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Authors: Cárdenas-Canales, Elsa M., Wolfe, Lisa L., Tripp, Daniel W., Rocke, Tonie E., Abbott, Rachel C., et al.

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Responses of Juvenile Black-tailed Prairie Dogs (*Cynomys ludovicianus*) to a Commercially Produced Oral Plague Vaccine Delivered at Two Doses

Elsa M. Cárdenas-Canales,[1] Lisa L. Wolfe,[1] Daniel W. Tripp,[1] Tonie E. Rocke,[2] Rachel C. Abbott,[2] and Michael W. Miller[1,3] [1]Colorado Division of Parks and Wildlife, Wildlife Health Program, Foothills Wildlife Research Facility, 4330 Laporte Ave., Fort Collins, Colorado 80521-2153, USA; [2]US Geological Survey, National Wildlife Health Center, 6006 Schroeder Rd., Madison, Wisconsin 53711, USA; [3]Corresponding author (email: mike.miller@state.co.us)

ABSTRACT: We confirmed safety and immunogenicity of mass-produced vaccine baits carrying an experimental, commercial-source plague vaccine (RCN-F1/V307) expressing *Yersinia pestis* V and F1 antigens. Forty-five juvenile black-tailed prairie dogs (*Cynomys ludovicianus*) were randomly divided into three treatment groups (n=15 animals/group). Animals in the first group received one standard-dose vaccine bait (5×10^7 plaque-forming units [pfu]; STD). The second group received a lower-dose bait (1×10^7 pfu; LOW). In the third group, five animals received two standard-dose baits and 10 were left untreated but in contact. Two vaccine-treated and one untreated prairie dogs died during the study, but laboratory analyses ruled out vaccine involvement. Overall, 17 of 33 (52%; 95% confidence interval for binomial proportion [bCI] 34–69%) prairie dogs receiving vaccine-laden bait showed a positive anti-V antibody response on at least one sampling occasion after bait consumption, and eight (24%; bCI 11–42%) showed sustained antibody responses. The STD and LOW groups did not differ ($P\geq0.78$) in their proportions of overall or sustained antibody responses after vaccine bait consumption. Serum from one of the nine (11%; bCI 0.3–48%) surviving untreated, in-contact prairie dogs also had detectable antibody on one sampling occasion. We did not observe any adverse effects related to oral vaccination.

Key words: Black-tailed prairie dog, *Cynomys ludovicianus*, plague, raccoonpox, vaccine, *Yersinia pestis*.

Over the last century, plague—a disease introduced to North America in the early 1900s—has caused significant harm to affected ecosystems. The damage is most evident in native prairie and shrub-steppe systems, where plague has depressed prairie dog (*Cynomys* spp.) abundance and hampered efforts to recover black-footed ferret (*Mustela nigripes*) populations from near extinction (Antolin et al. 2002). The conservation need for landscape-level plague control has been recognized for decades. Oral vaccination of prairie dogs has emerged recently as a potential tool for combating plague (Abbott et al. 2012).

The recombinant raccoonpox virus (RCN) plague vaccine, RCN-F1/V307 (also called "sylvatic plague vaccine"), developed by the US Geological Survey's (USGS's) National Wildlife Health Center and the University of Wisconsin appears to be safe and effective in prairie dogs (Rocke et al. 2014, 2015; Tripp et al. 2015). Promising preliminary results from field trials conducted throughout the western US have motivated interest in beginning sustained, large-scale field experiments to more fully assess oral vaccination as a prairie dog and black-footed ferret conservation tool (Johnson et al. 2014). Benchtop methods for producing vaccine and vaccine-laden baits could not supply these larger field experiments (Corro et al. 2017). Consequently, we secured approval from the US Department of Agriculture Center for Veterinary Biologics (CVB) and USGS to have some RCN-F1/V307 production transferred to a CVB-licensed bulk manufacturer. We also have used off-the-shelf technology to mass-produce vaccine-laden baits suitable for mechanized delivery (Corro et al. 2017).

Here we describe laboratory trials conducted to confirm the safety and immunogenicity of mass-produced baits carrying the experimental, commercial-source RCN-F1/V307 vaccine. We also measured black-tailed prairie dog (*Cynomys ludovicianus*) responses to a lower vaccine dose in order to begin exploring the potential for cost savings that could

enhance the feasibility and sustainability of long-term vaccination campaigns.

Our study was approved by the Colorado Parks and Wildlife (CPW) Animal Care and Use Committee (file 05-2016). Juvenile (<2 mo old) prairie dogs (n=45) were captured in Boulder County, Colorado, US, and transported to CPW's Foothills Wildlife Research Facility (Fort Collins, Colorado, USA) on 6 or 7 June 2016. Upon arrival, we treated animals with carbaryl dust (Sevin 5%, GardenTech, Atlanta, Georgia, USA) to kill fleas. We randomly divided prairie dogs into three groups. Each group was housed in an aluminum pen (122×88×215 cm) containing two stainless steel nest boxes (30×51×37 cm) with plastic pipe extensions to resemble burrows. Prairie dogs received a commercial diet specific for pups (Exotic Nutrition, Newport News, Virginia, USA), fresh produce (e.g., carrot, apple, and sweet potato chunks), timothy hay, and water ad libitum.

Vaccine-laden baits were manufactured as described by Corro et al. (2017; see Supplementary Material). Baits consisted of distilled water, peanut butter, FD&C Blue #1 food dye, a gelatin-based biopolymer matrix (Incortrix®, FoodSource Lure Corporation, Birmingham, Alabama, USA; FoodSource Lure Corporation 2013), and the experimental vaccine (Yersinia Pestis Vaccine, Live Raccoon Poxvirus Vector, Code 11Y2.R0, Colorado Serum Company, Denver, Colorado, USA). We produced baits carrying two target vaccine doses: a standard dose (5×10^7 plaque-forming units [pfu]; STD; Rocke et al. 2015) and a lower dose (1×10^7 pfu; LOW). The NHWC confirmed vaccine viability in a sample of finished vaccine-laden baits (Supplementary Material).

Each group (n=15) was randomly assigned to a vaccine dose treatment. Animals in the STD and LOW groups each received one bait containing their group's corresponding vaccine dose. Five animals in the double dose–contact group each received two standard-dose vaccine baits offered simultaneously and the remaining 10 animals were left untreated but housed in the same pen.

We offered placebo baits (identical to vaccine baits, but lacking vaccine) to all groups for about 1 wk prior to treatment in order to habituate prairie dogs to consuming these baits. To encourage vaccine bait uptake, we fasted all groups for 15–20 h prior to treatment. On day 0, prairie dogs were transferred to individual portable kennels and provided vaccine-laden bait(s) as assigned. The animals were left undisturbed for a period of 2–4 h, then returned to their pens. If the bait was not consumed on the first day then a second attempt was made the next day following the same procedures. All prairie dogs consumed baits offered on days 0–1, although one LOW animal ate only ~25% of its bait.

We allowed animals to acclimate for about 1 wk before sample collection began. We collected blood samples for serology (Tripp et al. 2015) at days –7 (7 d before vaccination; "baseline"), 14, 28, and 60. To assess vaccine safety, we physically examined each individual during sampling—including an oral examination—for lesions or other evidence of adverse effects potentially caused by vaccination (Tripp et al. 2015).

To test for seroconversion, we used a lateral flow assay that detects antibodies against *Yersinia pestis* V antigen as an index of humoral immune response to RCN-F1/V307 (Abbott et al. 2014; Rocke et al. 2014, 2015). The assay was run as described by Abbott et al. (2014), with reactions scored subjectively as 0 (no visible reaction), 0–1 (very faint, an added class), 1, 2, 3, or 4. As a means of further quantifying relative anti-V antibody titers, assay strips with a visible reaction were measured with a chromatographic reader (Holomic Rapid Diagnostic Reader or HRDR-200, Cellmic LLC, Los Angeles, California, USA) calibrated using reference prairie dog sera (Abbott et al. 2014) such that reported values equaled $\log_2$(reciprocal antibody titer). We regarded serum antibody responses as positive for samples wherein the postvaccination sample value was ≥2.2 (cutoff below which values were considered nonspecific; Supplementary Material Fig. S1)

after subtracting the respective values from the animal's baseline (day –7) sample.

We tallied the proportion of individuals seroconverting in respective treatment groups based on two definitions of seroconversion: positive antibody response in at least one sample ("positive response"), and positive responses in at least two successive samples ("sustained response"). We compared proportions of positive anti-V responses between the LOW and STD dose groups using a one-tailed (LOW<STD) Fisher's exact test (α=0.1). We also compared proportions of positive anti-V responses across all three vaccine-treated groups.

We did not observe any adverse effects after vaccine bait consumption. Two vaccine-treated and one untreated prairie dogs died during the course of the study (one each on days 11, 20, and 27). Gross and microscopic examinations revealed no lesions suggestive of RCN pathology or other vaccine effects. Moreover, screening with a polymerase chain reaction assay failed to detect RCN DNA in spleen or liver tissues from these individuals. Consequently, we regarded these deaths as likely arising from maladjustment to captivity rather than to vaccine bait consumption. Data from these individuals were not included in analyses of antibody responses. All other prairie dogs remained apparently healthy throughout the study.

Unexpectedly, seven of these 45 juvenile prairie dogs had baseline (day –7) serum antibody values above the anti-V threshold (range 2.8–4.7). The source colony had never been treated with vaccine baits and had shown no evidence of plague activity in >5 yr. At day 14 (27–28 d after capture), all seven had assay scores of 0. Based on estimated subject ages at the time of sampling and the observed pattern of titer decay in untreated animals, we suspected baseline values to be reactions from maternal antibodies. Maternal exposure to other cross-reacting *Yersinia* spp. seemed the most plausible explanation for these titers. None of the five vaccine-treated animals with baseline titers (three STD, two LOW) responded serologically after bait consumption, but it was unclear if the presence of anti-V reacting antibodies interfered with humoral responses in those individuals. We chose to include individuals with baseline titers in our analyses, believing this to be a conservative and potentially realistic representation of responses to the vaccine under field conditions.

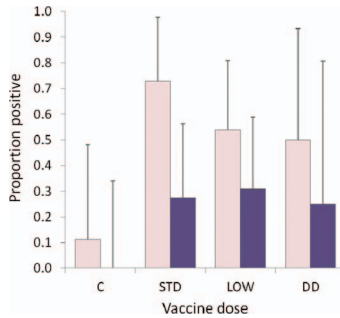

FIGURE 1. Proportion of juvenile black-tailed prairie dogs (*Cynomys ludovicianus*) showing a positive serum antibody response to *Yersinia pestis* V antigen on at least one sampling occasion (light shading) and on two or more sampling occasions (sustained responses; darker shading) after consuming bait containing an experimental, commercial-source recombinant plague vaccine (RCN-F1/V307). We compared serum antibody responses of prairie dogs that consumed a bait carrying 5×10^7 plaque-forming units (pfu; STD; n=14), 1×10^7 pfu (LOW; n=15), or two STD baits (DD; n=4) of RCN-F1/V307; unvaccinated prairie dogs (C; n=9) were housed in contact with DD individuals. Capped vertical lines are the upper 95% confidence intervals for each binomial proportion.

Overall, 17 of 33 (52%; 95% confidence interval for binomial proportion [bCI] 34–69%) prairie dogs consuming vaccine-laden bait(s) showed a positive anti-V antibody response (i.e., sample–baseline≥threshold) on at least one sampling occasion after treatment. Eight (24%; bCI 11–42%) showed sustained responses. The proportions of overall or sustained antibody responses did not differ between LOW and STD groups or across all three vaccine-treated groups ($P\geq0.78$; Fig. 1). The animal that consumed only a portion of its LOW bait was among those failing to seroconvert.

Expanding the capacity to produce vaccine and vaccine-laden baits affordably in large quantities was critical to undertaking the

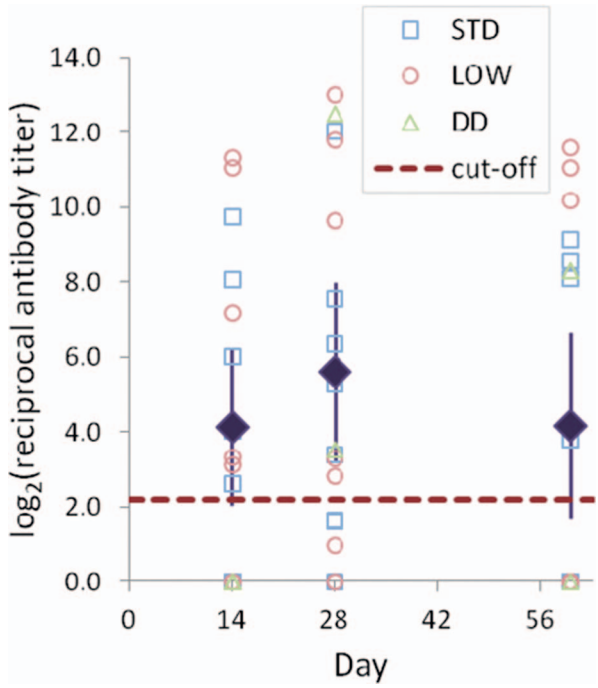

FIGURE 2. Serum antibody responses to *Yersinia pestis* V antigen in individual juvenile black-tailed prairie dogs (*Cynomys ludovicianus*) that seroconverted in at least one of three samples collected at 14, 28, and 60 d after consuming bait containing an experimental, commercial-source recombinant plague vaccine (RCN-F1/V307). Values shown are the differences between the animal's $\log_2$(reciprocal antibody titer) at each time point and that individual's baseline value that exceeded the threshold value established for anti-V antibodies (i.e., sample–baseline≥threshold) in order to be counted as positive. Squares represent individuals receiving 5×10^7 plaque-forming units (pfu; STD; n=8) of vaccine, circles represent individuals receiving 1×10^7 pfu (LOW; n=7), and triangles represent individuals receiving two STD baits (DD; n=2). Solid diamonds are the means of all values for that time point; the vertical lines span the 95% confidence intervals. The dashed horizontal line is the threshold value (2.2) for a positive response.

landscape-scale field assessments of RCN-F1/V307 launched in 2016 (US Fish and Wildlife Service 2016; Corro et al. 2017). The transition from benchtop to larger-scale production increased vaccine bait output by >10-fold from the outset (Corro et al. 2017), facilitating bait application across >2,500 ha. As part of the transition from custom to mass production, we wanted to assure that changes in vaccine or bait production had not inadvertently compromised immunogenicity. Seroconversion in response to mass-produced vaccine baits appeared equivalent to rates reported in captive prairie dogs from previous laboratory studies (37–65%; Rocke et al. 2014, 2015). Antibody responses to vaccine bait consumption varied among individuals (Fig. 2 and Supplementary Material Fig. S1) similar to responses observed in earlier studies with RCN-F1/V307 (Rocke et al. 2014, 2015).

One of the nine (11%; bCI 0.3–48%) untreated prairie dogs housed in contact with individuals that consumed vaccine baits showed a positive anti-V antibody response on day 28, but this was not a sustained response (Fig. 1). Close confinement may have facilitated exposure mechanically or during the brief vaccine shedding period (Tripp et al. 2015).

Applying either insecticide dust or oral vaccine baits to prevent plague outbreaks in prairie dog colonies remains expensive. Lowering the annual cost of plague management seems necessary in order to facilitate its sustained, large-scale use as a conservation tool. More efficient vaccine and bait manu-

facturing processes have helped make oral vaccination more affordable (Corro et al. 2017). Our data suggest that lowering vaccine dose could be explored as a means of further reducing the overall cost of oral vaccination for landscape-level plague control.

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SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/2017-02-033.

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