No Evidence of Persistent *Yersinia pestis* Infection at Prairie Dog Colonies in North-central Montana

Brian E. Holmes,[1,3] Kerry R. Foresman,[1] and Marc R. Matchett[2]

[1]Division of Biological Sciences, University of Montana, Missoula, Montana 59812, USA

[2]Charles M. Russell National Wildlife Refuge, Lewistown, Montana 59457, USA

[3]Corresponding author (phone: 406-829-6669, email: bholmes@tfn.net)

ABSTRACT: Sylvatic plague is a flea-borne zoonotic disease caused by the bacterium *Yersinia pestis* that can cause extensive mortality among prairie dogs (*Cynomys*) in western North America. It is unclear whether the plague organism persists locally among more resistant host species following epizootics or instead persists elsewhere. From June – August 2002 and 2003 we collected serum and flea samples from small mammals at prairie dog colonies with a history of plague. During this same period we also collected samples at prairie dog colonies with no history of plague and from off-colony sites where plague history was unknown. Sera were screened for antibody to *Y. pestis* by means of enzyme-linked immunosorbent assay or passive hemagglutination assay and fleas were screened by polymerase chain reaction. All material was negative for evidence of *Y. pestis* infection including 156 sera and 553 fleas from colonies with a known history of plague. This and other studies provide evidence that *Y. pestis* infection may not persist at prairie dog colonies following an epizootic.

KEY WORDS: *Cynomys*, fleas, prairie dogs, sylvatic plague, *Yersinia pestis*

Sylvatic plague is a flea-borne zoonotic disease of mammals caused by the bacterium *Yersinia pestis*. The disease primarily affects wild rodents, although many other groups of wild and commensal mammals can become infected (Gage et al., 1995). *Yersinia pestis* likely evolved in Asia and has since been introduced onto all continents except Antarctica and Australia. In North America, the presence of *Y. pestis* was first identified ~1900, having arrived in Pacific Coast ports via infected rats (*Rattus* sp.) on ships from Asia (Eskey and Haas, 1940). Today, the range of the disease in North America includes areas west of the 100th meridian (Cully et al., 2000).

The maintenance of plague infection in the wild depends on a complex set of interactions between host, vector, pathogen, and environmental factors that are still poorly understood. In

general, the sylvatic cycle of infection is characterized by relatively stable periods of enzootic activity where *Y. pestis* circulates at low levels within the "maintenance" host community, followed by explosive epizootics involving one or more species of "amplifying" host that often experience high mortality. In western North America, these epizootic hosts include species of prairie dogs (*Cynomys*) in which plague-associated die-offs can be particularly dramatic, with mortality often approaching 100% within colonies (Rayor, 1985; Menkens and Anderson, 1991).

Prairie dogs often repopulate colonies following epizootics (Menkens and Anderson, 1991; Cully et al. 1997) and these colonies may then persist for many years or experience a plague epizootic again. Barnes (1982) reported a recurrence of plague epizootics within four to five years and Cully et al. (1997) reported an epizootic again after three years. Whether these cases in which the same colonies experience plague again represent a continued presence of infection among hosts in that area or a reintroduction of *Y. pestis* from surrounding areas is not known. The objective of this study was to address two competing hypotheses regarding the interepizootic maintenance of sylvatic plague: 1) following an epizootic, *Y. pestis* infection persists in an area at low levels within the enzootic host community, or 2) following an epizootic, infection does not persist in a localized area and recurring plague epizootics result from a reintroduction of the disease (i.e. *Y. pestis* is absent during the period of recovery). To test this we collected serum and flea samples from small mammals at prairie dog colonies with a history of sylvatic plague epizootics and screened them for evidence of *Y. pestis*. In addition, we also collected samples from prairie dog colonies with no known history of plague and from off-colony sites where plague history was unknown.

The study took place in southern Phillips County, Montana, USA (47°35' to 47°50'N, 107°45' to 108°45'W) during June – August 2002 and 2003 (Figure 1). The area is characterized

by shrub and grassland habitats typical of the northern Great Plains, with big sagebrush (*Artemisia tridentata*), black greasewood (*Sarcobatus vermiculatus*), western wheatgrass (*Agropyron smithii*), and blue grama (*Bouteloua gracilis*) as common species. In addition, the southern margin of the county borders the Missouri River and consisted of forested "breaks" topography with ponderosa pine (*Pinus ponderosa*), Douglas-fir (*Pseudotsuga menziesii*), and Rocky Mountain juniper (*Juniperus scopulorum*). Elevations of study sites were between 740 and 1,050m. The area was a mosaic of federal, state, and private land ownership and has supported ~300 active black-tailed prairie dog (*C. ludovicianus*) colonies during the last 20 years. The majority of study sites were located on the Charles M. Russell National Wildlife Refuge (CMR) with the remainder located on adjacent Bureau of Land Management (BLM) lands north of the refuge.

We sampled 36 sites in 2002 and 60 sites (36 resampled from 2002 and 24 new) in 2003. In total, 15 sites were prairie dog colonies with a history of plague, 15 were prairie dog colonies with no history of plague, and 30 were off-colony sites. Sites with a history of plague were identified through regular mapping efforts by CMR and BLM personnel such that the location and year of epizootics among prairie dogs were known. Plague epizootics occurred between 1992 and 2001 at sites included in this study (Figure 1). We are confident that die-offs attributed to plague were in fact plague epizootics because no other disease has yet been identified that causes such high mortality in prairie dogs (Barnes, 1993) and antibody to *Y. pestis* has been consistently found in coyotes (*Canis latrans*) and badgers (*Taxidea taxus*) in the study area (M. R. Matchett, unpublished data). Off-colony sites occurred in a variety of habitats and were located >400m from the nearest prairie dog colony. Because we used prairie dogs as our sentinel species to indicate the presence or absence of plague epizootics over time and off-colony sites

had no prairie dogs present for at least the last 20 years, plague history at these sites was unknown.

Each study site consisted of a 10 x 10 grid of 100 Sherman live-traps (H.B. Sherman, Tallahassee, Florida, USA) with 10m spacing and 20 Tomahawk live-traps (Tomahawk Live Trap Co., Tomahawk, Wisconsin, USA) placed at prairie dog burrows on colony sites and systematically throughout the grid at off-colony sites. We anesthetized captured animals with isoflurane ("IsoFlo" Abbot Laboratories, North Chicago, Illinois, USA or "IsoSol" Halocarbon Laboratories, River Edge, New Jersey, USA) prior to blood and flea sampling. Fleas were collected from animals using a conventional flea comb as well as from prairie dog burrows using a swabbing technique described elsewhere (see Holmes, 2003). Fleas were stored in vials containing 2% NaCl solution with a small amount (<0.01%) of Tween 80, identified to species, and then frozen. We collected blood samples of ~200 µl from the retro-orbital sinus of small rodents using micro-hematocrit tubes (Chase Scientific, Rockwood, Tennessee, USA) and for larger animals such as prairie dogs and cottontails (*Sylvilagus audubonii*) we collected blood by clipping a hindfoot toenail to induce bleeding. In 2002, whole blood samples were stored in a conventional (-20°C) freezer upon return from the field. In 2003, most blood samples were centrifuged the day of collection to separate off serum which was then stored as above. The remainder of samples were collected onto individual Nobuto filter papers (Advantec MFS, Pleasanton, California, USA) which were air-dried, placed in paper envelopes, and stored at room temperature.

Laboratory diagnostics were performed at the Centers for Disease Control and Prevention, Division of Vector-Borne Infectious Diseases, Plague Section, Fort Collins, Colorado, USA. Serologic analyses followed protocols described by Chu (2000). We screened

serum samples for the presence of antibody against *Y. pestis*-specific Fraction 1 antigen using either a competitive enzyme-linked immunosorbent assay (cELISA) or a passive hemagglutination assay (PHA); all Nobuto strips were screened using PHA. Flea pools of one to 10 individuals (corresponding to the same species, host, date, and site of capture) were screened for the presence of *Y. pestis* using a multiplex polymerase chain reaction (PCR) assay described by Stevenson et al. (2003).

The number and source of samples screened for evidence of *Y. pestis* infection by serologic and PCR analysis is given in Tables 1 and 2, respectively. We screened a total of 156 serum samples and 553 fleas from small mammals trapped at prairie dog colonies with a history of plague over two summers and all were negative. Likewise, all material from prairie dog colonies with no history of plague (369 sera, 1,894 fleas) and from off-colony sites (439 sera, 603 fleas) was also negative for evidence of *Y. pestis* infection. Although we sampled an equal number of prairie dog colonies with and without a history of plague in each year of the study, the number of diagnostic samples collected at colonies with no history of plague was greater because capture rates were consistently higher at those sites than at colonies with a history of plague.

Two previous studies (Lechleitner et al., 1968; Cully et al., 1997) followed the progression of plague epizootics among prairie dogs and demonstrated that, in general, plague-positive fleas from prairie dogs, their burrows, and associated mammals are most likely to be collected during the course of an epizootic. These studies also illustrated that one year following the epizootic some plague-positive fleas may still be present in prairie dog burrows, but by two years following the epizootic there is little or no evidence of *Y. pestis* infection in the vector community. Serologic results from these two studies also failed to document evidence of persistent infection in the enzootic host community at the affected colonies. Lechleitner et al.

(1968) found only one of 108 deer mice (*Peromyscus maniculatus*) seropositive (the one seropositive came during the active epizootic) and Cully et al. (1997) only found prairie dogs seropositive. In both studies the epizootic appeared to diminish over the course of about a year. Interestingly, Davis et al. (2004) also found evidence of a one to two year "fade-out" period following plague epizootics among populations of the great gerbil (*Rhombomys opimus*) in Asia when evidence of *Y. pestis* infection was still detectable but after which the populations were apparently plague-free.

We found no evidence that *Y. pestis* infection persists at black-tailed prairie dog colonies with a history of plague, at least in the host and vector species that we sampled. The most conservative interpretation of these negative data is that *Y. pestis* infection was not widespread among small mammals in southern Phillips County. However, if infection occurred in isolated pockets or was present in only a small proportion of the small mammal community we may not have been able to detect it with our sampling effort. What these data also suggest is that prairie dog colonies *per se* are not ideal focal areas for the long-term maintenance of *Y. pestis* infection in our study area. The mechanism by which infection persists is still unclear, though.

One potential scenario is that the disease continually moves across the landscape, driven by new infection of susceptible hosts. However, the patchy distribution of plague-affected prairie dog colonies in Phillips County does not appear to support this hypothesis, although several colonies in close proximity to one another were often affected at once. Another possibility is that the disease persists in discrete enzootic foci that maintain the appropriate conditions for long-term persistence of the disease and may serve as sources for observed epizootics among highly susceptible species such as prairie dogs. In Phillips County, this may occur off of prairie dog colonies where the diversity of potential enzootic hosts is higher

(Holmes, 2003). Maintenance of *Y. pestis* infection is thought to be dependent upon this continued circulation among competent hosts (Barnes, 1993) and several authors (Olsen, 1981; Gage et al., 1995; Biggins and Kosoy, 2001) have proposed that the factors most likely to support permanent plague foci include several host species co-occurring in areas of diverse or patchy habitats. If so, areas where such diverse habitats occur in proximity to prairie dog colonies - that is, where both the proposed enzootic and epizootic components of the plague system coexist - may prove important in supporting permanent plague foci and perpetuating epizootics.

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TABLE 1. Number and source of serum samples collected in Phillips County, Montana during June – August 2002 and 2003. Samples are from prairie dog colonies with a history of plague (Plague), prairie dog colonies with no history of plague (No Plague), and off-colony sites (Off).

	2002			2003		
Host	Plague	No Plague	Off	Plague	No Plague	Off
Cynomys ludovicianus	22	36	-	17	32	-
Microtus ochrogaster	-	-	-	1	-	20
Neotoma cinerea	-	-	-	-	-	4
Onychomys leucogaster	1	1	1	12	6	3
Peromyscus maniculatus	45	84	98	53	205	302
Reithrodontomys megalotis	-	-	-	-	-	4
Sylvilagus audubonii	3	1	-	2	4	4
Tamias minimus	-	-	-	-	-	3
Total	71	122	99	85	247	340

TABLE 2. Number and source of fleas collected for plague testing in Phillips County, Montana during June – August 2002 and 2003. Samples are from prairie dog colonies with a history of plague (Plague), prairie dog colonies with no history of plague (No Plague), and off-colony sites (Off). The flea species and total number tested are given for each host.

	2002			2003		
Host	Plague	No Plague	Off	Plague	No Plague	Off
Cynomys ludovicianus [a]	67	683	-	83	310	-
Microtus ochrogaster [b]	-	-	-	4	-	44
Neotoma cinerea [c]	-	-	-	-	-	86
Onychomys leucogaster [d]	3	11	-	22	8	6
Peromyscus maniculatus [e]	125	233	119	129	402	328
Reithrodontomys megalotis [f]	-	-	-	-	-	3
Sylvilagus audubonii [g]	1	-	-	2	6	17
Prairie Dog Burrow [h]	48	145	-	69	96	-
Total	244	1,072	119	309	822	484

[a]*Oropsylla hirsuta* (n=208), *Oropsylla tuberculata* (n=68), *Pulex simulans* (n=867)

[b]*Aetheca wagneri* (n=4), *Malareus telchinus* (n=2), *Peromyscopsylla hesperomys* (n=2), *Orchopeas leucopus* (n=40)

[c]*Aetheca wagneri* (n=9), *Eumolpianus eumolpi* (n=1), *Orchopeas agilis* (n=76)

[d]*Aetheca wagneri* (n=22), *Foxella ignota* (n=5), *Malareus telchinus* (n=3), *Peromyscopsylla hesperomys* (n=20)

[e]*Aetheca wagneri* (n=1,145), *Callistopsyllus terinus* (n=4), *Epitedia wenmanni* (n=1), *Eumolpianus eumolpi* (n=1), *Foxella ignota* (n=1), *Malareus telchinus* (n=82), *Orchopeas leucopus* (n=11), *Peromyscopsylla hesperomys* (n=91)

[f]*Aetheca wagneri* (n=3)

[g]*Aetheca wagneri* (n=2), *Cediopsylla inaequalis* (n=24)

[h]*Aetheca wagneri* (n=11), *Oropsylla hirsuta* (n=147), *Oropsylla tuberculata* (n=40), *Peromyscopsylla hesperomys* (n=1), *Pulex simulans* (n=159)

FIGURE 1. Map of southern Phillips County, Montana showing the location of study sites including prairie dog colonies with a history of plague (asterisks), colonies with no history of plague (stars), and off-colony sites (circles). The year of plague epizootics is also indicated. Grey triangles are point locations of additional prairie dog colonies in the study area, some of which have been affected by plague, some not, and many where plague history is uncertain.