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Prevalence of *Yersinia pestis* in Rodents and Fleas Associated with Black-tailed Prairie Dogs (*Cynomys ludovicianus*) at Thunder Basin National Grassland, Wyoming

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ABSTRACT: Rodents (and their fleas) that are associated with prairie dogs are considered important for the maintenance and transmission of the bacterium (*Yersinia pestis*) that causes plague. Our goal was to identify rodent and flea species that were potentially involved in a plague epizootic in black-tailed prairie dogs at Thunder Basin National Grassland. We collected blood samples and ectoparasites from rodents trapped at off- and on-colony grids at Thunder Basin National Grassland between 2002 and 2004. Blood samples were tested for antibodies to *Y. pestis* F-1 antigen by a passive hemagglutination assay, and fleas were tested by a multiplex polymerase chain reaction, for the presence of the plague bacterium. Only one of 1,421 fleas, an *Oropsylla hirsuta* collected in 2002 from a deer mouse, *Peromyscus maniculatus*, tested positive for *Y. pestis*. Blood samples collected in summer 2004 from two northern grasshopper mice, *Onychomys leucogaster*, tested positive for *Y. pestis* antibodies. All three positive samples were collected from on-colony grids shortly after a plague epizootic occurred. This study confirms that plague is difficult to detect in rodents and fleas associated with prairie dog colonies, unless samples are collected immediately after a prairie dog die-off.

Key words: *Cynomys ludovicianus*, fleas, *Oropsylla hirsute*, plague, prevalence, rodents, *Yersinia pestis*.

Plague is a vector-borne zoonosis introduced in the United States, where it was first identified on the West Coast around 1900. *Yersinia pestis*, the etiologic agent of plague, is a gram-negative coccobacillus (Enterobacteriaceae) that is transmitted to animals by infected flea-bites, direct contact, or inhalation of respiratory droplets from an infected animal (Gage et al., 1995; Webb et al., 2006). Plague causes large population reductions in rodents of several species within its native and introduced ranges (Barnes, 1993). Sylvatic plague is thought to be primarily maintained among wild rodent populations and is transmitted by several flea species that occur on vertebrate hosts (Thomas, 1988; Anderson and Williams, 1997; Cully et al., 1997). Plague causes close to 100% mortality in infected colonies (Cully et al., 1997) of rodents such as prairie dogs and ground squirrels. All species of prairie dogs are susceptible to the disease (Perry and Fetherston, 1997) and their presence may amplify plague epizootics, resulting in high mortality. However, other rodent hosts are presumed important for enzootic maintenance and transmission by circulating low levels of the pathogen (Poland and Barnes, 1979; Cully, 1993; Biggins and Kosoy, 2001; Gage and Kosoy, 2005).

Plague was first identified in Wyoming in 1936 in ground squirrel fleas from Yellowstone National Park and, since then, has been identified in animals or fleas from 20 counties (Wyoming Department of Health). Prairie dog colonies underwent an 89% reduction between 2001 and 2002 at Thunder Basin National Grassland; although colonies grew in size between 2002 and 2004, eight colonies had decreased prairie dog activity in 2004 (Cully and Johnson, 2005), and these were attributed to plague epizootics (Byer, 2001; Cully and Johnson, 2005).

Interspecific transmission could be a key to maintaining plague within a community if more than one species act as enzootic hosts for the transmission of plague to prairie dogs. In this study, we tested rodents and their fleas for *Y. pestis*,

or for presence of antibodies to *Y. pestis*, to identify potential enzootic rodent hosts, flea vectors, and the rodent—flea complexes that might be important for the maintenance of plague among prairie dogs at Thunder Basin National Grassland, Wyoming, an area which has been undergoing an epizootic since 2000–2001 (Byer, 2001).

Thunder Basin National Grassland is located in northeastern Wyoming (105.0°W, 43.6°N) in the Powder River Basin between the Black Hills and the Big Horn Mountains. The area is interspersed with private, state, and federal lands. It covers 231,481 ha, and the elevation ranges between 1,000 m and 1,500 m, with a semiarid climate. Johnson (2005), Thiagarajan (2006), and Pauli et al. (2006) provide a detailed description of the study area. In 1999, prior to plague, prairie dog colonies covered at least 6,597 ha at Thunder Basin (Cully and Johnson, 2005).

At Thunder Basin, we trapped rodents on six grids located on prairie dog colonies, and on six grids positioned 500–2,000 m distant from the nearest prairie dog colony in the surrounding grasslands. Each grid consisted of 49 trap stations (7×7), with a single Sherman live trap (H. Sherman Traps, Tallahassee, Florida, USA) at each station. Stations were separated by 20 m. Traps were opened and baited with oatmeal in the late afternoon, checked for captures the next morning, and left closed during the daytime. The same grids were trapped for three consecutive nights, on two separate occasions, each year; once in May–June and once in July–August, from 2002–2004.

All captured rodents were moved to a processing site where they were ear-tagged, sexed, identified to species, and examined for ectoparasites. Animals recaptured within the same 3-day trapping session were not reprocessed. Rodents were anesthetized with a mixture of isofluorane and oxygen using a vaporizer (SurgiVet, Waukesha, Wisconsin, USA). After anesthetization, rodents were held over a white plastic tray and combed vigorously for fleas with a plastic toothbrush (Gage, 1999). Additional fleas were collected from the plastic bag used in handling the animal and from the anesthetizing jar. Fleas collected from each animal were preserved in a vial with 2% saline solution and sent to the Flea-Borne Disease Laboratory at the Centers for Disease Control and Prevention (CDC) in Fort Collins, Colorado, USA for identification. We collected blood samples (200 µl) from the retro-orbital sinus of rodents captured from 2002 to 2004; however, only samples from 2003 and 2004 were tested. We limited the number of rodents processed for fleas and blood to 10 individuals of each species obtained from each grid in 2003 and 2004; the sex of these 10 individuals was not considered as part of the selection process. Blood samples were coated onto a nobuto strip per the manufacturer's instructions (Advantec MFS, Dublin, California, USA), air-dried, and stored individually in marked envelopes prior to processing for serology. All rodents were released at their capture sites after data collection. The research methods were approved by the Institutional Animal Care and Use Committee of Kansas State University.

Fleas were identified to species (Lewis, 1993, 2000, 2002) and sexed under a dissecting microscope (Hubbard, 1947; Stark, 1958, 1970; Furman and Catts, 1982). Voucher specimens of all flea species were collected and stored at CDC. Flea samples were tested for the presence of the *Y. pestis* bacterium and *Bartonella* by a multiplex polymerase chain reaction (Stevenson et al., 2003). Blood samples from the nobuto strips were tested for the presence of antibodies to *Y. pestis*-specific F-1 antigen by a passive hemagglutination assay (Chu, 2000). All laboratory diagnostic tests were performed at CDC.

We captured 1,773 rodents in 3 yr, and of these, 529 were recaptures. The number of individual rodents of each species captured varied among years. We tested

TABLE 1. Number of fleas collected (number of rodents infested) from various rodent species that were tested for presence of *Yersinia pestis* by multiplex PCR, from off- and on-colony grids.

Rodent species	2002		2003		2004	
	Off-colony	On-colony	Off-colony	On-colony	Off-colony	On-colony
Dipodomys ordii		14 (8)		1 (1)	4 (2)	4 (3)
Lemmiscus curtatus					33 (1)	5 (3)
Microtus ochrogaster			1 (1)			
Onychomys leucogaster	37 (10)	51 (16)	12 (4)	63 (22)	13 (7)	26 (14)
Perognathus fasciatus					7 (2)	7 (2)
Peromyscus leucopus	4 (1)					
Peromyscus maniculatus	128 (45)	86 (41)	132 (64)	135 (51)	279 (82)	314 (83)
Reithrodontomys megalotis					2 (1)	
R. montanus		1 (1)				
Spermophilus tridecemlineatus	37 (6)	6 (2)	11 (6)	8 (4)	1 (1)	8 (4)

493 blood samples from 2003 and 2004, and 1,428 fleas from 2002–2004 for *Y. pestis*. Of the 493 blood samples tested, 241 samples were from rodents captured at off-colony grids, including *Onychomys leucogaster* ($n=27$), *Peromyscus maniculatus* ($n=198$), *Spermophilus tridecemlineatus* ($n=17$), *Dipodomys ordii* ($n=2$), and *Lemmiscus curtatus* ($n=3$); and 252 samples were collected from on-colony grids, including *O. leucogaster* ($n=43$), *P. maniculatus* ($n=181$), *S. tridecemlineatus* ($n=11$), *D. ordii* ($n=14$), and *L. curtatus* ($n=1$). Fourteen species of fleas were collected from rodents, and both *O. leucogaster* and *P. maniculatus* harbored a wide diversity of fleas at both off- and on-colony grids (Table 1). All of the prairie dog colonies on which we established trapping grids were active in 2002. However, in 2004, grids on PD10, PD13, and PD14 had no prairie dog activity; grids PD9 and PD11 were reduced in colony size from 2003, but were active. Among the fleas, an *Oropsylla hirsuta* female from a *P. maniculatus* male at an on-colony grid (PD11) in July 2002 tested positive for *Y. pestis*. From the serologic testing, two *O. leucogaster* from the same prairie dog colony (PD9) tested positive, with a 512 titer for *Y. pestis* antibodies. All other flea and serology samples were negative for *Y. pestis*.

Yersinia pestis-infected fleas have been collected from prairie dogs and rodents during epizootics or from prairie dog burrows for as long as a year after an epizootic (Lechleitner et al., 1968; Cully et al., 1997). Lechleitner et al. (1968) found one *Y. pestis* seropositive deer mouse, *P. maniculatus*, and Cully et al. (1997) found seropositive prairie dogs during plague epizootics. In the present study, we collected a *Y. pestis*-infected *O. hirsuta*, a common prairie dog flea, from a deer mouse in 2002 and captured two seropositve northern grasshopper mice, *O. leucogaster*, in 2004. The deer mouse with the *Y. pestis*-positive flea was captured on two more days within the same trapping session, and only one flea was present on this rodent. Given that this was the only flea (common to prairie dogs) collected from a different rodent host in 2002, and that it was positive for *Y. pestis* among 362 fleas tested from Thunder Basin in that year, it seems most probable that the flea changed hosts during the epizootic or was picked up from a prairie dog burrow. Presence of this infected flea on a deer mouse shows one potential mechanism and route for interspecific transmission of *Y. pestis* through fleas between prairie dogs and rodents, though prairie dog fleas were not common on other rodent hosts. We did not detect any *Y. pestis*-positive fleas in 2003 or 2004. Persistence of plague on the landscape since 2000–2001

TABLE 2. Flea species and the number of fleas collected, from *Onychomys leucogaster* and *Peromyscus maniculatus* that were tested for the presence of *Y. pestis*, from off- and on-colony grids. Refer to Table 1 for the numbers of *O. leucogaster* and *P. maniculatus* infested with fleas.

		2002		2003		2004	
Rodent species	Flea species	Off-colony	On-colony	Off-colony	On-colony	Off-colony	On-colony
O. leucogaster	*Atheca wagneri*	6	13	4	36	1	1
	Foxella ignota	25		4		2	
	Meringis parkeri	1	1		2		
	Oropsylla tuberculata		1			1	9
	Peromyscopsylla hesperomys	4	26		16		
	Pleochaetis exilis		7		1		
	Rhadinopsylla spp.		1				
	Thrassis fotus	1	2	4	8	6	5
P. maniculatus	*Atheca wagneri*	111	72	117	119	258	285
	Epitedia wenmanni	1				1	
	Callistopsyllus deuterus			2			
	Foxella ignota	2	1				
	Malareus telchinus	1		2	5	3	2
	Meringis parkeri				1	1	
	Oropsylla hirsuta	1	1				
	Orchopeas leucopus			1		9	3
	Orchopeas sexdentatus	1					
	Peromyscopsylla hesperomys	6	7	6	8	7	24
	Pleochaetis exilis	1					
	Thrassis fotus	4	5	4	2		

was confirmed by the seroconversion of the two *O. leucogaster* captured on the same prairie dog colony during June and July of 2004. The titer value of 512 probably indicates either an anamnestic immune response to repeated exposure or recent exposure to the *Y. pestis* antigen.

Onychomys leucogaster was more abundant on black-tailed prairie dog colonies at Thunder Basin, compared to off-colony grids, and had a wide diversity of flea species and high flea loads (Table 2; Thiagarajan, 2006). Grasshopper mice were the only species in our study that tested positive for *Y. pestis* antibodies; they have been considered as alternate hosts for plague by Thomas et al. (1988), Webb et al. (2006), and Stapp (2007), and the presence of resistant individuals at Thunder Basin indicates that they may be important for maintenance and transmission of plague in this area. Rodents such as deer mice may also play an important role in the maintenance and or transmission of plague; they were present in higher numbers than other rodent species and harbored very high flea loads and diversity. Because deer mice were also abundant and widespread away from prairie dog colonies, they provided continuity among populations on the landscape, a continuity that might support disease transmission locally. At other areas where plague is present but grasshopper mice are not, deer mice are considered as potential maintenance hosts (Cully et al., unpubl.).

There was widespread plague activity among prairie dog colonies in Thunder Basin from 2002–2004. However, evidence of plague was rare among other rodents and fleas. Holmes et al. (2006) found no evidence of persistent *Y. pestis* infection in small mammals on prairie dog colonies in Phillips County, Montana, and suggested that prairie dog colonies may not be the focal area for the maintenance

of the pathogen. As widespread plague activity has been seen among prairie dogs in Thunder Basin since 2001, our possible explanation for our few positive samples could be that enzootic hosts circulate *Y. pestis* in very low levels, with low susceptibility and a fast turn-over rate. Mathematic models developed by Webb et al. (2006) showed that a short-term reservoir is needed for the transmission of plague epizootics in prairie dogs, and that small mammals that are resistant to plague, including the grasshopper mouse, could be short-term reservoirs in our study area. Recently, Hanson et al. (2007) reported *Y. pestis* prevalence in low levels in prairie dog fleas in the absence of die-offs, and suggested that plague could be maintained among prairie dog colonies in an enzootic state. Our study was also limited by our sampling effort, in that we were not able to trap small mammals throughout the plague outbreak. Our study reaffirms that plague is difficult to detect in rodents and fleas, unless samples are collected immediately after a prairie dog die-off (Gage, 1999), and that the mechanisms involved in the maintenance and transmission of plague are not clearly understood.

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