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SEASON AND APPLICATION RATES AFFECT VACCINE BAIT CONSUMPTION BY PRAIRIE DOGS IN COLORADO AND UTAH, USA

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ABSTRACT: Plague, a zoonotic disease caused by the bacterium *Yersinia pestis*, causes high rates of mortality in prairie dogs (*Cynomys* spp.). An oral vaccine against plague has been developed for prairie dogs along with a palatable bait to deliver vaccine and a biomarker to track bait consumption. We conducted field trials between September 2009 and September 2012 to develop recommendations for bait distribution to deliver plague vaccine to prairie dogs. The objectives were to evaluate the use of the biomarker, rhodamine B, in field settings to compare bait distribution strategies, to compare uptake of baits distributed at different densities, to assess seasonal effects on bait uptake, and to measure bait uptake by nontarget small mammal species. Rhodamine B effectively marked prairie dogs' whiskers during these field trials. To compare bait distribution strategies, we applied baits around active burrows or along transects at densities of 32, 65, and 130 baits/ha. Distributing baits at active burrows or by transect did not affect uptake by prairie dogs. Distributing baits at rates of ≥65/ha (or ≥1 bait/active burrow) produced optimal uptake, and bait uptake by prairie dogs in the autumn was superior to uptake in the spring. Six other species of small mammals consumed baits during these trials. All four species of tested prairie dogs readily consumed the baits, demonstrating that vaccine uptake will not be an obstacle to plague control via oral vaccination.

Key words: Black-tailed prairie dog, *Cynomys* spp. Gunnison's prairie dog, plague, rhodamine B, Utah prairie dog, vaccine, white-tailed prairie dog.

INTRODUCTION

Plague, a zoonotic disease caused by *Yersinia pestis*, is well-established in wild rodents throughout western North America. Plague causes high rates of mortality in prairie dogs (*Cynomys* spp.) and other species as well as cascading ecologic effects (Gage and Kosoy, 2005; Augustine et al., 2008; Biggins et al., 2010). Plague-related declines in prairie dog abundance jeopardize the long-term persistence of their populations and of multiple wildlife species that depend on prairie dogs for habitat or prey, including the endangered black-footed ferret (*Mustela nigripes*; Antolin et al., 2002). An effective means of protecting prairie dogs from plague would aid in reducing population declines, in recovering black-footed ferrets and imperiled prairie dog species, and in reducing the estimated 14% of human plague cases in the USA attributed to exposure to prairie dogs or their fleas (Seery et al., 2003).

Efforts to contain or control plague epizootics have primarily focused on the reactive use of insecticides to control fleas, the primary plague vector (Seery et al., 2003; Biggins et al., 2010). These efforts are labor intensive and expensive and are often applied too late to prevent large-scale mortality (Griebel, 2012; Jachowski et al., 2012). Consequently, a more proactive, effective, efficient, and sustainable long-term approach for controlling plague has been identified as a conservation need (Seglund and Schnurr, 2010; Abbott et al., 2012).

To this end, a raccoonpox virus-vectored oral vaccine against plague has been developed for prairie dogs (Rocke et al.,

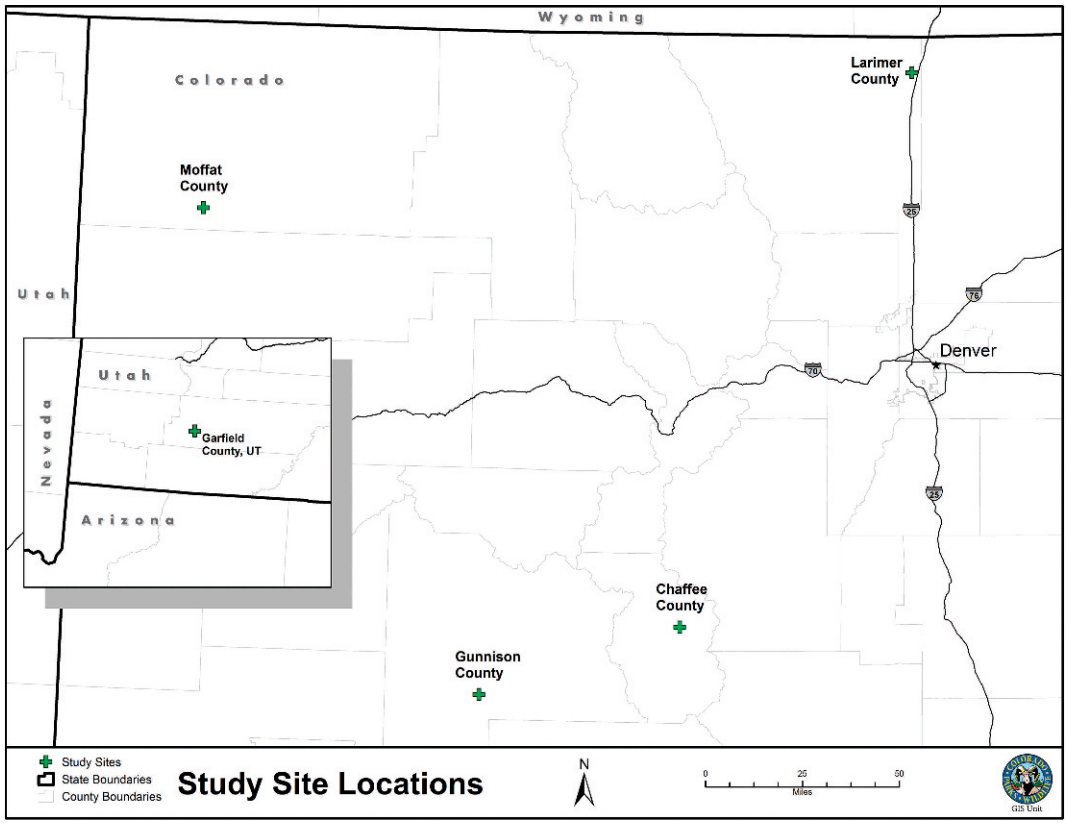

FIGURE 1. Field trials were conducted between September 2009 and September 2012 to measure bait uptake by white-tailed (*Cynomys leucurus*), Gunnison's (*Cynomys gunnisoni*), and black-tailed prairie dogs (*Cynomys ludovicianus*) in Moffat, Gunnison, Chaffee, and Larimer counties, Colorado, and by Utah prairie dogs (*Cynomys parvidens*) in Garfield County, Utah, USA.

2010). Laboratory studies have demonstrated vaccine safety and efficacy (Mencher et al., 2004; Rocke et al., 2008; Rocke et al., 2010). A bait formulation for delivering the vaccine has been identified along with a biomarker for tracking bait consumption (Fernandez and Rocke, 2011). Given these laboratory advances in bait, biomarker, and vaccine development, complementary field trials were undertaken to develop recommendations for efficient and cost-effective bait distribution systems to deliver plague vaccine to prairie dogs.

Here, we describe observations and key recommendations on plague vaccine bait applications from work on four free-ranging prairie dog species. The study objectives were to: evaluate the use of the biomarker to effectively mark prairie dogs in field settings; compare bait distribution strategies; compare uptake of baits distributed at different densities to estimate minimum bait densities needed to assure >50% uptake; assess seasonal effects on bait uptake by prairie dogs; and measure uptake by nontarget small mammals.

MATERIALS AND METHODS

We conducted 22 bait uptake trials in four species of prairie dogs in Colorado and Utah, USA, between September 2009 and September 2012 (Fig. 1). Bait distribution methods, bait densities, and seasonal timing of distribution were examined. An adaptive approach was used between trials to capitalize on the findings and maximize efficiency; therefore, study methods are described chronologically by prairie dog species.

Study areas

Field trials involving white-tailed (*Cynomys leucurus*), Gunnison's (*Cynomys gunnisoni*), and black-tailed prairie dogs (*Cynomys ludovicianus*) were conducted in Colorado (Fig. 1). White-tailed prairie dog study areas were located on land administered by the US Bureau of Land Management (BLM) in southern Moffat and northern Rio Blanco counties. Study areas for Gunnison's prairie dogs included a series of colonies on BLM-administered lands, state-owned land in Gunnison County, and privately owned lands in Chaffee County. Black-tailed prairie dog study areas were located on properties owned by the City of Fort Collins in northern Larimer County. The study area for Utah prairie dogs (*Cynomys parvidens*) was located on US Forest Service land in Garfield County, Utah (Fig. 1).

Placebo and vaccine baits

The bait used in all field trials consisted of an edible polymer containing rhodamine B as a biomarker (Fernandez and Rocke, 2011) and peanut butter as an attractant. Placebo baits (no vaccine included) were used at 20 sites while vaccine-laden baits were used at the remaining two. All baits were prepared by Animal Health Technologies, Abingdon, Virginia, USA or by the US Geological Survey (USGS) National Wildlife Health Center (NWHC) where noted.

Regulatory compliance

All field trials in Colorado were conducted under study protocols reviewed and approved by the Colorado Division of (Parks and) Wildlife, Animal Care and Use Committee (file numbers 05-2009, 05-2010, 06-2010, 03-2011 and 05-2012). The field trial in Utah was conducted under a study protocol reviewed and approved by the USGS NWHC Animal Care and Use Committee (EP090803) with a permit issued by the US Fish and Wildlife Service (TE-047266). Experimental use of vaccine during field safety trials was granted by the US Department of Agriculture, Center for Veterinary Biologics.

Animal capture and handing

Prairie dog and nontarget small mammal capture and handling followed methods outlined by Tripp et al. (2009) and Stapp et al. (2008). Briefly, prairie dogs were captured with live traps placed at active burrows and anesthetized with isoflurane in oxygen via precision vaporizers. Nontarget small mammals were captured with live traps arranged in four transects (25 traps spaced 10 m apart) and either manually restrained or anesthetized with isoflurane vaporizers as above. About 20 guard hairs and three whiskers with follicles were plucked to determine rates of bait uptake. In early trials each animal was inspected for staining associated with rhodamine B under natural light or under a portable ultraviolet (UV) light in a darkened area, and feces were collected to assess bait exposure. All individuals were weighed, classified by sex and age, and examined for signs of adverse effects to bait or biomarker such as lethargy, ataxia, intoxication, and digestive upset. We released recovered animals at the site of original capture.

Field trials in white-tailed prairie dogs

Exploratory field trials were conducted in September 2009 on white-tailed prairie dog colonies to compare two bait-distribution techniques following Creekmore et al. (2002). The two treatment plots were 6.9 ha and 7.7 ha in area with similar habitat and prairie dog density. We counted the number of active prairie dog burrows (Biggins et al., 1993) within each plot and then distributed baits (8.5 g) containing 0.35% rhodamine B at a rate of four baits per active burrow in each plot as described below.

On day 0 in plot 1, baits were distributed around active burrows similar to the method used by Creekmore et al. (2002; three baits placed in a circle 1 m from the burrow entrance and a fourth placed just inside the entrance). In plot 2, baits were distributed at a rate of one bait every 7.5 m in transects spaced every 10 m over the plot; we calculated this rate by summing the length of all transects and dividing by the total number of baits for plot 1 (four/burrow). On days 3 and 4, prairie dogs on both plots were captured and sampled. Plot 2 was sampled again on day 27; any recaptured individuals were sampled again.

In May 2010 an additional trial in plot 3 was conducted to assess seasonal differences in bait uptake. Plot 3 was 8 ha in area and we distributed baits along transects at a rate equivalent to 2.3 baits per active burrow. We evaluated bait consumption by both prairie dogs and nontarget species.

Field trials in Gunnison's and black-tailed prairie dogs

Field trials were conducted between June 2010 and October 2011 on Gunnison's and black-tailed prairie dog colonies to assess effects of bait density and season on bait uptake as well as consumption by nontarget

small mammal species. Baits (5 g) containing 0.25% rhodamine B were distributed in transects as above. Minor changes in bait size and formulation from the white-tailed prairie dog field trials were based on additional laboratory data (Fernandez and Rocke, 2011). We compared "high" (130 baits/ha, the mean bait density used in the initial trials), "medium" (65 baits/ha), and "low" (32 baits/ha) bait-density treatments applied to entire Gunnison's prairie dog colonies (1.6 ha to 18 ha) or to 8-ha plots within larger colonies in June 2010. We applied only the high and medium bait-density treatments to 8 ha plots within black-tailed prairie dog colonies. Field trials were replicated in August and September of 2010 at nearby study sites for both species to assess seasonal differences in bait uptake. However, only a subset of bait densities were replicated (Table 1).

Three to eight days after bait distribution, we captured and sampled prairie dogs and nontarget small mammal species as described. Visual counts of prairie dogs and their burrows were conducted to assess prairie dog abundance and activity levels (Biggins et al., 1993; Severson and Plumb, 1998). We regarded counts of active burrows as the most consistent proxy of prairie dog density across all study sites; therefore, only those methods are described. Burrow counts were conducted within a grid overlaid on the plot (50 m/side). Every burrow within each grid cell was counted and recorded as "active" (based on presence of fresh feces, digging, and tracks) or "inactive" (based on presence of spider webs and vegetation in the burrow entrance).

Field trials in Utah prairie dogs

A single trial was conducted on a Utah prairie dog colony in late August–early September 2010 to assess bait uptake. Placebo baits were placed at 125 active burrow entrances as above. Forty treated entrances were chosen at random, flagged for direct observation of bait uptake, and observed daily until all baits disappeared. On days 3 and 4 post bait distribution, prairie dogs and other small mammals were trapped and sampled using the methods described above.

Field safety trials with vaccine-laden baits in Gunnison's and black-tailed prairie dogs

In August and September 2012, as part of a separate experiment, field safety trials with vaccine were conducted in Gunnison's and black-tailed prairie dogs (Rocke et al., 2010; D.W.T. and T.E.R., unpubl. data). We paired Gunnison's prairie dog colonies (10.4–10.9 ha) or 8-ha plots within larger black-tailed prairie dog colonies and treated one site from each pair with a placebo and the other with vaccine-laden baits (prepared by NWHC) applied via transect at 130 baits/ha as described above. Measures of bait uptake and prairie dog abundance were as described.

Laboratory analyses

To assess bait uptake, hairs and whiskers from sampled species were examined under a fluorescence microscope (excitation wavelength =540 nm; emission wavelength=625 nm; Fernandez and Rocke, 2011). If at least one whisker or hair sample fluoresced under UV light microscopy, it was considered positive for rhodamine and indicative of bait consumption.

Data analyses

The R statistical package (Version 2.15.2) was used for all analyses (R Development Core Team, 2012). We compared bait uptake in the high, medium, and low bait-density distribution areas and between seasons and study sites using chi-square (χ^2) tests with probabilities adjusted for multiple tests by the Dunn-Sidak procedure (Sokal and Rohlf, 1995). Generalized linear models (GLM) with binomial error distributions were fit using the GLM procedures in R. Model fitting was carried out using stepwise procedures based on Akaike's information criterion and analysis of deviance via χ^2 tests implemented using the analysis of variance procedure. Statistical significance was set at $P \leq 0.05$. Covariates for comparing bait uptake included prairie dog species, bait density (high, medium, and low), season (spring and autumn), and burrow density.

The relationship between bait uptake by prairie dogs and burrow density was examined by calculating Pearson's correlation coefficient after calculating the number of baits distributed per burrow. Utah prairie dog data were excluded from all but descriptive analyses because only one trial was conducted in that species. White-tailed prairie dog data were excluded from analyses of uptake and bait density because only the high bait density was used in these trials.

RESULTS

Biomarker detection

We explored several approaches for biomarker detection. Evidence of rhodamine B in whiskers (94%; 95% confidence

TABLE 1. Summary of bait uptake by four species of prairie dogs during 22 field trials in Colorado and Utah, USA using high (130 baits/ha), medium (65 baits/ha), and low (32 baits/ha) bait-density treatments during spring and autumn of 2009–2012. AB = Trials in which baits were distributed around active burrows. In all other trials baits were distributed in transects.

Year	Season	Site	Bait density (baits/hectare)	Species [a]	Percent uptake (95% CI)	Whisker samples (positive/total)[b]
2009	Autumn	Moffat County (AB)	High (130)	WTPD	90.5 (68.1–98.3)	19/21
2009	Autumn	Moffat County	High (130)	WTPD	96.6 (80.4–99.8)	28/29
2010	Spring	Moffat County	High (130)	WTPD	100 (83.4–100)	25/25
2010	Autumn	Garfield County (AB)	Active Burrow	UTPD	94.8 (81.4–99.1)	37/39
2010	Spring	Chaffee County	Low (32)	GUPD	21.9 (9.9–40.4)	7/23
2010	Spring	Chaffee County	Med (65)	GUPD	45.5 (28.5–63.4)	15/33
2010	Spring	Chaffee County	High (130)	GUPD	70.4 (49.7–85.5)	19/27
2010	Autumn	Chaffee County	Med (65)	GUPD	96.9 (82.0–99.8)	31/32
2010	Autumn	Chaffee County	High (130)	GUPD	84.8 (67.3–94.2)	28/33
2010	Spring	Gunnison County	Low (32)	GUPD	30.6 (16.9–48.3)	11/36
2010	Spring	Gunnison County	Med (65)	GUPD	71.0 (51.8–85.1)	22/31
2010	Spring	Gunnison County	High (130)	GUPD	40.0 (17.5–67.1)	6/15
2010	Autumn	Gunnison County	Low (32)	GUPD	54.3 (36.9–70.8)	19/35
2010	Autumn	Gunnison County	Med (65)	GUPD	75.0 (57.5–87.2)	27/36
2012	Autumn	Gunnison County	High (130)	GUPD	98.0 (88–99.9)	49/50
2012	Autumn	Gunnison County	High (130)	GUPD	100 (91.1–100)	50/50
2011	Spring	Larimer County	Med (65)	BTPD	56.1 (39.9–71.2)	23/41
2011	Spring	Larimer County	High (130)	BTPD	65.0 (48.3–78.9)	26/40
2011	Autumn	Larimer County	Med (65)	BTPD	86.5 (70.4–94.9)	32/37
2011	Autumn	Larimer County	High (130)	BTPD	95.3 (82.9–99.1)	41/43
2012	Autumn	Larimer County	High (130)	BTPD	70.0 (55.2–81.7)	35/50
2012	Autumn	Larimer County	High (130)	BTPD	92.0 (79.9–97.4)	46/50

[a] WTPD = white-tailed prairie dog (*Cynomys leucurus*), UTPD = Utah prairie dog (*C. parvidens*), GUPD = Gunnison's prairie dog (*C. gunnisoni*), BTPD = black-tailed prairie dog (*C. ludovicianus*).

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

interval [CI] 83–98%), hair (92%; 95% CI 80–97%), and feces (80%; 95% CI 66–89%) was found in white-tailed prairie dogs sampled on days 3 and 4 after bait distribution (Table 1); none of the 19 individuals captured before bait distribution had rhodamine B staining or confounding background fluorescence. Microscopy was necessary to consistently detect whisker and hair fluorescence because rhodamine B was visible on the body in only a proportion of marked individuals under either natural (59%; 95% CI 44–72%) or UV light (57%; 95% CI 42–70%). Feces positive for rhodamine B were visibly red and fluoresced under UV light. Seven prairie dogs resampled 27 days after bait distribution were all positive for biomarker. Based on these observations and other data (Fernandez and Rocke, 2011), we relied on hair and whisker sampling to estimate bait uptake in all subsequent trials. A 0.25% concentration of rhodamine B in bait appeared sufficient for marking all four species.

Bait palatability

The edible polymer baits with 0.25–0.35% rhodamine B were apparently palatable to all four species of prairie dogs (Table 1). Estimated bait uptake by prairie dogs ranged from 22% (95% CI 10–40%) to 100% (95% CI 91–100%) across 22 separate trials and was >50% in 18 trials. A variety of nontarget small mammals including deer mice (*Peromyscus maniculatus*) and northern grasshopper mice (*Onychomys leucogaster*) also consumed baits.

Palatability was inferred by the rapid removal of baits from study plots. In all

TABLE 2. Analysis of deviance of differences in bait uptake by prairie dogs at high (130 baits/ha), medium (65 baits/ha), and low (32 baits/ha) bait-density treatments during the spring and autumn seasons and burrow density from a general linear model with binominal errors, during field trials in Colorado, USA, 2009–2012.

Prairie dog species[a]	Bait density, burrow density, and season effects	df	Test of deviance (χ^2)	P
All species	Bait density	2	77.75	<0.0001
	Season	1	54.37	<0.0001
	Burrow density	1	0.030	=0.8600
	Bait density × burrow density	2	3.74	=0.0500
Gunnison's	Bait density	2	66.95	<0.0001
	Season	1	38.72	<0.0001
	Burrow density	1	0.070	=0.7900
	Season × burrow density	1	11.14	=0.0008
Black-tailed	Bait density	1	3.277	=0.0700
	Season	1	16.12	<0.0001
	Burrow density	1	11.76	=0.0010

[a] Gunnison's prairie dog (*Cynomys gunnisoni*), black-tailed prairie dog (*Cynomys ludovicianus*), White-tailed prairie dog (*Cynomys leucurus*).

trials ≥90% of the monitored baits disappeared from the plots within 1–8 days after distribution. In eight trials ≥95% of the monitored baits disappeared within 1–3 days.

Presence of vaccine did not appear to affect prairie dogs' willingness to consume baits. Analyses of hair and whiskers marked with rhodamine B revealed that 98% (CI 88–100%) of Gunnison's prairie dogs consumed vaccine and 100% (CI 91–100%) consumed placebo baits; similarly, 92% (CI 82–97%) of black-tailed prairie dogs consumed vaccine and 70% (CI 55–82%) consumed placebo baits (Table 1). Consequently, we included these data in our analyses.

No evidence of mortality, intoxication, digestive upset, or other adverse effects associated with the bait formulation or biomarker consumption in prairie dogs or other small mammal species was observed in any trial. Neither sex nor age (juvenile vs. adult) appeared to influence bait uptake by prairie dogs during autumn trials at bait densities of ≥65/ha. Uptake was 89% (95% CI 84–92%) in males and 90% (95% CI 85–94%) in females (χ^2=0.1019, 1 df, P>0.749). Uptake was 89% (95% CI 85–92%) in adults and 87% (95% CI 75–94%) in juveniles (χ^2= 0.0322, 1 df, P>0.857).

Bait distribution

Bait uptake was 90% (95% CI 68–98%) when baits were distributed around active white-tailed prairie dog burrows and 97% (95% CI 81–100%) when distributed in transects (Table 1). Given the minimal apparent difference in uptake between the methods (χ^2=0.102, 1 df, P>0.749), we distributed baits along transects in all subsequent trials.

Season and bait density effects

Both season (P<0.001) and density of baits distributed (P<0.001) strongly influenced uptake by prairie dogs (Table 2; Fig. 2). For data pooled across all trials and including all three prairie dog species in Colorado, bait uptake during May–June (spring) trials was 26% (95% CI 17–39%), 57% (95% CI 47–67%), or 73% (95% CI 64–81%) at bait densities of 32, 65, or 130/ha, respectively (Fig. 2). Uptake was considerably higher (χ^2=28.8, 1 df, P<0.0008) during August–October (autumn) trials (54% [95% CI 37–71%], 86% [95% CI 77–92%], or 91% [95% CI 87–94%]) at the respective bait densities (Fig. 2). The same pattern held for Gunnison's prairie dogs, where sufficient data for all six combinations of season and bait density were available for a within-species analysis (season: P<0.001;

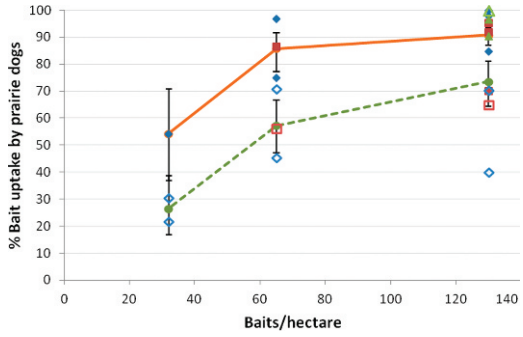

FIGURE 2. Bait uptake by three species of prairie dogs during spring and autumn and at three bait densities (32, 65, and 130 baits/ha). The trend lines (solid orange=fall, dashed green=spring) show the mean uptake at each bait density when trials are pooled across species. Error bars are 95% confidence intervals. Bait uptake during each trial is shown as symbols (black-tailed prairie dogs [*Cynomys ludovicianus*] = red squares, Gunnison's [*Cynomys gunnisoni*] = blue diamonds, and white-tailed [*Cynomys leucurus*] = green triangles). Solid symbols represent trials conducted in the autumn while open symbols represent trials conducted in the spring.

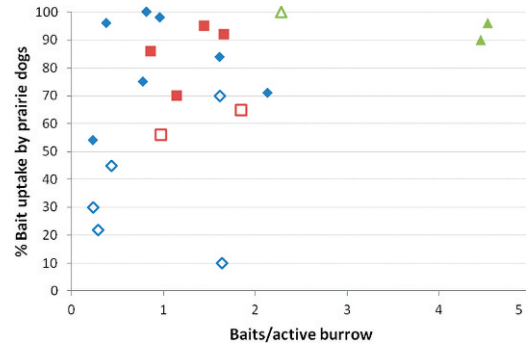

FIGURE 3. Bait uptake by three species of prairie dogs during spring and autumn was moderately correlated with the number of baits distributed per active burrow (Pearson's correlation coefficient = 0.45). Bait uptake during each trial is shown as symbols (black-tailed prairie dogs [*Cynomys ludovicianus*] = red squares, Gunnison's [*Cynomys gunnisoni*] = blue diamonds, and white-tailed [*Cynomys leucurus*] = green triangles). Solid symbols represent trials conducted in the autumn while open symbols represent trials conducted in the spring.

bait density: $P<0.001$). Uptake was similar for white-tailed prairie dogs ($\chi^2=0.009$, 1 df, $P<0.9244$) during spring (100%; 95% CI 83–100%) and autumn (97%; 95% CI 80–100%) at the high bait density with transect distribution. The high observed uptake (95%; 95% CI 81–99%) in the single autumn trial involving Utah prairie dogs appeared consistent with the patterns described.

Prairie dog density

Visual counts of prairie dogs were originally conducted to estimate prairie dog abundance at Colorado sites; however, visual counts at sites in the shrub steppe ecosystem were unreliable because tall vegetation screened many animals from view. Consequently, we used counts of active burrows as an index of prairie dog abundance and density (Biggins et al., 1993) in our analyses to account for the likely influence of prairie dog abundance on bait uptake.

General linear models demonstrated that bait uptake was not solely influenced by burrow density but by the interaction between burrow density and bait density (Table 2; Fig. 3). This trend was observed for data pooled across Gunnison's prairie dogs and black-tailed prairie dogs (burrow density: $P=0.86$; bait density*burrow density: $P=0.05$). For Gunnison's prairie dogs uptake was influenced by the interaction between burrow density and season (burrow density: $P=0.79$; season*burrow density: $P=0.0008$). Burrow density alone affected uptake in black-tailed prairie dogs ($P=0.001$). Bait uptake by all species was moderately correlated with the number of baits distributed per burrow (Pearson's correlation coefficient=0.45; Fig. 3)

Nontarget small mammals

Baits also appeared attractive to nontarget small mammals. Among 19 trials where small mammals were sampled in sufficient numbers, bait uptake pooled across all species sampled within a site ranged from 27% (95% CI 17–39) to 90% (95% CI 67–98) (Table 3; Fig.4). Deer mice ($n=533$), the dominant species captured and sampled at sites in Moffat, Gunnison, Chaffee, and Garfield counties, exhibited estimated bait uptakes from 28%

TABLE 3. Summary of bait uptake by small mammals other than prairie dogs (pooled across species) during field trials in Colorado and Utah, USA using high (130 baits/ha), medium (65 baits/ha), and low (32 baits/ha) bait-density treatments during spring and autumn of 2010–2012. AB = Trials in which baits were distributed around active prairie dog (*Cynomys* spp.) burrows. In all other trials baits were distributed in transects.

Year	Season	Site	Bait density (baits/hectare)	Percent uptake (95% CI)	Whisker samples (positive/total)[a]
2010	Spring	Moffat County	High (130)	68.4 (58.0–77.4)	65/95
2010	Autumn	Garfield County (AB)	Active burrow	66.7 (12.5–98.2)	2/3
2010	Spring	Chaffee County	Low (32)	85.7 (62.6–96.2)	18/21
2010	Spring	Chaffee County	Med (65)	90.0 (66.9–98.2)	18/20
2010	Spring	Chaffee County	High (130)	54.5 (24.6–81.9)	6/11
2010	Autumn	Chaffee County	Med (65)	68.0 (53.2–80.1)	34/50
2010	Autumn	Chaffee County	High (130)	81.8 (63.9–92.3)	27/33
2010	Spring	Gunnison County	Low (32)	39 (24.6–55.5)	16/41
2010	Spring	Gunnison County	Med (65)	83.9 (71.2–91.9)	47/56
2010	Spring	Gunnison County	High (130)	41.7 (16.5–71.4)	5/12
2010	Autumn	Gunnison County	Low (32)	26.9 (17.1–39.3)	18/67
2010	Autumn	Gunnison County	Med (65)	80.0 (63.9–90.4)	32/40
2012	Autumn	Gunnison County	High (130)	69.0 (56.8–79.2)	49/71
2012	Autumn	Gunnison County	High (130)	86.4 (72.0–94.3)	38/44
2011	Spring	Larimer County	Med (65)	90.0 (66.9–98.2)	18/20
2011	Spring	Larimer County	High (130)	89.5 (65.5–98.2)	17/19
2011	Autumn	Larimer County	Med (65)	60.0 (17.0–92.3)	3/5
2011	Autumn	Larimer County	High (130)	100 (36.9–100)	4/4
2012	Autumn	Larimer County	High (130)	66.7 (24.1–94.0)	4/6
2012	Autumn	Larimer County	High (130)	100 (77.1–100)	17/17

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

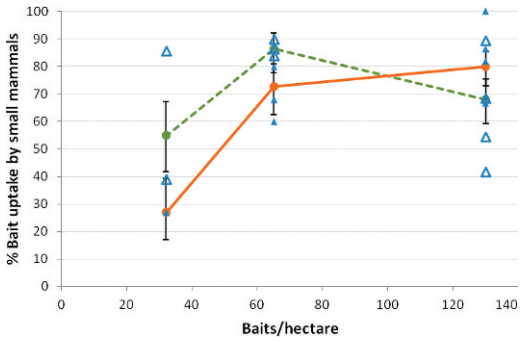

FIGURE 4. Bait uptake by small mammals other than prairie dogs (*Cynomys* spp.) during spring and fall and at three bait densities (32, 65, and 130 baits/hectare). The trend lines (solid orange = autumn, dashed green = spring) show the mean uptake at each bait density when trials are pooled across all species: deer mouse (*Peromyscus maniculatus*), northern grasshopper mouse (*Onychomys leucogaster*), least chipmunk (*Tamias minimus*), thirteen-lined ground squirrel (*Spermophilus tridecemlineatus*), meadow vole (*Microtus pennsylvanicus*), and golden-mantled ground squirrel (*Spermophilus lateralis*). Error bars are 95% confidence intervals. Bait uptake by small mammals during each trial is shown as blue triangles (solid = autumn, open = spring).

(95% CI 18–41%) to 90% (95% CI 76–97%). Among northern grasshopper mice (n=52), the dominant species captured and sampled in Larimer County, estimated uptake ranged from 60% (95% CI 17–93) to 100% (95% CI 77–100). Bait consumption was detected in captured least chipmunks (*Tamias minimus*; 9/18), thirteen-lined ground squirrels (*Spermophilus tridecemlineatus*; 13/14), meadow voles (*Microtus pennsylvanicus*; 4/10), and golden-mantled ground squirrels (*Spermophilus lateralis*; 5/7). For all species, bait uptake in spring was 73% (95% CI 66–79%) when data from low, medium, and high bait densities were pooled but declined (χ^2=7.00, 1 df, P=0.008) to 59% (95% CI 52–66) during autumn; this trend was opposite that observed among prairie dogs.

Some nontarget small mammals were captured with fresh biomarker staining around their mouths, on their bodies, and in their urine, indicating consumption of bait in the 24–48 hr prior to capture.

During one trial in Moffat County and another in Larimer County, 33% (5/15) of deer mice and 66% (6/9) of grasshopper mice, respectively, were freshly stained with biomarker 22 days after the last bait was observed on the plot, suggesting that baits may have been cached and consumed at least 20 days after distribution.

DISCUSSION

Free-ranging prairie dogs of all four studied species readily consumed baits intended to deliver plague vaccine, thereby offering promise that vaccine uptake will not be an obstacle to plague control via oral vaccination. Based on estimates of the reproductive value of plague (Lorange et al., 2005; Eisen et al., 2006; Salkeld et al., 2010), we hypothesize that a minimum effective vaccination rate of 50% of prairie dogs in a colony may be necessary to achieve a level of herd immunity that would control enzootic plague and prevent epizootics (Fine et al., 2011). However, striving for higher bait uptake rates (≥80%) by prairie dogs may be critical for a successful vaccination program because of imperfect immunization efficacy demonstrated in the laboratory (Rocke et al., 2010; T.E.R., unpubl. data). During 22 field trials, we estimated bait uptake by prairie dogs at ≥50% in 18 trials and at ≥80% in 11 trials. Beyond this general encouragement, our findings facilitate refinement and optimization of vaccination strategies because these trials were designed to identify factors anticipated to hamper uptake.

Both the rate and the season of bait application appear critical to maximizing uptake by prairie dogs (Fig. 2). We conducted only three trials at bait densities below 65/ha because in early trials, lower bait densities tended to yield insufficient uptake (Table 1). In contrast, estimated uptake was ≥80% in 11 of the 19 trials with bait densities ≥65/ha (Fig. 2). The importance of bait density seemed clearer when considered in relation to estimated prairie dog density because application rates resulting in ≥1 bait/active burrow appeared necessary to achieve the 80% target for uptake (Fig. 3). More bait may be needed where prairie dog densities are extremely high, and estimating active burrow densities should provide a simple and relatively economical metric for guiding such decisions. Although results suggest that bait application rates of 65–130/ha will achieve the 80% uptake target, further work to improve resolution of the relationship between bait density, prairie dog density, and uptake may help improve the overall efficiency of vaccination.

Autumn bait application, defined in the context of each species' unique ecology, is perhaps most important in consistently assuring high uptake in prairie dogs. Among the 11 trials conducted in August–October with bait densities ≥65/ha, estimated uptakes were 70–100% (Fig. 2) and averaged 88% (95% CI 85–91); in contrast, among seven spring trials conducted in May–June with bait densities ≥65/ha estimated, uptakes were all ≤72% and averaged 61% (95% CI 55–68) (Fig. 2). We hypothesize that prairie dogs may be more motivated to seek out and consume baits after juveniles have first emerged from burrows and when forage quality declines or their foraging activities increase in late summer and autumn; such tendencies may be accentuated in hibernating species. Late summer or early autumn bait application may offer benefits such as ensuring higher per capita vaccine exposure to surviving juveniles as opposed to vaccinating emergent juveniles that may only have a 50% annual survival (Hoogland, 1995). In light of the apparent importance of juvenile vaccination in protecting prairie dogs from plague (T.E.R., unpubl. data), strategies favoring vaccine uptake by juvenile prairie dogs should be emphasized when devising control programs. Rapid bait removal at two vaccine sites and high vaccine uptake suggested no avoidance of the vaccine baits compared to placebos.

In contrast to the marked influences of rate and season, bait distribution strategy did not appear to measurably affect uptake. Based on our and a previous study's findings (Creekmore et al., 2002), it appears that high vaccine uptakes can be achieved with some form of transect or broadcast distribution. Such approaches should be relatively cost-effective compared to individual burrow treatments and will lend more readily to aerial or landscape-level vaccine applications. From our experience working with these gelatin-like baits under a variety of field conditions, a drier bait formulation with a pellet-like structure may aid in the transition to management-scale applications. Although broadcast applications seem most generally useful in plague management, delivering baits directly to burrows may be more efficient in cases where prairie dog densities are low or colonies are small, as illustrated in our Utah prairie dog trial.

Six species of nontarget small mammals consumed baits during these trials with pooled uptakes as high as 90% (95% CI 67–98%). Deer mice and grasshopper mice have been hypothesized to be important components of a complex cycle of plague transmission or likely reservoirs of the disease between more-visible epizootics (Gage and Kosoy, 2005; Salkeld et al., 2010). Further research is needed on the efficacy of this vaccine in these species to understand more fully how the community ecology of pathogen transmission may be impacted by vaccination.

As previously demonstrated in a wide variety of mammalian species (Lindsey, 1983; Fichet-Calvet, 1999; Jacob et al., 2002) and in captive prairie dogs (Fernandez and Rocke, 2011), rhodamine B appeared to be a safe and effective biomarker in prairie dogs. We observed good staining of whisker and hair follicles in all four prairie dog species as well as in a variety of nontarget small mammal species.

Our recommendations to managers are to apply vaccine after juvenile emergence and in the late summer or autumn when forage quality declines. Distribution of vaccine-laden baits in transects and at rates of ≥65/ha or ≥1 bait/active burrow is likely to produce optimal uptake with an efficient method of distribution. Further research to improve resolution of the relationship between bait density, prairie dog density, and uptake may help improve the overall efficiency of vaccination. Additional work is needed to understand better how nontarget small mammals may influence vaccine uptake by prairie dogs and how plague transmission in all rodent communities is impacted by vaccination.

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LITERATURE CITED

Abbott RC, Osorio JE, Bunck CM, Rocke TE. 2012. Sylvatic plague vaccine: A new tool for conservation of threatened and endangered species? *Ecohealth* 9:243–250.

Antolin MF, Gober P, Luce B, Biggins DE, Pelt WEV, Seery DB, Lockhart M, Ball M. 2002. The influence of sylvatic plague on North American wildlife at the landscape level, with special emphasis on black-footed ferret and prairie dog conservation. *Trans of the 67th N Am Wildl and Nat Resour Conf* 67:104–127.

Augustine DJ, Dinsmore SJ, Wunder MB, Dreitz VJ, Knopf FL. 2008. Response of mountain plovers to plague-driven dynamics of black-tailed prairie dog colonies. *Landscape Ecol* 23:689–697.

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

Biggins DE, Miller BJ, Hanebury LR, Oakleaf B, Farmer AH, Crete R, Dood A. 1993. A technique for evaluating black-footed ferret habitat. In: *Management of prairie dog complex-*

es for the re-introduction of the black-footed ferret, Oldemeyer Jl, Biggins DE, Miller BJ, Crete R, editors. US Fish and Wildlife Service Biological Report 13, Washington, DC, pp. 73–88.

Creekmore TE, Rocke TE, Hurley J. 2002. A baiting system for delivery of an oral plague vaccine to black-tailed prairie dogs. *J Wildl Dis* 38:32–39.

Eisen RJ, Bearden SW, Wilder AP, Montenieri JA, Antolin MF, Gage KL. 2006. Early-phase transmission of *Yersinia pestis* by unblocked fleas as a mechanism explaining rapidly spreading plague epizootics. *Proc Natl Acad Sci* U S A 103:15380–15385.

Fernandez JR, Rocke TE. 2011. Use of rhodamine B as a biomarker for oral plague vaccination of prairie dogs. *J Wildl Dis* 47:765–768.

Fichet-Calvet E. 1999. Persistence of a systemic labeling in fur and guard hairs by ingestion of rhodamine B in *Myocastor coypus* (Rodentia). *Mammalia* 63:241–244.

Fine P, Eames K, Heymann DL. 2011. "Herd Immunity": A rough guide. *Vaccines* 52:911–916.

Gage KL, Kosoy MY. 2005. Natural history of plague: Perspectives from more than a century of research. *Annu Rev Entomol* 50:505–528.

Griebel RL. 2012. *Conata Basin/Badlands Area 2012 plague management report*. Nebraska National Forest, Buffalo Gap National Grasslands, Wall Ranger District, Wall, South Dakota, 10 pp.

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

Jachowski DS, Brown N, Wehtje M, Tripp DW, Millspaugh JJ, Gompper ME. 2012. Mitigating plague risk in Utah prairie dogs: Evaluation of a systemic flea control product. *Wildl Soc Bull* 36:167–175.

Jacob J, Jones DA, Singleton GR. 2002. Retention of the bait marker rhodamine B in wild house mice. *Wildl Res* 29:159–164.

Lindsey GD. 1983. Rhodamine B: A systemic fluorescent marker for studying mountain beavers (*Aplodontia rufa*) and other animals. *Northwest Sci* 57:16–21.

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

Mencher JS, Smith SR, Powell TD, Stinchcomb DT, Osorio JE, Rocke TE. 2004. Protection of black-tailed prairie dogs (*Cynomys ludovicianus*) against plague after voluntary consumption of baits containing recombinant raccoon poxvirus vaccine. *Infect Immun* 72:5502–5505.

R Development Core Team. 2012. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org. Accessed December 2013.

Rocke TE, Smith SR, Stinchcomb DT, Osorio JE. 2008. Immunization of black-tailed prairie dog against plague through consumption of vaccine-laden baits. *J Wildl Dis* 44:930–937.

Rocke TE, Pussini N, Smith SR, Williamson J, Powell B, Osorio JE. 2010. Consumption of baits containing raccoon pox-based plague vaccines protects black-tailed prairie dogs (*Cynomys ludovicianus*). *Vector-Borne and Zoonotic Dis* 10:53–58.

Salkeld DJ, Salathe M, Stapp P, Jones JH. 2010. Plague outbreaks in prairie dog populations explained by percolation thresholds of alternate host abundance. *Proc Natl Acad Sci* U S A 107:14247–14250.

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

Seglund AE, Schnurr PM. 2010. *Colorado Gunnison's and white-tailed prairie dog conservation strategy*. Colorado Division of Wildlife, Denver, Colorado, 218 pp.

Severson KE, Plumb GE. 1998. Comparison of methods to estimate population densities of black-tailed prairie dogs. *Wildl Soc Bull* 26:859–866.

Sokal RR, Rohlf FJ. 1995. *Biometry: The principals and practice of statistics in biological research*. 3rd Ed. WH Freeman and Co., New York, New York, 887 pp.

Stapp P, Salkeld DJ, Eisen RJ, Pappert R, Young J, Carter LG, Gage KL, Tripp DW, Antolin MF. 2008. Exposure of small rodents to plague during epizootics in black-tailed prairie dogs. *J Wildl Dis* 44:724–730.

Tripp DW, Gage KL, Montenieri JA, Antolin MF. 2009. Flea abundance on black-tailed prairie dogs (*Cynomys ludovicianus*) increases during plague epizootics. *Vector-Borne and Zoonotic Dis* 9:313–321.

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