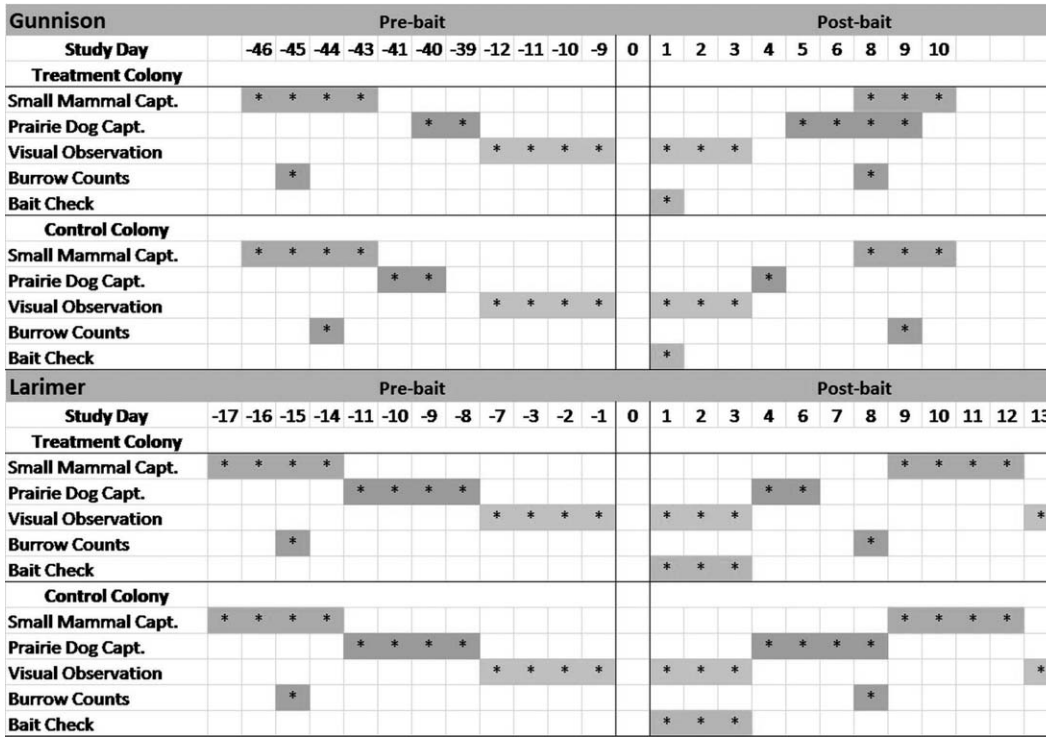

FIGURE 1. Study design and schedule of field trials to assess the safety of a sylvatic plague vaccine in prairie dogs (*Cynomys* spp.) in Gunnison and Larimer counties, Colorado, USA in 2012. Vaccine and placebo baits were distributed on study day 0 at treatment and control colonies of Gunnison's (*Cynomys gunnisoni*; 5 August) and black-tailed prairie dogs (*Cynomys ludovicianus*; 7 September). Prairie dogs and nontarget small mammals were captured, and counts of prairie dogs and their burrows were conducted before and after bait distribution. Randomly selected baits were checked until all baits were removed from the sites.

marked decline (≥25%) in local prairie dog abundance. We assumed that smaller, vaccine-related losses not detected by our counts still might be detected by encountering sick or moribund prairie dogs during physical exams. Prairie dog health and activity were also monitored during the postvaccination counts with specific observations for abnormal behavior, morbidity, and mortality.

Small rodents and prairie dogs were captured on the study areas on four consecutive days prior to the study start to estimate pretreatment diversity and abundance (Fig. 1). All individuals were marked by ear- or PIT-tagging for recapture analysis. For physical examinations, we captured and sampled Gunnison's prairie dogs on days −41 to −39 and again on days 4 to 6, 8, and 9; small rodents were captured on days −46 to −43 and again on days 8 to 10. We captured and sampled black-tailed prairie dogs on days −11 to −8 and again on days 4 to 8; small rodents were captured and sampled on days −17 to −14 and again on days 9 to 12.

We compared postvaccination recapture rates and trap success (total captures/total trap days) to pretreatment and control plot data to determine if recapture rate or trap success decreased after vaccination.

Visual and burrow counts

We counted prairie dogs as well as active and inactive burrows on study plots to estimate prairie dog abundance and activity (Biggins et al. 1993; Severson and Plumb 1998). Visual counts were made on at least three consecutive days from 0700 until 1100 hours. Two observers per plot were stationed on opposite corners of the plot or on elevated areas offering the best vantage point. Plots were divided in half along natural sight lines based on topography, and boundaries were marked using pin flags. The observers counted all prairie dogs above ground every 20 min using 10×50 binoculars or spotting scopes; the maximum count was considered the minimum number known alive. Burrow counts were

conducted within a grid overlaid on the plot (50 m/side) following the methods of Biggins et al. (1993). Individual burrows within each grid cell were counted and recorded as being "active" or "inactive" based on their condition. We used counts of active burrows to estimate prairie dog density within each plot and to assess changes in prairie dog activity levels (Biggins et al. 1993).

Animal capture and handling

Prairie dog and small rodent capture and handling followed methods described elsewhere (Stapp et al. 2008; Tripp et al. 2009, 2014). For prairie dog capture, 100–150 Tomahawk traps (Tomahawk Live Trap Co., Tomahawk, Wisconsin, USA) were placed individually at randomly selected active burrows and were not moved between pre- and posttreatment capture sessions. Prior to trapping, prairie dogs were acclimated by leaving traps wired open and prebaited for 1–2 wk with 2–4 applications of 8% 3-way sweet feed (Manna Pro Corp, St. Louis, Missouri, USA). During active trapping we baited and set traps between 0500 and 0700 hours and checked for captures every 1 to 2 hr. We anesthetized captured prairie dogs using isoflurane (Halocarbon Industries, River Edge, New Jersey, USA) administered in oxygen via precision vaporizers (Seven-Seven Anesthesia Inc., Fort Collins, Colorado, USA). Prior to anesthetization, we observed captured prairie dogs for lethargy, ataxia, tremors, nasal or ocular discharge, and unkempt appearance and then fully examined them while anesthetized for oral lesions, ulcers, and blisters consistent with poxvirus infection. Whiskers and hair with follicles were plucked to determine bait uptake by prairie dogs and small rodents (Fernandez and Rocke 2011; Tripp et al. 2014). In addition, we collected oral swabs stored in viral transport media and immediately frozen on dry ice and stored frozen for poxvirus screening. Prairie dogs were weighed, classified by sex, age (based on body condition and pelage), and reproductive status and then ear-tagged (Monel tag 1005-1; National Band and Tag Co., Newport, Kentucky, USA). After full recovery from anesthesia, we released each individual at the site of its capture.

Sherman live traps (8×9×33 cm; H.B. Sherman Traps, Inc., Tallahassee, Florida, USA) were used to capture small rodents on study plots. Traps were distributed 10 m apart along four, evenly spaced transects of 25 traps each (100 total traps). We trapped at each site on four consecutive nights. Traps were opened and baited with peanut butter and rolled oats at dusk and checked at first light. For handling, we transferred small rodents from the trap to a plastic, zip-top bag; marking and sampling was done using manual restraint or under anesthesia provided via an isoflurane vaporizer. Hair and whiskers with follicles were plucked from each animal to determine bait uptake. Each animal also was examined under natural light for evidence of pox lesions or other illness (as described earlier for prairie dogs) as well as for red or pink staining of the hair associated with rhodamine B. Individuals were ear-tagged, weighed, and classified by sex, age, and reproductive status. Oral swabs were collected for virology as recently described. Once the animal was recovered from anesthesia, we released each individual at the site of capture. Prairie dogs or nontarget small rodents found dead were submitted to a veterinary pathologist for necropsy.

Laboratory analyses

To assess bait uptake, hairs and whiskers from prairie dogs and other sampled species were examined under a fluorescence microscope (excitation wavelength=540 nm; emission wavelength=625 nm; Fernandez and Rocke 2011). Oral swabs from captured animals were sent to USGS NWHC on dry ice for processing and virus isolation. Samples were thawed at 4 C, vortexed briefly, and serial 10-fold dilutions were performed in M199 medium with Earle's salts (Sigma, St. Louis, Missouri, USA). Vero cells at about 75% confluence on 96-well plates were inoculated with 100-µL volumes of both the undiluted sample and the subsequent dilutions. Plates were incubated at 37 C, in 5% CO_2, for 72 hr. After incubation, plates were stained with crystal violet to visualize viral cytopathic effects (CPE). All positive samples were further tested by PCR analysis with primers (Rocke et al. 2014) that amplify part of the RCN genome (G9R-F: 5′-YGG-ACC-RGG-AGG-TCT-TTC-TGC-ATT-3′ and G9R-R: 5′TCT-GGC-CAA-CAT-GAT-TCT-AAT-ACT-GCR-TC-3′) and the F1/V gene insert (V-F: 5′-CGC-TGG-CTT-TAA-AAA-TCT-CTT-3′ and V-R: 5′-ATT-GTG-TAT-TCG-GCG-ATG-AT-3′). DNA extractions were performed on swab suspensions using QIAmp DNA extraction kits (Qiagen, Valencia, California, USA). Real-time PCR was performed using SYBR® Green master mix (Applied Biosystems, Foster City, California, USA) in 50-µL reactions and amplified 40 times with a profile of 95 C for 10 min, 95 C for 15 s, and 60 C for 1 min. The cutoff value was set at 2 SDs above the average of cycles 3–10 (minus blanks).

Data analyses

The R statistical package (Version 2.15.2) was used for all analyses (R Development Core Team 2012). Confidence intervals (CI; 95%) for proportions were calculated using the prop.test function in R. We compared bait uptake and trap success in the treatment and placebo plots, and between pre- and postbaiting sessions, using chi-squared (χ^2) tests with probabilities adjusted for multiple tests by the Dunn-Sidak procedure (Sokal and Rohlf 1995). Statistical significance was set at $P \leq 0.05$.

RESULTS

Bait removal and uptake

We observed prairie dogs eating vaccine baits within 20 min of distribution. All marked baits had been removed from both Gunnison's prairie dog colonies by day 1 and from both black-tailed prairie dog study plots by day 3, suggesting high, rapid uptake (>95% removal, CI 95–100%) and no aversion to the vaccine. Additionally, we observed no unmarked baits remaining at the Gunnison County sites after day 1 or at the black-tailed prairie dog sites after day 3. Biomarker-stained prairie dog feces were observed on all four study sites during surveys conducted 1–3 days after bait distribution, indicating that many prairie dogs had consumed baits.

Visual observations provided no evidence that nontarget species consumed or interacted with baits at any study area, and no biomarker-stained feces from nontarget species were observed. However, deer mice (*Peromyscus maniculatus*) subsequently captured at Gunnison County sites and northern grasshopper mice (*Onychomys leucogaster*) captured at Larimer County sites showed hair, urine, or perineal staining consistent with rhodamine B exposure during the 8–12 days after baiting.

Analyses of plucked hair and whiskers revealed that ≥99% (CI 94–100%) of Gunnison's prairie dogs and 76% (CI 67–84%) of associated nontarget small rodents were marked with rhodamine B (Table 1). Similarly, ≥70% (CI 55–82%) of black-tailed prairie dogs and 91% (CI 70–98%) of associated nontarget small rodents were marked with rhodamine B (Table 2). Rapid bait removal at both vaccine and control sites and high bait exposure suggested most (90%; CI 85–94%) of the prairie dogs and small rodents (78%; CI 71–85%) on these colonies had consumed bait material.

Prairie dog activity and abundance

Visual counts revealed no evidence of prairie dog declines after bait distribution on either study site. In Gunnison County, the maximum count on the treatment colony before and after vaccine distribution was 137 and 152 prairie dogs, respectively, while on the control colony the maximum counts were 122 and 108. In Larimer County, the maximum count on the treatment colony before and after vaccine distribution was 133 and 127 prairie dogs, respectively, while on the control colony the maximum counts were 165 and 170.

Active burrow counts revealed an increase in burrow activity on the Gunnison County sites and a slight decrease on Larimer County sites after bait distribution. In Gunnison County, the burrow count on the treatment colony was 1,223 before vaccine distribution and 1,489 after, while the control colony counts were 1,339 and 1,679 burrows, respectively. In Larimer County, the burrow count on the treatment colony was 695 before vaccine distribution and 640 after, while the control colony counts were 943 and 925 burrows, respectively.

Prairie dog and small rodent capture

For Gunnison's prairie dogs, trap success on the treatment colony before and after bait distribution was 53% and 71% ($\chi^2=13.17$, 1 df, $P=0.0002$), respectively, while on the control colony trap success was 53% and 35%, respectively ($\chi^2=8.57$, 1 df, $P=0.0034$). After baiting, the recapture rate of previously marked individual prairie dogs on the treatment and control colonies was 38% (CI 25–54%) and 22% (CI 12–37%), respectively ($\chi^2=2.09$, 1 df,

TABLE 1. Summary of animal capture, sampling, and vaccine or placebo bait uptake on Gunnison's prairie dog (*Cynomys gunnisoni*) colonies during field safety trials conducted in 2012. Gunnison's prairie dog, deer mouse (*Peromyscus maniculatus*), meadow vole (*Microtus pennsylvanicus*), golden-mantled ground squirrel (gs) (*Spermophilus lateralis*), and least chipmunk (*Tamias minimus*) were captured. ns = not sampled; na = not applicable.

Sample collection by species	Animals captured	Trap-side exams	Oral exams	Oral swab	Virus-positive samples	Hair, whisker samples (positive/total)[a]	Percent uptake (95% confidence interval)
Treatment colony							
Prevaccine							
Prairie dog[b]	150	100	50	0	0	ns	na
Deer mouse[c]	135	4	131	0	0	ns	na
Meadow vole[c]	2	0	2	0	0	ns	na
Postvaccine							
Prairie dog[b]	131	81	50	50	2	49/50	98 (88–100)
Deer mouse[c]	177	0	175	32	2	38/42	90 (76–97)
Meadow vole[c]	2	0	2	1	0	0/1	0
Control colony							
Prebait							
Prairie dog[b]	78	28	50	0	0	ns	na
Golden-mantle gs[b]	1	0	1	0	0	ns	na
Deer mouse[c]	217	7	210	0	0	ns	na
Postbait							
Prairie dog[b]	52	2	50	50	0	50/50	100 (91–100)
Golden-mantle gs[b]	5	0	5	5	0	4/5	80 (30–99)
Least chipmunk[b]	3	0	3	3	0	1/3	33 (2–82)
Deer mouse[c]	256	226	253	30	0	42/59	71 (58–82)
Least chipmunk[c]	8	0	8	5	0	2/4	50 (15–85)

[a] Number of samples positive for rhodamine B staining/total number of samples examined.

[b] Animals captured using a Tomahawk Live Trap during the prairie dog capture sessions.

[c] Animals captured using a Sherman Live Trap during the small rodent capture sessions.

$P=0.1475$). For black-tailed prairie dogs, trap success on the treatment colony before and after bait distribution was 10% and 15% ($\chi^2=4.76$, 1 df, $P=0.029$), respectively, while on the control colony trap success was 8% and 28%, respectively ($\chi^2=59.61$, 1 df, $P<0.0001$). After baiting, the recapture rate of previously marked individual prairie dogs on the treatment and control colonies was 44% (CI 28–66%) and 60% (CI 41–77%), respectively ($\chi^2=1.22$, 1 df, $P=0.2675$).

For small rodent capture on Gunnison's prairie dog plots, trap success on the treatment colony before and after bait distribution was 48% and 63% ($\chi^2=12.414$, 1 df, $P=0.0004$), respectively, while on the control colony trap success was 55% and 94%, respectively ($\chi^2=115.85$, 1 df, $P=<0.0001$). After baiting, the recapture rate for individual deer mice on the treatment and control colonies was 45% (CI 32–58%) and 55% (CI 44–65%), respectively ($\chi^2=1.03$, 1 df, $P=0.309$). For small rodent capture on black-tailed prairie dog plots, trap success on the treatment colony both before and after bait distribution was 2% ($\chi^2=0.0$, 1 df, $P=1.0$), while on the control colony trap success was 9% and 11%, respectively ($\chi^2=0.207$, 1 df, $P=0.6491$). After baiting, the recapture rate for individual grasshopper mice on the treatment and control colonies was 20% (CI 1–70%) and 73% (CI 45–91%), respectively ($\chi^2=2.5$, 1 df, $P=0.1138$).

Morbidity and mortality

No vaccine-related lesions or morbidity were observed at any study site. We fully examined 50 prairie dogs at each of the

TABLE 2. Summary of animal capture, sampling, and vaccine or placebo bait uptake on black-tailed prairie dog (*Cynomys ludovicianus*) colonies during field safety trials conducted in 2012 in Colorado, USA. Black-tailed prairie dogs, thirteen-lined ground squirrels (gs) (*Spermophilus tridecemlineatus*), northern grasshopper mice (*Onychomys leucogaster*), and eastern cotton-tailed rabbits (*Sylvilagus floridanus*) were captured. ns = not sampled; na = not applicable.

Sample collection by species	Animals captured	Trap-side exams	Oral exams	Oral swab	Virus-positive samples	Hair, whisker samples (positive/total)[a]	Percent uptake (95% confidence interval)
Treatment colony							
Prevaccine							
Prairie dog[b]	57	18	39	16	0	ns	na
Thirteen lined gs[b]	1	0	1	0	0	ns	na
Grasshopper mouse[c]	6	0	6	1	0	ns	na
Postvaccine							
Prairie dog[b]	89	39	50	50	15	46/50	92 (80–97)
Thirteen-lined gs[b]	1	0	1	1	0	1/1	100 (5–100)
Cotton tail rabbit[b]	1	0	1	1	0	0/1	0
Grasshopper mouse[c]	5	0	5	5	0	3/4	75 (22–99)
Control colony							
Prebait							
Prairie dog[b]	50	20	30	16	0	ns	na
Grasshopper mouse[c]	34	0	34	9	0	ns	na
Postbait							
Prairie dog[b]	82	32	50	50	0	35/50	70 (55–82)
Grasshopper mouse[c]	40	0	40	17	0	17/17	100 (77–100)

[a] Number of samples positive for rhodamine B staining/total number of samples examined.

[b] Animals captured using a Tomahawk Live Trap during the prairie dog capture sessions.

[c] Animals captured using a Sherman Live Trap during the small rodent capture sessions.

four study sites for signs of a negative response to the vaccine. Additional Gunnison's ($n=81$) and black-tailed prairie dogs ($n=39$) were visually inspected while in traps prior to release. Other small rodents visually inspected at the Gunnison's ($n=177$) and black-tailed prairie dog ($n=7$) treatment sites also showed no signs of adverse effects.

All minor scars or abrasions appeared related either to capture and handling or to natural causes and were encountered at all study sites. All deaths (deer mouse, $n=11$, Gunnison's prairie dog, $n=2$, least chipmunk [*Tamias minimus*], $n=1$) occurred on sites in Gunnison County and all appeared related either to capture or to incidental, natural causes—no vaccine-related gross or microscopic lesions or evidence of bait consumption (bait in stomach contents or external biomarker staining) were observed on necropsy for the few carcasses encountered in the course of field work.

Virus isolation from oral swabs

Viruses were not isolated from any oral swab samples collected from prairie dogs or other rodents prior to bait distribution or on sites that received placebo baits (Gunnison County $n=93$; Larimer County $n=92$). On the Gunnison County vaccine colony, two of 15 prairie dogs sampled on day 6 were positive for viral CPE; other prairie dogs captured on days 5, 8, and 9 were all negative (Table 1). Two of 32 deer mice were also positive and one meadow vole (*Microtus pennsylvanicus*) was negative; these animals were captured on days 8 and 9. At the Larimer County vaccine site, 6 of 21, 5 of 9, and 4 of 13 prairie dogs captured on days 4, 6, and 7, respectively, were positive for viral CPE; seven animals captured on day 8 were all

negative (Table 2). Four grasshopper mice, one thirteen-lined ground squirrel (*Spermophilus tridecemlineatus*), and one cottontail rabbit (*Sylvilagus floridanus*) captured on days 9–13 were negative. PCR confirmed the virus isolated was our vaccine virus and contained the *Y. pestis* insert.

DISCUSSION

Free-ranging prairie dogs and nontarget small rodents readily consumed baits containing SPV with no apparent adverse effects. Our findings are consistent with those from laboratory studies demonstrating the safety of this vaccine for prairie dogs (Rocke et al. 2010a) and other wild rodents (T.E.R. and D.W.T. unpubl. data). In light of the high exposure to vaccine in the prairie dogs (95% CI 88–98%) and other rodent species (86% CI 72–94%) we sampled, the lack of observed adverse effects suggests that this vaccine is safe for targeted field application to prairie dog colonies.

There were no significant differences in the recapture rates of marked prairie dogs between treatment and control plots in either Gunnison ($P=0.1475$) or Larimer County ($P=0.2675$). Additionally, significant differences were not detected in the recapture rates of marked small rodents on treatment and control plots in either Gunnison ($P=0.309$) or Larimer County ($P=0.1138$). The only significant decrease in prairie dog trap success was on the Gunnison County placebo site ($P=0.0034$). At all other sites, including both vaccinated sites, trap success increased significantly after treatment ($P\leq0.029$). The stable recapture rates and increasing trap success on treatment and control colonies are strong evidence for the absence of vaccine-related mortality in these species.

The abundance of prairie dogs observed during the study demonstrated the relative stability of populations at both sites. Minor differences in visual counts (5–11%) were likely because of seasonal fluctuation in weather and predator or human activity and were below the ≥25% reduction we could have detected had there been vaccine- or placebo-related mortality. On both Gunnison's prairie dog colonies, active burrow counts increased during the posttreatment session compared to pretreatment. On both black-tailed prairie dog plots, the active burrow counts decreased slightly posttreatment (2–8%). Some fluctuation in the numbers of active burrows was expected, and we regarded this as consistent with healthy colonies and below the ≥25% reduction detectable had there been treatment-related mortality. Seasonal colony expansion and contraction in response to increased foraging needs during drought conditions likely accounted for the changing number of active burrows.

Vaccine-laden baits appeared to be stable in the field environment and capable of transmitting the vaccine virus to prairie dogs as desired. At the Larimer County treatment site, 30% of prairie dogs captured post bait distribution had detectable virus, with the highest proportion (5/9) on day 6. At the Gunnison County treatment site, only two of 50 animals captured were positive for the vaccine virus; both were among 15 individuals captured on day 6. In a previous laboratory study (Rocke et al. 2014) in which prairie dogs consumed a single, high-titer bait (1×10^8 pfu) and oral swabs were collected daily, oral shedding of the vaccine virus was first detected on day 6 postconsumption in 30% of the animals. Peak shedding occurred on day 13 and, in many animals, shedding was intermittent. Thus, by trapping and sampling on days 4–8 in Larimer County and on days 5–9 in Gunnison County, we may have collected oral swabs prior to the peak of oral virus replication in most animals. Nonetheless, detection of virus in the mouths of prairie dogs provides strong evidence that the vaccine virus replicated and expressed vaccine antigens.

In summary, our field trials revealed no evidence of adverse effects in free-ranging prairie dogs and other small rodents that consumed SPV-laden baits. As seen in other locations (Tripp et al. 2014), bait uptake in prairie dogs was high (81–99%), and no morbidity or mortality was associated with either vaccine or placebo bait consumption in prairie dogs or associated small rodents. Work is underway to evaluate the efficacy of large-scale SPV application to prairie dog colonies for reducing the risk of plague outbreaks and to determine optimal methods and timing for bait distribution of SPV as a prospective plague management tool for prairie dogs and other shrub-steppe and grassland species.

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