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Moderate Susceptibility to Subcutaneous Plague (*Yersinia pestis*) Challenge in Vaccine-treated and Untreated Sonoran Deer Mice (*Peromyscus maniculatus sonoriensis*) and Northern Grasshopper Mice (*Onychomys leucogaster*)

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ABSTRACT: The variable response of wild mice to *Yersinia pestis* infection, the causative agent of plague, has generated much speculation concerning their role in the ecology of this potentially lethal disease. Researchers have questioned the means by which *Y. pestis* is maintained in nature and also sought methods for managing the disease. Here we assessed the efficacy of a new tool, the sylvatic plague vaccine (SPV), in wild-caught northern grasshopper mice (*Onychomys leucogaster*) and commercially acquired Sonoran deer mice (*Peromyscus maniculatus sonoriensis*). More than 40% of the animals survived a subcutaneous *Y. pestis* challenge of 175,000 colony forming units (over 30,000 times the white mouse 50% lethal dose) in both vaccine-treated and control groups. Our results indicate that SPV distribution is unlikely to protect adult mice from plague infection in field settings and corroborate the heterogeneous response to *Y. pestis* infection in mice reported by others.

Key words: *Onychomys leucogaster*, *Peromyscus maniculatus*, sylvatic plague vaccine, *Yersinia pestis*.

Plague, caused by the bacterium *Yersinia pestis*, can reduce mammal abundance and distribution dramatically. The pathogen is associated with rodents and primarily transmitted by fleas that acquired infection from a moribund infectious host (Hinnebusch 2005). In North America, the disease can decimate colonies of prairie dogs (*Cynomys* spp.; Lechleitner et al. 1968), a keystone species of grassland ecosystems (Kotliar et al. 1999). Maintenance of *Y. pestis* in natural settings is still poorly understood. The heterogeneous response of wild mice to *Y. pestis* infection has generated numerous hypotheses regarding their role in plague maintenance (Gage and Kosoy 2005). Highly susceptible species generally experience rapid die-offs and are, therefore, poor reservoir and maintenance hosts, whereas moderately susceptible hosts could conceivably maintain and spread the pathogen. The search for maintenance hosts and alternative reservoirs for *Y. pestis* and for novel plague management tools has been ongoing (McCoy and Smith 1910; Quan and Kartman 1962; Thomas et al. 1988; Salkeld et al. 2007). Recently, bait-delivered sylvatic plague vaccine (SPV), distributed on small, paired plots (1–59 ha in size) within or adjacent to untreated prairie dog colonies, increased the odds of prairie dog survival 1.1 to 1.5 times on plots treated with SPV compared to their paired placebo-treated plots (Rocke et al. 2017). Although prairie dog populations declined on study sites that experienced plague outbreaks (Rocke et al. 2017; Tripp et al. 2017), the odds of survival were 1.8 (adult) to 2.4 (juvenile) times higher on SPV-treated plots than on paired placebo plots (Rocke et al. 2017). The bait was consumed at high rates by deer mice (*Peromyscus maniculatus*), a nontarget species, during these field trials. On paired plots where *Y. pestis* was detected and mice were trapped, deer mouse abundance was higher on three of five vaccine-treated plots compared to their paired placebo plots (Bron et al. 2018). However, it is unclear if mice are directly protected by the vaccine. We assessed SPV efficacy in laboratory challenge experiments in two mouse species, wild-caught northern grasshopper mice (GHM; *Onychomys leucogaster*) and commercially acquired

TABLE 1. Numbers and age group of northern grasshopper mice (GHM; *Onychomys leucogaster*) and commercially acquired Sonoran deer mice (DM; *Peromyscus maniculatus sonoriensis*) that consumed plague vaccine-laden baits (vaccine-treated) or placebo baits (control), had an antibody response to V proteins following vaccination, survived challenge with *Yersinia pestis*, and had an antibody response to V or Fraction 1 (F1) proteins after challenge. NT = not tested.

			After vaccination			After challenge		
				No. positive/ no. tested			No. positive/ no. tested[a]	
Species	Treatment	Age at vaccination (d)	*n* vaccinated	Anti-V	Anti-F1	*n* survived	Anti-V	Anti-F
GHM	Vaccinated	>72	16	3/15	0/15	10	2/3	3/3
	Control	>72	16	NT	NT	9	2/3	3/3
DM	Vaccinated	<50	8	2/8	0/8	6	3/3	3/3
		72–100	7	3/7	0/7	0	0	0
	Control	72–102	15	NT	NT	7	1/3	3/3

[a] Positive antibody levels to V proteins were 1 for animals in the control group and 1 (n=1), 2 (n=1), or 3 (n=3) in the vaccine group. All antibody levels to F1 proteins were 3 (n=1) or 4 (n=11).

Sonoran deer mice (DM; *Peromyscus maniculatus sonoriensis*).

Laboratory animal procedures (EP110504) were reviewed and approved by the Institutional Animal Care and Use Committee of the US Geological Survey National Wildlife Health Center. The GHM (adults and subadults) were captured in Colorado (permit 12TR2077), transported to National Wildlife Health Center, and housed individually in conventional mouse cages with enrichment (e.g., nest material and shelter); 16 animals were randomly assigned to one of two treatment groups, vaccine-treated (SPV baits) or control (placebo baits). All were bled prior to treatment and serum was collected. Sonoran DM (Peromyscus Genetic Stock Center, University of South Carolina, Columbia, South Carolina, USA) were group-housed (three to four animals of the same sex) in conventional mouse cages with enrichment, and were similarly assigned to one of two treatment groups (n=15 each). Ages at vaccination of DM ranged from 43 to 100 d in the vaccine group and 72 to 102 d in the control group (Table 1).

Individually caged DM were fed 4-g baits with SPV or placebo (Rocke et al. 2017) overnight and returned to group housing the next morning. Nine grasshopper mice that had not consumed the entire bait overnight were rebaited 6 d later. Animals were bled from the saphenous vein and serum collected at 26 (GHM) or 41 (DM) d postvaccination. Survivors were bled at termination of the study, 27 to 29 d postchallenge; serum was stored at –20 C. We diluted serum 1:100 for use in protein G-based lateral flow tests (IDxDI, Carlsbad, California, USA) for the detection of antibodies to Fraction 1 (F1) and V proteins (Abbott et al. 2014). Optical scores of the test ranged from 0 (negative) to 4 (strong positive). Very faint bands with optical scores of less than 1 were considered negative.

Animals were challenged 63 d (DM) and 92 d (GHM) posttreatment, via subcutaneous injection with 175,000 colony forming units (CFU) of *Y. pestis* CO92. At the same time, the lethality of the inoculum was determined in white mice (*Mus musculus*; strain ICR) and the inoculum had a 50% lethal dose (LD50) of 3–5 CFU. Animals were monitored for morbidity and were euthanized if signs were severe. Kaplan-Meier survival curves were compared using log-rank statistics for survival differences between groups in R Statistical Computing Software (R Core Team 2020), using packages *survival* (Therneau and Grambsch 2000) and *survminer* (Kassambara et al. 2020).

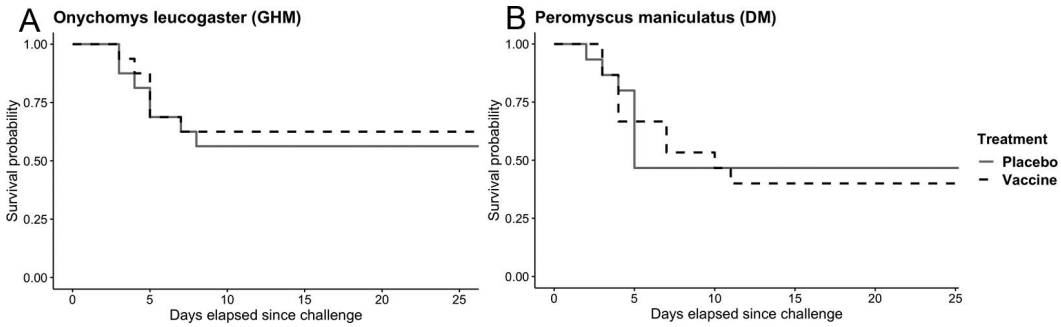

FIGURE 1. Survival curves of mice following *Yersinia pestis* laboratory challenge. A. Northern grasshopper mice (*Onychomys leucogaster*), wild-caught in Colorado, USA (n=16 per group). B. Sonoran deer mice (*Peromyscus maniculatus sonoriensis*) acquired commercially (n=15 per group). Mice received a subcutaneous injection of 175,000 colony-forming units of *Y. pestis* CO92 at 41 (deer mice) or 26 d (grasshopper mice) after vaccine (vaccine-treated) or placebo bait consumption (control).

The probability of survival did not differ significantly ($P>0.05$) between vaccine-treated and control groups for either GHM (62.5% vs. 56.3%; P=0.716, n=16 each) or DM (40% vs. 46.7%; P=0.811, n=15 each; Fig. 1). Three of 15 (one blood sample was not available) GHM in the vaccine-treated group had detectable antibodies to V and all three survived plague challenge (optical scores: 1, 3, and 4), as did six animals without detectable antibodies after vaccine treatment (Table 1). Five of 15 DM developed antibodies to V in the vaccine-treated group (optical scores: 2, 2, 2, 3, and 4), but only two of these survived the challenge (optical scores: 3 and 4). Four other survivors had no detectable antibodies before challenge. None of the animals developed antibodies to F1 posttreatment. Notably, in the vaccine-treated group, younger DM (<50 d, n=8) were more likely to survive (75% vs. 0%; $P<0.001$) than older animals (72–100 d, n=7; Table 1). Age effects were not assessed for GHM, because the animals had been in captivity for more than 50 d before bait consumption.

Pretreatment sera from GHM were negative for antibodies to F1 and V, indicating that these wild-caught mice were not recently exposed to *Y. pestis* prior to capture. Postchallenge anti-F1 scores were high (3 and 4, n=12) in blood samples from three survivors of each treatment group of both species. In contrast, anti-V scores from survivors were low, ranging from 0 to 1 in the control groups and from 0 to 3 in the vaccine-treated groups. Taken together, the postvaccination and terminal antibody scores indicated that antibodies to V were more likely to be associated with vaccine consumption and antibodies to F1 with *Y. pestis* exposure. However, despite the absence of detectable antibodies pretreatment, prior exposure to *Y. pestis* cannot be excluded (Tollenaere et al. 2010).

The location and mode of *Y. pestis* inoculation, needle or flea, yield different disease progressions (Sebbane et al. 2005; Shannon et al. 2015). We challenged mice via subcutaneous injection, a common practice in laboratory studies to achieve a standardized challenge dose. Even though this is not a natural route of plague transmission, the low mortality rate after plague challenge in our laboratory study illustrates that GHM from plague-endemic areas and naïve DM, even commercially bred strains, are partly resistant to moderately susceptible to plague. Oral consumption of SPV did not increase plague survival of either species, and seroconversion after bait consumption was low (20–30%).

The absence of a protective immune response in mice after consumption of SPV could be due to their low susceptibility to the vaccine vector (raccoon poxvirus [RCN]) or due to an inefficient immune response to *Y. pestis* antigens. Susceptibility to RCN is known to vary among laboratory mice strains

(Domenico et al. 2012), and poxviruses might induce a stronger immune response in younger individuals (Lane et al. 1970). For example, young Gunnison's prairie dogs (*Cynomys gunnisoni*) were more likely to survive plague challenge after SPV consumption than adults, whereas plague susceptibility was similar among ages (Rocke et al. 2015). In a recent study, seven 46–75-d-old white-footed mice (*Peromyscus leucopus*) seroconverted after oral exposure to RCN (J. Mandli, pers. comm., 7 March 2020). High survival and seropositivity in younger animals indicate that RCN might be more suitable as a vaccine vector for younger animals. Although we observed higher survival in the younger vaccinated animals, implying a possible age-dependent susceptibility to the vaccine, we could not compare this to an age-matched control group. Thus, this observation might be due to lower plague susceptibility in this age group, although age-related differences in susceptibility have not been reported previously.

Nearly 50% of the naive DM survived, which was surprising even in light of great variability in mouse susceptibility to *Y. pestis* in previous studies. For our study, we chose a challenge dose of 175,000 CFU (white mice LD50; 3–5 CFU), representing the median *Y. pestis* load of a single flea (Engelthaler and Gage 2000), and we expected high mortality in DM at this dose. In other studies, only one of four DM from a plague-free area survived challenge at 10,000 CFU, and at 10 bacilli, eight of eight DM died (Holdenried and Quan 1956). In comparison, three of eight GHM from plague-free areas succumbed after exposure to 14 bacilli and all of them died at exposure to 14,000–14,000,000 CFU (Thomas et al. 1988). Perhaps the captive-bred Sonoran DM we used are less susceptible than other *Peromyscus* species or free-ranging mice, because they originated from California where plague has been present since the early 1900s. Thomas et al. (1988) found that GHM from plague-endemic areas were more resistant than animals from plague-free areas; only one out of eight and three out of eight GHM succumbed at 18,000 and 180,000 CFU, respectively. This is similar to the mortality rate of our wild-caught GHM (seven of 16) from an endemic area.

The heterogeneous response of GHM and DM to *Y. pestis* in our study indicates that mice could survive *Y. pestis* spillover from prairie dogs during plague outbreaks. These observations reiterate that mice could be involved in plague dynamics because they do not appear to experience rapid die-offs like highly susceptible species. However, it remains unlikely that a fast turnover species can maintain the bacterium in the wild through flea-borne transmission, because high and likely lethal bacteremia are needed to infect fleas (Boegler et al. 2016). More interesting might be the survival of *Y. pestis* in rodents without signs of disease; *Y. pestis* was recovered from three *P. maniculatus rufinius* at 25–34 d postchallenge (Holdenried and Quan 1956) and nonencapsulated *Y. pestis* persisted for 9 wk in white mice and 56 wk in rats after subcutaneous inoculation (Williams and Cavanaugh 1983). If bacteria become available later in time, via late onset bacteremia or after the animal dies and is consumed, mice could be a reservoir for the pathogen. However, these alternatives have not been investigated in wild rodents. Repeating the vaccine and challenge study, preferably using flea inoculations in mice of known age, with prolonged observations, would help researchers assess age susceptibility to both SPV and *Y. pestis*. The results of our study can help guide future research efforts in assessing the role of mice in plague dynamics on US grasslands.

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