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Source: Journal of Wildlife Diseases, 44(3): 724-730

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-44.3.724

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Exposure of Small Rodents to Plague during Epizootics in Black-tailed Prairie Dogs

Paul Stapp,^{1,5,6} Daniel J. Salkeld,^{1,2,5} Rebecca J. Eisen,³ Ryan Pappert,³ John Young,³ Leon G. Carter,³ Kenneth L. Gage,³ Daniel W. Tripp,⁴ and Michael F. Antolin⁴ ¹Department of Biological Science, California State University, Fullerton, CA 92831, USA; ²IUCN-The World Conservation Union, 1630 Connecticut Avenue, Washington, DC 20009, USA; ³Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, CO 80522, USA; ⁴Department of Biology, Colorado State University, Fort Collins, CO 80523, USA; ⁵Authors contributed equivalently; ⁶Corresponding author (email: pstapp@fullerton.edu)

ABSTRACT: Plague, caused by the bacterium Yersinia pestis, causes die-offs of colonies of prairie dogs (Cynomys ludovicianus). It has been argued that other small rodents are reservoirs for plague, spreading disease during epizootics and maintaining the pathogen in the absence of prairie dogs; yet there is little empirical support for distinct enzootic and epizootic cycles. Between 2004 and 2006, we collected blood from small rodents captured in colonies in northern Colorado before, during, and for up to 2 yr after prairie dog epizootics. We screened 1,603 blood samples for antibodies to Y. pestis, using passive hemagglutination and inhibition tests, and for a subset of samples we cultured blood for the bacterium itself. Of the four species of rodents that were common in colonies, the northern grasshopper mouse (Onychomys leucogaster) was the only species with consistent evidence of plague infection during epizootics, with 11.1-23.1% of mice seropositive for antibody to Y. pestis during these events. Seropositive grasshopper mice, thirteen-lined ground squirrels (Spermophilus tridecemlineatus), and deer mice (Peromyscus *maniculatus*) were captured the year following epizootics. The appearance of antibodies to Y. pestis in grasshopper mice coincided with periods of high prairie dog mortality; subsequently, antibody prevalence rates declined, with no seropositive individuals captured 2 yr after epizootics. We did not detect plague in any rodents off of colonies, or on colonies prior to epizootics, and found no evidence of persistent Y. pestis infection in blood cultures. Our results suggest that grasshopper mice could be involved in epizootic spread of Y. pestis, and possibly, serve as a short-term reservoir for plague, but provide no evidence that the grasshopper mouse or any small rodent acts as a long-term, enzootic host for *Y. pestis* in prairie dog colonies.

Key words: Cynomys ludovicianus, epizootics, Onychomys leucogaster, plague, Yersinia pestis.

Although known typically by its effects on human populations, plague is primarily a disease of wild rodents. Plague is caused by the bacterium Yersinia pestis and spread by the bites of infectious fleas, direct contact with infected tissues, or rarely, inhalation of infectious respiratory droplets (Gage and Kosoy, 2005). In wild rodents, plague cycles are often characterized by short epizootics that cause widespread host die-offs, followed by long periods with little or no evidence of disease. Hypotheses to explain inter-epizootic (enzootic) maintenance of Y. pestis include persistence in fleas, soil, or in carcasses in burrows; or circulation in populations of rodents that contain both susceptible and resistant individuals (Gage and Kosoy, 2005). For this study, the term 'susceptible' refers to animals that die as a result of Y. pestis infection, whereas resistant individuals are those that develop detectable antibody responses and survive infection.

One of the most conspicuous consequences of the introduction of Y. pestis to North America is the extinction of entire colonies of black-tailed prairie dogs (Cynomys ludovicianus; Cully and Williams, 2001; Stapp et al., 2004; Pauli et al., 2006). Because plague rapidly kills most prairie dogs in a colony, and colonies are often several kilometers apart, it has been hypothesized that other small rodents act as reservoirs that help spread the bacterium within colonies during epizootics and allow it to persist in the absence of prairie dogs (Barnes, 1993). Several species, notably the deer mouse, *Peromyscus* maniculatus, have been suggested as a reservoir; deer mice with antibodies to Y.

pestis have been reported and Y. pestisinfected deer mouse fleas have been found in prairie dog colonies after die-offs (Lechleitner et al., 1968; Fitzgerald, 1970; Anderson and Williams, 1997). Others (Ubico et al., 1988; Cully et al., 1997; Holmes et al., 2003), however, have found no serologic evidence of Y. pestis exposure of small rodents during or after epizootics, and in general, there is little direct evidence that small rodents are required to maintain enzootic plague cycles (Gage and Kosoy, 2005).

Plague epizootics occur frequently in prairie dog colonies in shortgrass steppe in northern Colorado (Stapp et al., 2004). We collected and tested blood from small rodents, on and off colonies, before, during, and after epizootics of plague in black-tailed prairie dog colonies to determine whether small rodents are exposed to Y. pestis. Blood samples from a subset of individuals were also cultured to investigate whether rodents maintain persistent infections that would suggest a possible enzootic role. We focused on the northern grasshopper mouse (Onychomys *leucogaster*), for several reasons. Grasshopper mice are the most common nocturnal rodent in prairie dog colonies in the region (Stapp, 2007). They are wide-ranging, regularly use burrows of other rodents (Stapp, 1997), and harbor an unusually diverse assemblage of fleas, including known vectors of plague (Thomas, 1988; Cully et al., 2000). In the laboratory, grasshopper mice, from areas where plague is widespread, are much more resistant to Y. pestis-induced mortality than mice from areas without plague (Holdenreid and Quan, 1956; Thomas et al., 1988). Last, because grasshopper mice eat other rodents, infection may also occur via consumption of plague-infected prey (Thomas et al., 1989). A few seropositive grasshopper mice have been collected in retrospective surveys (Hudson and Kartman, 1967; Gage et al., 1994), but to date, there have been no field studies of plague exposure in wild grasshopper mouse populations. Our study is unique in that we sampled rodents in colonies before, during, and after three epizootic periods, as well as in grassland areas without prairie dogs.

We studied rodent populations in a network of colonies on the Pawnee National Grasslands (PNG) and Central Plains Experimental Range (CPER), Colorado, USA (40°48'N; 104°45'W), approximately 60 km south of Cheyenne, Wyoming.

The climate is semiarid, and the vegetation is shortgrass steppe, with large expanses of relatively homogeneous grass cover. Rodents were sampled in PNG and CPER colonies where plague was confirmed or suspected in 2004, 2005, and 2006 (Table 1). We also sampled in prairie dog colonies that remained active throughout the study period, in colonies that had been abandoned for ≥ 6 yr, and in nearby grassland areas without colonies.

Rodents were trapped on grids on colonies before, during, and after prairie dog epizootics. Each site had a single grid of 60 Sherman traps (1.35 ha) in May–July 2004; all grids were expanded to 100 traps (2.25 ha) for subsequent trapping sessions. Most of the larger colonies had three grids to maximize the number of captures. Each site was trapped 1–3 times between May and September in 2004 and 2005, and once in May or June 2006. Trap sessions consisted of four consecutive nights of trapping, with traps reset the following morning to capture diurnal thirteen-lined ground squirrels (Spermophilus tridecem*lineatus*). The CPER colonies were only sampled in June and July 2006, with grids of either 100 (CPER127) or 49 traps.

Captured rodents were anesthetized with isoflurane. Blood was collected from the retro-orbital sinus and placed on a Nobuto strip, separately placed in an Eppendorf tube, and then stored at -80 C. All animals were released at their capture location. Handling procedures were approved by the Institutional Animal

Epizootic year/colony (ha) ^a	Epizootic date	$Evidence \ of \ plague^b$	
2004			
PNG74 (23)	December 2003–February 2004	2	
PNG3 (52)	December 2003–February 2004	2, 3	
PNG41 (3)	April–May 2004	2, 3	
PNG62 (65)	May–July 2004	1, 2, 3	
2005			
PNG8 (13)	May–July 2005	1, 2, 3	
PNG5 (176)	May–July 2005	1, 2, 3	
PNG82 (25)	July–September 2005	1, 2, 3	
PNG35 (130)	July–September 2005	3	
PNG84 (18)	October–November 2005, April–May 2006	1, 2, 3	
2006			
PNG83/CPER135 (54)	March–May 2006	1, 3	
CPER127/128 (48)	May–June 2006	2, 3	
CPER122 (38)	June–August 2006	0	

TABLE 1. Black-tailed prairie dog colonies in northern Colorado that were extirpated by plague from 2004 through July 2006. Only colonies from which small rodent blood samples were collected are included.

^a Values in parentheses are the approximate active areas of the colony in 2003. Epizootic date is our best estimate of timing of mortality in prairie dogs.

^b Indicates whether the presence of plague was confirmed by: 1) postmortem examination of prairie dog carcasses at Centers for Disease Control (CDC); 2) positive PHA tests of blood of other rodents at CDC; or 3) presence of *Y. pestis*infected fleas, as determined by polymerase chain reaction tests (Stevenson et al., 2003); or was suspected based on rapid and near-complete mortality of prairie dogs and plague-confirmed mortality of nearby colonies (0).

Care and Use Committee at California State University Fullerton.

Blood on Nobuto strips was tested at the Centers for Disease Control and Prevention in Fort Collins, Colorado for the presence of antibodies to Y. pestis Fraction 1 (F1) antigen, using a passive hemagglutination test with corresponding inhibition tests (Chu, 2000). Titers of \geq 32 were considered seropositive for Y. pestis, indicating that individuals were exposed to, and survived, disease; laboratory studies (Thomas et al., 1988) suggest that antibodies are detectable in grasshopper mice as early as 10 days after exposure and for at least 90 days postinfection, depending on the amount of bacteria inoculated. For a subset of grasshopper mice captured on colonies undergoing epizootics, blood was tested for Y. pestis. Blood samples were vortexed briefly, and 4 µl of whole blood were streaked on blood agar plates containing 6% sheep blood. Plates were incubated at 25 C for 48 hr and examined for bacterial growth. From any samples that produced colony forming units with morphology consistent with Y. pestis, a subculture was streaked for isolation on blood agar plates, incubated at 37 C for 48 hr, and screened by direct immunofluorescent antibody tests targeting the F1 antigen (Chu, 2000). Bacterial or fungal growth was overwhelming on some plates; to avoid overlooking Y. pestis in these cases, all cultures yielding microbial growth were streaked on Yersinia-selective CIN (cefsulodin, irgasan, novobiocine) agar, incubated at 25 C for 48 hr, and examined for bacterial growth.

Ten rodent species were tested but four species made up >98% of captures. Of the 1,603 samples tested, 42.6% were from grasshopper mice, 28.5% were from ground squirrels, 21.1% were from deer mice, and 6.4% were from kangaroo rats (*Dipodomys ordii*); this roughly mirrors the order of abundance of these species in colonies (Stapp, 2007). Except for one instance, grasshopper mice were the only seropositive species detected during epizootics (Table 2). The exception was one ground squirrel (titer=32) that was

Species/colony ^a		2004		2005	
	May–July	August–September	April–July	August–September	May–July
Onychomys leucogaster					
2004 epizootic	$13 (3)^{c}$	51(1)	46(1)	0	60
2005 epizootic	37	11	73	49 (10)	64 (4)
2006 epizootic	14	0	28	0	27 (3)
Active colonies	61	23	36	3	0
Extinct/grasslands	56	12	16	2	0
Peromyscus maniculatus					
2004 epizootic	18	30	29(1)	2(2)	2
2005 epizootic	14	16	60	8	14
2006 epizootic	8	0	22	0	40
Active colonies	15	7	13	3	0
Extinct/grasslands	28	5	3	0	0
Spermophilus tridecemlineatu	s				
2004 epizootic	3	18	42(5)	0	33
2005 epizootic	9	2	46	15	89 (1)
2006 epizootic	7	0	22	0	13(1)
Active colonies	35	2	11	0	0
Extinct/grasslands	85	13	12	0	0
Dipodomys ordii					
2004 epizootic	2	12	11	0	13
2005 epizootic	0	1	9	0	9
2006 epizootic	0	0	0	0	4
Active colonies	6	16	13	0	0
Extinct/grasslands	4	3	0	0	0

TABLE 2. Numbers of rodent blood samples that were tested for antibody to *Yersinia pestis* in prairie dog colonies before, during, and after epizootics in northern Colorado.

^a Samples were also collected on active colonies (i.e., where no epizootics occurred) and in grassland areas and colonies that had been inactive for ≥ 6 years. Samples from other species (*Chaetodipus hispidus*, 10; *Perognathus flavus*, 3; *Reithrodontomys megalotis*, 4; *R. montanus*, 1; *Microtus ochrogaster*, 2; *Mus musculus*, 2) were all negative.

^b Samples from PNG84 were included in the 2006 epizootic results because samples were not taken during the epizootic until 2006.

^c Values in parentheses indicate number of seropositive samples (titer \geq 32).

trapped in May 2006 in an area of colony PNG84, where plague had extirpated prairie dogs the previous fall. During epizootics between 2004 and 2006, antibody prevalence in grasshopper mice ranged from 23% (3/13) in 2004, to 20% (10/49) in 2005, to 11% (3/27) in 2006 (Fig. 1). Higher antibody prevalence rates in grasshopper mice were observed in some colonies after peak prairie dog mortality; for example, in 29% (5/17) of individuals on PNG8 in August and September 2005; 33% (3/9) of individuals on CPER127 in June and July 2006; and in 60% (3/5) of individuals on PNG3 and PNG62 in May 2004.

Combining all years, 8.0% (17/213) of

grasshopper mice in colonies undergoing epizootics were seropositive. The year following an epizootic, 4.5% (5/110) of grasshopper mice, 7% (3/45) of deer mice, and 4.6% (6/131) of ground squirrels were seropositive. No seropositive animals were captured on colonies prior to, or 2 yr after, epizootics, or on sites without prairie dogs (Table 2). Antibody prevalence estimates in ground squirrels (11.9%, 5/42) and deer mice (9.7%, 3/31), sampled the year after the 2004 epizootic, were relatively high (Table 2). Two of the individuals (one grasshopper mouse, one deer mouse) captured in 2005, on the 2004 epizootic colonies, had elevated antibody titers (2,048 and 1,024, respectively), implying

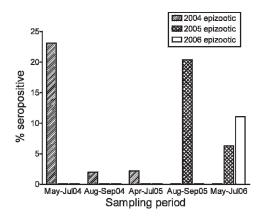


FIGURE 1. Antibody prevalence (% seropositive) of northern grasshopper mice (*Onychomys leuco-gaster*) for antibody to *Yersinia pestis* on prairie dog colonies, showing changes in exposure before, during, and after prairie dog epizootics in 2004, 2005, and 2006. Sample sizes are given in Table 2.

recent infection. These individuals were young-of-the-year, which suggests that Y. pestis persisted in the environment over winter and for >11 mo after the last prairie dogs died. Infectious fleas may have survived over this period (Kartman et al., 1962; Fitzgerald, 1970), or Y. pestis may have circulated in small rodent and flea populations independently of prairie dogs. It is not clear why seropositive deer mice or ground squirrels were found only after epizootics; individuals of these species may have died before sampling or they may have been infected by questing fleas over the following year.

During epizootics, antibody titers in grasshopper mice ranged from 32 to 4,096 (geometric mean[GM]=195.5). The year after an epizootic, titers of seropositive grasshopper mice ranged from 32 to 2,048 (GM=147.0). Ground squirrels generally had lower titers (GM= 64.0; range 32 to 128). One grasshopper mouse was seropositive when captured on 3 August 2004 (128) and again on 21 September (128), 7 wk later. Seven other individual grasshopper mice were tested multiple times during the study period; in all cases, the seropositive result came from the last capture. Five of these cases were from 2005 epizootics (PNG5, PNG8), with

39–44 days separation between a negative and positive result, corresponding to the period immediately after peak prairie dog mortality.

We were unable to culture Y. pestis from the blood of 43 grasshopper mice that were captured during epizootics in 2005, including from nine individuals that were seropositive, suggesting that mice do not maintain persistent, detectable infections. Sample size for these analyses was relatively small; however, and we do not know what fraction of these mice was actually exposed to Y. pestis. We also were not able to test for presence of Y. pestis in other tissues such as the spleen, kidneys, or bone marrow, from which Y. pestis has been recovered in asymptomatic individuals of other rodents (e.g., deer mice, Holdenreid and Quan, 1956; Microtus californicus, Goldenberg et al., 1964).

Our results suggest that grasshopper mice may be involved in epizootics in prairie dog colonies in shortgrass steppe. They were the only small rodents that consistently showed serologic evidence of Y. pestis infection during epizootics, and the appearance of antibodies to *Y. pestis* in grasshopper mice coincided with periods of high prairie dog mortality. Grasshopper mice may help spread the pathogen within, and among, colonies by consuming infected tissue (Thomas et al., 1989) or, possibly, by transporting and providing maintenance feedings for competent flea vectors. It is also possible that Y. pestis persists over the short term in resistant grasshopper mouse populations, which may serve as a source of new infections in the absence of prairie dogs following epizootics. However, antibody prevalence in grasshopper mouse populations declined after epizootics, with no seropositive individuals captured 2 yr afterward, and we did not detect plague in any rodents off colonies, or on colonies, prior to epizootics. In shortgrass steppe, plague epizootics therefore do not appear to occur independently of prairie dogs, suggesting that plague exposure in grasshopper mice might simply be a result of incidental 'spillover' from prairie dogs, which appear to be the primary amplifying host for *Y. pestis* in our study area.

We thank the National Science Foundation for support through the Emerging Infectious Disease program (EID-0327052) and the Shortgrass Steppe Long-Term Ecological Research project (DEB-0217631). We are indebted to M. Schriefer, H. Franklin, J. Kraft, D. Kite, J. Holm, C. Cannon, A. Benson, H. Houghton, C. Knox, C. Wermager, E. Humphrey, and M. Lindquist for their assistance.

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Received for publication 12 August 2006.